ISSN 2413-6077
e-ISSN 2414-9985

I. Horbachevsky Ternopil National Medical University

International Journal of Medicine and Medical Research

Scientific-Practical Journal

Founded in 2015
Frequency: semiannually

Volume 10, No. 2

Ternopil – 2024

INTERNATIONAL JOURNAL OF MEDICINE AND MEDICAL RESEARCH

Founder:
I. Horbachevsky Ternopil National Medical University

Year of foundation: 2015

Recommended for printing and distribution via the Internet by the Academic Council I. Horbachevsky Ternopil National Medical University (Minutes No. 13 of November 26, 2024)

State Registration:
Media identifier R30-02201.
Decision of the National Council of Television and Radio Broadcasting of Ukraine No. 1551, Minutes No. 28, dated 23.11.2023.

The journal is included in the list of Professional Scientific Publications of Ukraine
Category "B" Specialties: 0511 – Biology; 0912 – Medicine; 0916 – Pharmacy; 0512 – Biochemistry according to the Order of Ministry of Education and Science No. 612, 7 May 2019 and 25 November 2019

The journal is presented international scientometric databases, repositories and scientific systems: Google Scholar, Journal TOCs, ROAD, Base, DOAJ, Polska Bibliografia Naukowa, Ulrich's Periodicals Directory, EuroPub, Professional publications of Ukraine, National Library of Ukraine named after V.I. Vernadskyi, UCSB Library, Dimensions, German Union Catalogue of Serials, University of Oslo Library, University of Hull Library, SOLO – Search Oxford Libraries Online, European University Institute, Leipzig University Library, Cambridge University Library, Open Ukrainian Citation Index, Worldcat, CORE

International Journal of Medicine and Medical Research / Ed. by M. Korda. (Editor-in-Chief) et al. Ternopil: I. Horbachevsky Ternopil National Medical University, 2024. Vol. 10, No. 2. 90 p.

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International Journal of Medicine and Medical Research

Journal homepage: https://ijmr.com.ua/
Volume 10, No. 2. 2024

UDC 616.379-008.64-08:616.8-008.64
DOI: 10.61751/ijmmr/2.2024.06

Article's History:
Received: 21.08.2024; Revised: 01.11.2024; Accepted: 26.11.2024

Prevalence of depression among diabetic patients attending outpatient and inpatient

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Abstract. *Diabetes mellitus* is a collection of metabolic illnesses characterised by persistent hyperglycemia, resulting in consequences affecting several organ systems. Depression is a common and impactful psychosocial condition in diabetic patients, further hindering glycemic control and negatively affecting overall health outcomes. The present study investigated the depression prevalence among *diabetes mellitus* patients in outpatient treatment and its impact on diabetes management. This hospital-based cross-sectional observational study was conducted over 18 months. A sample of 125 *diabetes mellitus* patients was selected based on a 9% prevalence rate with a 5% margin of error and a 95% confidence interval. Depression was evaluated via the 9-item Patient Health Questionnaire Depression Screening Tool. Clinical evaluation comprised HbA1c, fasting plasma glucose, and postprandial glucose measurements. Statistical analysis was conducted utilising SPSS software. The study determined that 29.6% of diabetic patients were diagnosed with depression, with varying degrees of severity. Depression was associated with poorer glycemic control, evidenced by significantly higher levels of HbA1c, fasting plasma glucose, and postprandial glucose. Patients with depression also had a higher prevalence of diabetes-related complications, such as nephropathy and neuropathy. The study concluded that depression is prevalent among patients with diabetes and has a significant impact on diabetes management. The practical value of this study is that integrated care, including screening and treatment of mental disorders, is crucial to the improvement of the outcomes of diabetes patients

Keywords: *diabetes mellitus*; glycemic control; hyperglycemia; diabetes complications

Introduction

Diabetes mellitus (DM) is a collection of metabolic illnesses marked by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. These metabolic abnormalities significantly impact the processing of carbohydrates, proteins, and fats, often accompanied by vascular damage, leading to complications affecting multiple organ systems, as highlighted by F.J. Snoek [1]. These complications, including cardiovascular disease,

Suggest Citation:
Agaz K, Khan A, Kaur A. Prevalence of depression among diabetic patients attending outpatient and inpatient. Int J Med Med Res. 2024;10(2):6–15. DOI: 10.61751/ijmmr/2.2024.06

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neuropathy, and retinopathy, are the leading causes of morbidity and mortality among individuals with diabetes, as noted by F.S. Yen *et al.* [2]. B.K. Jha *et al.* [3] noted that Type 2 *diabetes mellitus* (T2DM) is a major challenge due to the prolonged asymptomatic phase of hyperglycemia that precedes diagnosis, complicating the treatment of both glucose and insulin. Furthermore, B.K. Jha *et al.* [3] emphasised that the interplay between hyperglycemia and insulin resistance significantly increases the risk of cardiovascular diseases, contributing to the complexity of managing T2DM.

The high prevalence of depression among individuals with diabetes is a significant psychosocial aspect of diabetes management, as described by S. Akshatha & U.B. Nayak [4]. H. Abuhegazy *et al.* [5] reported that nearly one-third of T2DM patients experience depression. J.A. Agyekum *et al.* [6] determined that depression among diabetic patients is correlated with poor quality of life, a higher risk of complications, and inadequate glycemic control. S. Akshatha & U.B. Nayak [4] also noted that the burden of diabetes management, combined with the stigma and social challenges associated with chronic illnesses, increases the risk of depression.

P. Saeedi *et al.* [7] projected a 51% increase in the global prevalence of diabetes, rising from 9.3% in 2019 to 10.9% in 2045. P. Ranasinghe *et al.* [8] documented a dramatic rise in diabetes prevalence in India, increasing from 3.3 to 19% in urban areas and from 2.4 to 15% in rural areas between 1972 and 2015-2019. Furthermore, P. Ranasinghe *et al.* [8] emphasised that diabetes accounts for approximately 10% of global mortality and ranks as the fourteenth leading cause of disability-adjusted life years. D. Carrozzino *et al.* [9] highlighted the utility of the Hamilton Depression Rating Scale (HAMD) in assessing the severity of depressive symptoms among diabetic patients. Accurate assessment and timely intervention for depression in this population are crucial for improving overall health outcomes and quality of life.

Understanding the incidence and influence of depression among DM patients, particularly in India, is vital for the development of effective healthcare policies and interventions. This research aimed to explore the occurrence of depressive disorders among diabetic patients in outpatient settings in India to enhance mental health care integration into diabetes management protocols.

Materials and Methods

This hospital-based cross-sectional observational study was conducted over 18 months, with an additional 6 months allocated for thesis writing, at the Department of General Medicine and Department of Psychiatry, F.H. Medical College and Hospital. The study was conducted based on the 022 ADA criteria [10]. The sample size was determined to be 125 patients, calculated on a prevalence rate of 9%, with a 5% margin of error and a 95% confidence interval. Participants included patients with established DM who were willing to follow up for one year at six-month intervals. Exclusion criteria were strictly applied to ensure the focus on DM-related depression, excluding patients with uncontrolled hypertension, active infections, other chronic illnesses, or psychiatric conditions other than depression.

Data was gathered via organised face-to-face interviews conducted by trained healthcare professionals utilising standardised, validated questionnaires. The surveys comprised the Patient Health Questionnaire-9 (PHQ-9) [11] to screen for depression, along with additional demographic and clinical data. This approach ensured both reliability and consistency in the data collection process. Participants were followed up at 6-month intervals over the course of a year. During each follow-up, patients were re-evaluated using the PHQ-9 to monitor changes in depressive symptoms. Additional clinical parameters related to diabetes control, such as HbA1c levels, were also recorded. Criteria for ongoing participation included regular follow-up visits, adherence to prescribed treatment for DM, and completion of follow-up assessments. Participants were excluded from the study if two consecutive follow-up visits were missed.

Participant selection was accomplished using a convenience sample approach, with diabetic patients attending both outpatient and inpatient departments, used as the subject pool. This method was selected based on the availability of patients willing to participate and meet the inclusion criteria. Inclusion criteria consisted of patients with established DM as per ADA 2022 criteria [10], who were able to participate in follow-up for one year. Exclusion criteria included patients with uncontrolled hypertension, active infections, other chronic illnesses, or psychiatric conditions other than depression. Ethical clearance and informed consent were obtained before the study. The study was conducted per the Declaration of Helsinki [12] and was approved by the Institutional Ethics Committee of F.H. Medical College and Hospital. Informed permission was acquired from all individuals before their involvement in the study. The confidentiality of all participants was ensured during the testing, and patients retained the autonomy to withdraw at any time without repercussions on care.

The statistical analysis was conducted using SPSS software (SPSS Inc., Chicago, IL, USA), version 26.0, on the Windows platform. Descriptive statistics were employed to summarise socio-demographic, clinical, therapeutic, and lifestyle aspects. Frequencies and percentages were calculated for categorical data, whilst continuous variables were expressed as means and standard deviations. The chi-square test was used to assess the associations between categorical variables, including depression, and covariates such as gender, marital status, and employment status. Continuous variables, such as HbA1c, fasting plasma glucose, and postprandial glucose levels, were analysed between patients with and without depression utilising independent t-tests. To analyse the correlation between depression severity (measured by PHQ-9 scores) and glycemic control indicators, including HbA1c, fasting plasma glucose, and 2-hour postprandial glucose, alongside the duration of diabetes, correlation analyses were performed, employing Spearman's rank correlation and point-biserial correlation. A p-value below 0.05 was considered statistically significant for all analyses.

The accuracy of the estimates was demonstrated by the 95% confidence intervals for the correlation coefficients.

Results

This study provides a comprehensive analysis of the socio-demographic, clinical, treatment, and psychological profiles of diabetic patients, with a particular focus on the prevalence and severity of depression. Most participants were over 40 years old, with a slight predominance of males (56.8%) and a high proportion of married individuals (80.0%). Educational levels were varied, with nearly equal representation among university, secondary, and primary levels (Table 1).

Table 1. Socio-demographic data of the enrolled patients among the groups

	Frequency (n = 125)	Percentage (%)
Age		
18-40 years	8	6.4
>40 years	117	93.6
Mean ± SD	44.63±5.37	
Gender		
Male	71	56.8
Female	54	43.2
Marital status		
Married	100	80.0
Widow	16	12.8
Divorced	6	4.8
Single	3	2.4
Employment status		
Employed	28	22.4
Non-employed	55	44.0
Retired	42	33.6
Education level		
University	35	28.0
Secondary	35	28.0
Primary	24	19.2
Illiterate	31	24.8

Source: compiled by the authors

The socio-demographic data of the study demonstrates the diverse background of the diabetic population, which could influence both disease management and mental health outcomes. Clinically, a significant portion of patients exhibited elevated systolic and diastolic blood pressure (35.20 and 30.40%, respectively) and abnormal glucose levels, including HbA1c, fasting plasma glucose, and postprandial glucose (Table 2).

Table 2. Clinical parameters of the enrolled patients

Clinical parameter	Frequency (n = 125)	Percentage (%)
Systolic blood pressure (SBP)		
Normal (<140 mm Hg)	81	64.8
Abnormal (≥140 mm Hg)	44	35.2
Diastolic blood pressure (DBP)		
Normal (<90 mm Hg)	87	69.6
Abnormal (≥90 mm Hg)	38	30.4
Haemoglobin glycosylated (HbA1c)		
Normal (<7%)	71	56.8
Abnormal (≥7%)	54	43.2
Fasting plasma glucose (FPG)		
Normal (<126 mg/dL)	79	63.2
Abnormal (≥126 mg/dL)	46	36.8
2-hour postprandial glucose (2-H PG)		
Normal (<180 mg/dL)	70	56.0
Abnormal (≥180 mg/dL)	55	44.0

Source: compiled by the authors

A significant portion of patients had elevated blood pressure, with 35.2% showing abnormal systolic levels (≥140 mm Hg) and 30.4% with abnormal diastolic levels (≥90 mm Hg). Moreover, a notable proportion of patients had poor glycemic control, as 43.2% had elevated HbA1c levels (≥7%), 36.8% had high fasting plasma glucose (≥126 mg/dL), and 44.0% had abnormal postprandial glucose levels (≥180 mg/dL). These findings suggest that many patients have both hypertension and glycemic control abnormalities, key factors in managing diabetes effectively. Notably, 29.6% of the patients were found to have depression, with varying degrees of severity (Fig. 1).

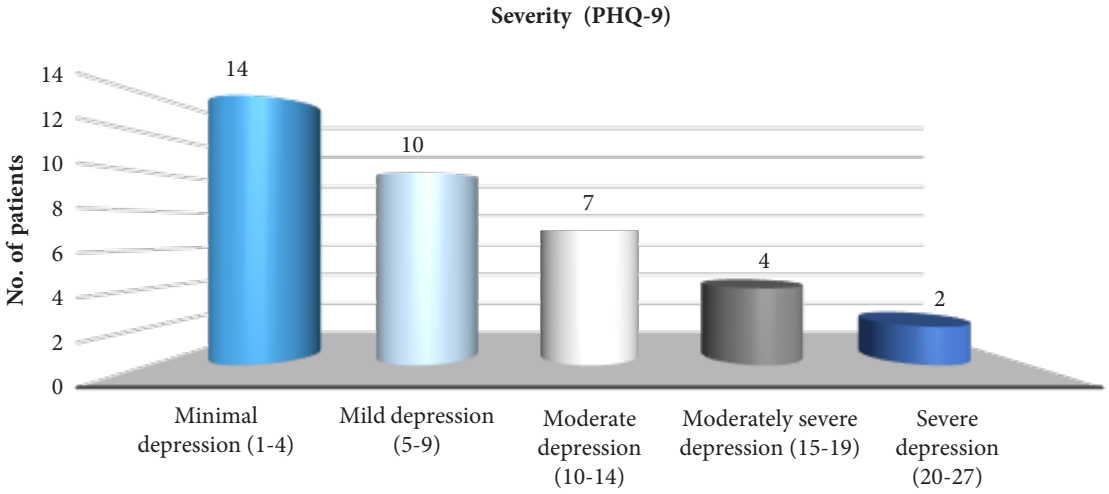

Figure 1. Graphical representation of the distribution of the severity of depression among enrolled patients
Source: compiled by the authors

The depression severity data show that most patients with depression experienced minimal (37.8%) or mild (27%) symptoms. Moderate depression affected 18.9% of patients, while more severe forms, including moderately severe (10.8%) and severe depression (5.4%), were less common. This indicates that most diabetic patients with depression in the study experienced milder forms, but a smaller proportion faced more severe mental health challenges.

The study highlights a substantial correlation between depression and poorer glycemic control, as evidenced by higher rates of abnormal glucose levels among depressed patients. Specifically, depressed individuals showed significantly higher levels of HbA1c, fasting plasma glucose, and postprandial glucose, indicating a potential link between depression and diabetes management difficulties (Table 3).

Table 3. Bivariate table: clinical parameter of diabetic patients with and without depression

Clinical parameter	With depression (n = 37)		Without depression (n = 88)		p-value
	Number	%	Number	%	
Systolic blood pressure (SBP)					
Normal (<140 mm Hg)	20	54.10	61	69.30	0.1503
Abnormal (≥140 mm Hg)	17	45.90	27	30.70	
Diastolic blood pressure (DBP)					
Normal (<90 mm Hg)	21	56.80	66	75.00	0.0556
Abnormal (≥90 mm Hg)	16	43.20	22	25.00	
Haemoglobin glycosylated (HbA1c)					
Normal (<7%)	11	29.70	60	68.20	0.0001
Abnormal (≥7%)	26	70.30	28	31.80	
Fasting plasma glucose (FPG)					
Normal (<126 mg/dL)	13	35.10	66	75.00	<0.0001
Abnormal (≥126 mg/dL)	24	64.90	22	25.00	
2-hour postprandial glucose (2-h PG)					
Normal (<180 mg/dL)	10	27.00	60	68.20	<0.0001
Abnormal (≥180 mg/dL)	27	73.00	28	31.80	
Duration of diabetes					
<5 years	8	21.62	28	31.82	0.2861
≥5 years	29	78.38	60	68.18	

Source: compiled by the authors

The above bivariate analysis shows significant associations between depression and several clinical parameters in diabetic patients. Those with depression had higher rates of abnormal glucose levels, including fasting plasma glucose (64.9 vs. 25.0%, $p < 0.0001$), postprandial glucose (73.0 vs. 31.8%, $p < 0.0001$), and HbA1c (70.3 vs. 31.8%, $p = 0.0001$) compared to non-depressed patients. Depression was also linked to abnormal diastolic blood pressure levels, although the latter showed borderline significance ($p = 0.0556$). These findings suggest that depression in diabetic patients is associated with poorer glycemic control.

Additionally, depression was associated with a higher prevalence of diabetes-related complications, such as nephropathy and neuropathy, further emphasising the complex interplay between mental and physical health in diabetic patients. Treatment patterns revealed a substantial reliance on oral drugs (49.6%), but a notable proportion of depressed patients used insulin with higher frequency. Lifestyle factors such as physical activity were also lower among depressed individuals, suggesting the need for targeted interventions to promote exercise and improve mental health (Table 4).

Table 4. Bivariate table: treatment and lifestyle factors of diabetic patients with and without depression

Treatment/Lifestyle factor	With depression (n = 37)		Without depression (n = 88)		p-value
	Number	%	Number	%	
Type of medication					
Insulin	18	48.6	24	27.3	$X^2 = 5.770$ 0.0559
Oral drugs	13	35.1	49	55.7	
Both	6	16.2	15	17.0	
Diabetes-related complication					
Yes	28	75.7	39	44.3	0.0016
No	9	24.3	49	55.7	
Workout					
Yes	8	21.6	35	39.8	0.0640
No	29	78.4	53	60.2	

Notes: X^2 – chi-square value
Source: compiled by the authors

The analysis of treatment and lifestyle factors reveals that diabetic patients with depression were more likely to use insulin (48.6 vs. 27.3%, $p = 0.0559$) and had a significantly higher prevalence of diabetes-related complications (75.7 vs. 44.3%, $p = 0.0016$) compared to those without depression. Additionally, depressed patients were less likely to engage in physical activity (21.6 vs. 39.8%, $p = 0.0640$), though this difference was not statistically significant. These findings suggest that depression in diabetic patients is correlated to more severe disease management challenges and higher complication rates. Higher HbA1c levels, fasting plasma glucose, and 2-hour postprandial glucose are significantly associated with higher PHQ-9 depression scores, while neuropathy, nephropathy, and retinopathy show no significant correlation with PHQ-9 scores (Table 5).

Table 5. Correlation of depression with severity of DM

Variable	Spearman's p	95% confidence interval	p-value
PHQ-9 score	1.000	N/A	-
HbA1c level	0.287	[0.104, 0.452]	0.006
Fasting plasma glucose	0.201	[0.027, 0.364]	0.047
2-hour postprandial glucose	0.318	[0.151, 0.476]	0.002
Neuropathy	0.125	[-0.043, 0.282]	0.214
Nephropathy	0.043	[-0.125, 0.206]	0.664
Retinopathy	0.182	[-0.013, 0.359]	0.082

Source: compiled by the authors

The correlation analysis shows that higher PHQ-9 depression scores are associated with worse glycemic control, as indicated by positive correlations with HbA1c levels ($r = 0.287$, $p = 0.006$), fasting plasma glucose ($r = 0.201$, $p = 0.047$), and 2-hour postprandial glucose ($r = 0.318$, $p = 0.002$). However, no significant correlation between depression and diabetes-related complications such as neuropathy, nephropathy, or retinopathy, was noted, suggesting that depression has a substantial correlation with poor glycemic control rather than complications. Higher PHQ-9 depression scores are significantly associated with lower values of a binary variable (Table 6).

Table 6. Correlation of depression with duration of DM

Variable	Point-biserial r	95% CI	p-value
PHQ-9 score	-0.231	[-0.389, -0.059]	0.018

Source: compiled by the authors

The analysis shows a significant negative correlation between PHQ-9 depression scores and the duration of diabetes ($r = -0.231$, $p = 0.018$), indicating that patients with longer durations of diabetes tend to have lower depression scores. This suggests that patients with long-term diabetes possibly possess coping mechanisms or have adapted to new conditions, leading to lower levels of depression. The findings of the study highlight the necessity of addressing both psychological and physiological components when managing diabetes to improve the outcomes for patients.

Discussion

DM affects 9.3% of the global population (463 million) as of 2019 and is projected to rise to 10.9% (700 million) by 2045. Urban areas (10.8%) and high-income countries (10.4%) demonstrate higher prevalence compared to rural areas (7.2%) and low-income countries (4.0%). Type 2 DM accounts for 90% of cases. In 2019, Sudan was among the countries with a DM prevalence exceeding 12%, according to the International Diabetes Federation [13]. The disparity in co-morbid depression prevalence among T2DM patients can be attributed to a difference in assessment methods, sociocultural and behavioural influences, and varied cutoff scores for depression diagnosis using identical techniques. A more detailed analysis of depression severity using PHQ-9 scores from 37 people demonstrates that most people with depression fit into the minimal (37.8%) or mild (27.0%) depression category. But a significant fraction also suffers from moderate (18.9%), fairly severe (10.8%), and severe (5.4%) depression, indicating the population's range of psychological distress levels. A study by M. Ismail *et al.* [14] reported that the incidence of depression with a PHQ-9 cutoff value of more than 4 was 20.1% with moderate depression accounting for most cases (70.8%), which is marginally lower than the results of the present study investigation.

Another study by M. Ebrahim *et al.* [15] revealed that 48.9% of depression was developed by diabetic outpatients. This outcome correlates with similar research findings from other regions, indicating a high prevalence of depression among diabetic patients. In Saudi Arabia, a study by A.A. El Mahalli [16] reported a depression prevalence of 49.6% among diabetic patients, emphasising the global nature of the mental health burden associated with diabetes. Similarly, research in Pakistan [17] determined a 43.5% prevalence of depression in diabetic populations, further underscoring the significant psychological challenges that accompany diabetes in diverse settings. Evidence suggests a bi-directional relationship between depression and type 2 diabetes (T2DM): patients are twice as likely to experience depression, while individuals with depression face a 60% higher risk of developing T2DM [18-20]. The prevalence of T2DM was extremely high in the context of the study by P. Ranasinghe *et al.* [8]. From 1972 to 2019, it increased from 3.3 to 19% in urban areas and from 2.4 to 15% in rural areas in India. The development of prevalent comorbidities and disability-adjusted life years (DALYs) in diabetic patients is significantly influenced by depression [21].

Research conducted in the population of India by S. Siddiqui *et al.* [22] aimed to ascertain the prevalence of depressive disorder among patients with T2DM and compare it with that of individuals without the disease. Findings indicated that the prevalence of depression in patients with known T2DM is nearly twice that of cases (35.38%) and controls (20%) without the disease. In a different study [23], it was discovered that of the 210 study participants, 103 (or 49%) had a PHQ-9 score of 10 or more, indicating that over half of them were depressed. The findings of this study are consistent with those of B. Kamble *et al.* [24], who reported an overall prevalence of depression at 48.1% among patients with diabetes and hypertension, using the same scale and cut-off score as in the present study. Similarly, N. Taneja *et al.* [25] determined a 42.6% prevalence of depression in individuals with both conditions. S. Khullar *et al.* [26] reported that 32% of patients experienced severe depression, while 25.3% had mild to moderate depression. These comparable results across studies reinforce the high burden of depression among patients managing chronic conditions such as diabetes and hypertension. These differences are attributable to variations in the study time, participants' sociodemographic profile, and study setting.

The age distribution of participants in the current study shows that although 94.6% of those with depression are older than 40, the difference with those without depression (93.2%) is not statistically significant. The study by O.B. Albasheer *et al.* [27] is comparable to most studies conducted in other nations worldwide, also revealing no significant correlation with length of diabetes, age, or gender. Additionally, a study by P. Aschner *et al.* [28] revealed that among T2DM patients, depression was associated with being female, being older, having lesser education, and having poorer glycemic control. Another study by V.G. Ashok & S.S. Ghosh [29] also demonstrated that depression in hypertension patients was predicted by the female gender, low socioeconomic level, and family support. A conflicting study revealed that depression in hypertensive individuals is markedly affected by variables such as gender, socioeconomic position, marital status, low educational attainment, regular physical activity, duration of hypertension, and uncontrolled blood pressure [30].

The results of a study conducted in Qatar [14] indicated that male gender was correlated with the development of depression. This finding is similar to the findings of a

study conducted in Ethiopia [15]. The gender distribution of those without depression shows a slightly higher representation of males (59.1%) than females (40.9%), although the difference is not statistically significant [31]. Nonetheless, additional research [32] revealed that female T2DM patients experienced a greater prevalence of depression. M.A.K. Khiro *et al.* [33] stated that correlating with previous research, female diabetic patients in this study were more likely to develop depression than male patients. Several aspects, including a lack of social support and a higher sensitivity to negative life experiences due to constitution, could account for this while in the present study males were slightly higher.

No substantial variations in marital status were noted between persons with depression and those without. Additionally, no notable associations were found between depressive state and employment status or educational attainment. However, the study revealed that individuals who were single, divorced, or widowed had higher rates of depression compared to those who were married. A study carried out in Sri Lanka [34] revealed a similar conclusion. Married individuals benefit from social and psychological support, which can alleviate depressive symptoms. Partner support also improves adherence to antidiabetic treatment, thereby reducing the risk of depression and other complications related to diabetes. Similarly, Y. Wang *et al.* [35] demonstrated that social support acts as a protective factor against depression.

In the present study, a comparison of clinical parameters between individuals with and without depression revealed certain significant correlations. Although individuals with depression showed a higher prevalence of abnormal diastolic blood pressure (DBP) compared to those without depression, the difference was non-significant. In both groups, systolic blood pressure (SBP) showed non-significant difference. Similarly, a recent Singapore study found that over 95% of DM patients had T2DM. Glycated haemoglobin and fasting blood glucose levels, as shown by H. Abuhegazy *et al.* [5], are associated with an increased chance of depression. In the present study, depression exhibited a significantly higher proportion of abnormal glucose levels across all metrics, including overall glucose, HbA1c, fasting plasma glucose, 2-hour postprandial glucose, and random plasma glucose levels. R. Das *et al.* [36] indicated that the development of depression in diabetic patients is significantly influenced by the HbA1c level, a measure of long-term glucose control there may be a connection between depression and inadequate glycemic control.

Several studies found that the mean HBA1c was considerably higher in the group of depressed patients compared to non-depressed patients in a sample of T2DM patients and that the intensity of depression symptoms was independently linked with HBA1c in a sample of T2DM patients [37]. There are several noteworthy correlations in a comparison of treatment/lifestyle characteristics between people with depression and people without depression. The type of medication had no significant effect on the difference between the two groups. According to a study by R. Maimaitituerxun *et al.* [38], 75.40% of participants received insulin treatment; in comparison to other individuals, this group had a 1.86-fold increased chance of getting depression. According to a study by P.S. Ciechanowski *et al.* [39], depression in adult diabetics is possibly correlated with a reduced adherence to diet, exercise, and medication. Studies by C.E. Lloyd *et al.* [40] have shown a correlation between low physical activity and increased depression in diabetic patients, as well as poor diet and physical activity compliance, but no correlation between medicine and depression was obtained in the present study.

According to the current study, patients with depression are significantly more likely than those without depression to report diabetes-related complications (75.7%). The present study data suggests that higher depression scores are associated with HbA1c levels, even though depression was found to be marginally correlated with poor glycemic control in patients at a high significant level in a study by S.J. Mahan & M.M. Mahammad [41]. The research demonstrates a substantial positive correlation among PHQ-9 scores, fasting plasma glucose, and 2-hour postprandial glucose, suggesting an association between increased glucose levels and heightened depression scores. A study by S. Sharif *et al.* [42] identified a significant disparity in fasting blood glucose levels between patients with and without depression. The study also revealed no substantial correlation between PHQ-9 scores and nephropathy or neuropathy; however, clinical depression was more prevalent among patients with diabetic retinopathy, nephropathy, and neuropathy. A substantial negative connection was found in the current study connection of Depression with Duration of DM between PHQ-9 depression scores and another unidentified variable. However, longer diabetes duration was related to higher depression in a study by P. Reddy *et al.* [43]. Diabetes duration was related to PHQ-9 scores. PHQ-9 was associated with more diabetes complications.

The current study determined that depression among diabetes patients at F.H. Medical College was slightly less prevalent than in other studies. However, depression remains a critical factor affecting the quality of life in chronic disease patients, with diabetes contributing to premature mortality, disability, and economic losses for individuals and healthcare systems.

Conclusions

This study highlighted a high prevalence of depression (29.6%) among patients with DM, emphasising the importance of routine depression screening in both outpatient and inpatient settings. Depression significantly impacts diabetes management, as evidenced by poorer glycemic control among depressed patients, including elevated HbA1c levels (70.3% of patients with depression had HbA1c ≥7% compared to 31.8% in non-depressed patients, $p = 0.0001$). Moreover, fasting plasma glucose levels were abnormal (≥126 mg/dL) in 64.9% of depressed patients compared to 25.0% of those without depression ($p < 0.0001$), while

postprandial glucose levels were abnormal (≥180 mg/dL) in 73.0% of depressed patients versus 31.8% in non-depressed patients ($p < 0.0001$).

Additionally, the study found that 75.7% of depressed patients had diabetes-related complications such as nephropathy and neuropathy, compared to 44.3% of non-depressed patients ($p = 0.0016$), underscoring the critical link between mental health and physical outcomes in diabetic patients. Depressed patients were also more likely to use insulin (48.6 vs 27.3% in non-depressed patients, $p = 0.0559$) and were less likely to engage in regular physical activity (21.6 vs 39.8%, $p = 0.0640$).

These findings strongly support the need for integrated care approaches that address both mental and physical health, requiring collaboration between endocrinologists, psychiatrists, and primary care providers. Future research should address the mechanisms linking diabetes and depression and develop effective interventions that manage both conditions simultaneously. The study also highlights potential methodological inconsistencies, such as the reliance on self-report questionnaires and the categorical treatment of depression and HbA1c, which may have influenced the findings. Additionally, the impact of antidepressant medications on glycemic control warrants further exploration. Overall, incorporating mental health into diabetes care is crucial for improving patient outcomes.

Acknowledgements

The authors would like to express their sincere gratitude to the Department of General Medicine and the Department of Psychiatry at F.H. Medical College and Hospital for their invaluable support throughout this study. Special thanks to the mentors and colleagues for their guidance and encouragement. The authors are also deeply thankful to the patients who participated in this study for their cooperation.

Conflict of Interest

None.

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Поширеність депресії серед хворих на цукровий діабет, які перебувають на амбулаторному та стаціонарному лікуванні

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Анотація. Цукровий діабет – це сукупність метаболічних захворювань, які характеризуються стійкою гіперглікемією, що призводить до наслідків, які впливають на кілька систем органів. Депресія є поширеним і впливовим психосоціальним станом у пацієнтів з діабетом, який ще більше ускладнює контроль глікемії та негативно впливає на загальний стан здоров'я. Дане дослідження вивчало поширеність депресії серед пацієнтів з цукровим діабетом, які перебувають на амбулаторному лікуванні, та її вплив на перебіг діабету. Це лікарняне перехресне спостереження проводилося протягом 18 місяців. Вибірка з 125 пацієнтів з цукровим діабетом була відібрана на основі 9 % показника поширеності з 5 % похибкою та 95 % довірчим інтервалом. Депресію було оцінено за допомогою 9-пунктного опитувальника для скринінгу депресії. Клінічне оцінювання включало вимірювання рівня HbA1c, глюкози в плазмі крові натще та постпрандіальної глюкози. Статистичний аналіз проводився за допомогою програмного забезпечення SPSS. Дослідження показало, що у 29,6 % пацієнтів з діабетом діагностували депресію різного ступеня тяжкості. Депресія асоціювалася з гіршим глікемічним контролем, про що свідчили достовірно вищі рівні HbA1c, глюкози плазми крові натще та постпрандіальної глюкози. Пацієнти з депресією також мали вищу поширеність пов'язаних з діабетом ускладнень, таких як нефропатія та нейропатія. У дослідженні зроблено висновок, що депресія поширена серед пацієнтів з цукровим діабетом і має значний вплив на управління діабетом. Практична цінність цього дослідження полягає в тому, що інтегрована допомога, включаючи скринінг та лікування психічних розладів, має вирішальне значення для покращення результатів лікування пацієнтів з діабетом

Ключові слова: *diabetes mellitus*; контроль глікемії; гіперглікемія; ускладнення діабету

International Journal of Medicine and Medical Research

Journal homepage: https://ijmr.com.ua/
Volume 10, No. 2. 2024

UDC 618.19-006.6-085.277.3
DOI: 10.61751/ijmmr/2.2024.16

Article's History:
Received: 30.06.2024; Revised: 17.09.2024; Accepted: 26.11.2024

Clinicopathological response and breast conservation in locally advanced breast cancer cases treated with neoadjuvant chemotherapy

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Abstract. Neoadjuvant chemotherapy is crucial for enhancing surgical outcomes and enabling breast conservation in locally advanced breast adenocarcinoma, which is often inoperable. This prospective cohort study aimed to evaluate the clinic-pathological response and breast conservation rates in patients with locally advanced breast cancer undergoing neoadjuvant chemotherapy. The patients were administered chemotherapy according to a standard protocol and were followed until their surgical outcome. Out of a total of 90 patients with locally advanced breast cancer, 78 were eligible for neoadjuvant therapy. These patients, with a mean (SD) age of 47.5 (9.4) years, were included in the study. Nearly half (52.6%) were post-menopausal; 55% (n = 43) had right breast involvement, and 57.7% (n = 45) had invasive lobular carcinoma. Approximately 47.43% of patients demonstrated a complete clinical response. In comparison, only 37% achieved a pathological complete response, which was not associated with the oestrogen receptor, progesterone receptor, or human epidermal growth factor receptor-2 status ($p > 0.05$). Patients who achieved a complete clinical response had a higher likelihood of undergoing breast conserving surgery ($p < 0.05$). The study observed that breast conservation rates were improved with neoadjuvant chemotherapy. These findings may assist clinicians in improving treatment outcomes for patients with locally advanced breast cancer

Keywords: advanced cancer; invasive breast carcinoma; oncological outcomes; breast-conserving surgery

Suggest Citation:
Gupta V, Gupta R, Aggrawal R, Chhatre A. Clinicopathological response and breast conservation in locally advanced breast cancer cases treated with neoadjuvant chemotherapy. Int J Med Med Res. 2024;10(2):16–25. DOI: 10.61751/ijmmr/2.2024.16

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Introduction

The advanced stage and unpredictable presentation of locally advanced breast cancer (LABC) make it a formidable challenge for treatment and prone to surgical difficulties, requiring a multidisciplinary approach for optimal management [1]. The diagnosis of LABC is characterised by a primary breast tumour (TM) that is larger than 5 cm or classified as T3, along with fixed (matted) axillary lymph nodes, skin or chest wall involvement (T4), or spread of the disease to ipsilateral internal mammary nodes or supraclavicular nodes, without distant metastases [2].

Among Indian women, breast cancer is the most frequent malignancy, with an age-adjusted incidence rate of 25.8 per 1,000,000 [3]. LABC represents a subset of breast cancer patients who present with unique clinical characteristics and treatment considerations. LABC accounts for 5% of breast cancer cases in developed countries [4], while its prevalence is notably higher in resource-limited countries such as India [1]. LABC constitutes nearly 40% of breast cancer cases, with a survival rate of 13-24% following surgical intervention, and a recurrence rate of approximately 27% [3, 5].

L. Cuniolo *et al.* [1] and M.E. Akbari *et al.* [6] revealed that LABC is a complex tumour that requires a multi-pronged approach. They highlighted that optimal management necessitates well-coordinated care by medical, surgical, and radiation oncologists, involving radiation therapy, surgery, and chemotherapy.

Neoadjuvant chemotherapy (NACT) has been instrumental in managing LABC, as noted in studies by L.A. Korde *et al.* [7] and M. Dhanushkodi *et al.* [3]. NACT has recently been employed to reduce pre-operative tumours. A study by V. Pandurangappa *et al.* [8] demonstrated that NACT may increase the likelihood of achieving a pathologic complete response (pCR) in 91% of patients and reduce the mean tumour size; however, it may not lead to a significant increase in overall survival. Additionally, a partial or complete response to NACT results not only in a better prognosis but also in improved disease-free survival (DFS), distant disease-free survival (DDFS), and overall survival (OS), as evidenced by a case report by L. Cuniolo *et al.* [1]. Thus, patients who achieve a pCR after NACT exhibit a significantly better prognosis compared to those who do not.

Despite these advancements, several questions remain regarding the prognostic significance of NACT response and its implications for clinical practice. Furthermore, the impact of different chemotherapy regimens on treatment outcomes remains an area of ongoing investigation. In addressing these questions, this study was undertaken to gain insights into optimising treatment strategies and improving outcomes for patients with LABC. Therefore, this study aimed to assess the clinicopathological response of NACT in LABC patients and to evaluate the effect of different chemotherapy regimens on patient outcomes.

Materials and Methods

This was a prospective cohort study conducted among female breast cancer patients registered at the institute between 2021 and 2023. A total of 90 female patients diagnosed with LABC, according to the criteria established by the American Joint Committee on Cancer (8th edition) [9], were included in the study. Of these, only 78 were eligible for neoadjuvant therapy. The follow-up of the patients was conducted during their regular hospital visits for chemotherapy on a monthly basis. Data were recorded up to the final outcome (i.e. surgery) as per the study objective.

Patients diagnosed with LABC, as defined below, were included in the study. Exclusion criteria were patients who did not give consent and those with metastases. All patients diagnosed with LABC were informed about neoadjuvant chemotherapy and were given the regimen as decided by the oncologist. All patients received external beam radiation therapy (EBRT) as adjuvant therapy, followed by hormonal therapy depending on their hormonal status.

Diagnosis. The patients were diagnosed based on relevant clinical history, mammography, and a pathologically confirmed biopsy. Chest X-rays, abdominal ultrasonography, bone scans, and PET-CT scans were performed to identify distant metastases. Immunohistochemistry (IHC) was used to determine the hormonal and HER2/neu receptor status.

Pre-operative therapy. Neoadjuvant chemotherapy is employed to achieve local control and enable surgery with clear resection margins. The response to neoadjuvant chemotherapy includes tumour downstaging by reducing the size of the tumour and the extent of local disease [9-11], improving surgical outcomes by increasing the chances of achieving clear surgical margins, identifying non-response to initial chemotherapy, reducing the likelihood of axillary lymph node involvement, thereby facilitating surgery, and achieving a pathological complete response [12, 13]. Response to neoadjuvant chemotherapy was monitored through clinical assessments such as changes in tumour size and symptoms [14], mammography for baseline assessments, ultrasound for real-time tumour size monitoring, magnetic resonance imaging (MRI) for detailed imaging of tumour response, and positron emission tomography (PET) imaging studies for evaluating metabolic response. Pathological evaluations were conducted with needle biopsies to assess histological response. In addition, biomarker analysis and patient-reported outcomes were undertaken [14, 15].

The 78 patients involved in the study were given different chemotherapy regimens such as AC followed by Taxol, Taxol followed by AC, Cyclophosphamide, Methotrexate, and 5-Fluorouracil (CMF regimen), and Fluorouracil, doxorubicin, and cyclophosphamide (FAC regimen) based on clinical discretion. The four most common regimens used are listed in Table 1.

Table 1. Chemotherapy regimens for LABC

Sr. No.	Chemotherapy regimen
1	Doxorubicin (Adriamycin 40 mg/m^2) and Cyclophosphamide (200 mg/m^2/day) also called AC. The patients received chemotherapy every four weeks for four cycles. After this, surgery was performed, followed by four cycles of Taxol.
2	In this regimen, four cycles of Doxorubicin (Adriamycin) and Cyclophosphamide were followed by four cycles of Taxol (175 mg/m^2). After these eight cycles, surgery was performed.
3	Cyclophosphamide 600 mg/m^2 IV infusion, Methotrexate 40 mg/m^2 IV bolus, and 5-fluorouracil 600 mg/m^2 IV bolus. The regimen was administered every four weeks for six to eight cycles.
4	Fluorouracil 500 mg/m^2, Doxorubicin 40 mg/m^2, and Cyclophosphamide 500 mg/m^2. This regimen was administered every four weeks for six months.

Source: compiled by the authors

Radiotherapy. Radiotherapy involved standard fractionation over 25-28 days, delivering a cumulative dose of 45-50.4 Gy. The standard fractionated dose was given five days a week, from Monday to Friday, with sessions lasting 15 to 30 minutes, varying from patient to patient. The most common fractionation schedule was 1.8 to 2.0 Gray (Gy) per fraction, with a total cumulative dose ranging from 45 to 50.4 Gy. The cumulative dose was chosen to be high enough to target and kill cancer cells, but not high enough to damage surrounding healthy tissues. Smaller fractions of these doses would also allow effective treatment by taking advantage of the differential repair rates of cancer and normal cells [16, 17].

Monitoring and evaluation of the radiotherapy were conducted through clinical assessments, imaging studies including CT scans, MRI, PET scans, and the response evaluation criteria in solid tumours (RECIST) [16, 18] criteria to assess tumour size and classify response as complete, partial, stable disease, or progressive disease. Blood tests and biomarkers were also used to evaluate the tumour's response to treatment. Patient-reported outcomes and feedback were also considered [19].

Local therapy (surgery). Following NACT, the tumour was surgically removed, thereby eliminating any locoregional spread. Depending on the patient's condition, various surgical techniques were employed, including modified radical mastectomy (MRM), total mastectomy, and breast-conserving surgery (BCS).

Adjuvant systemic therapy. Patients received post-operative systemic therapy tailored to their individual needs following surgery. Adjuvant endocrine therapy was administered to patients with expressed oestrogen and/or progesterone receptors. Patients requiring specialised therapy had harmful germline abnormalities in either BRCA1 or BRCA2. Overall, the strategy included a combination of adjuvant systemic therapy, radiotherapy, chemotherapy, surgery, and diagnostic imaging, all customised to the specific requirements and tumour characteristics of each patient.

Statistical analysis. The collected data were entered into MS Excel using passwordprotected files and analysed using SPSS software. Quantitative variables were described as the mean and SD after checking the normality of the data. Qualitative variables were described as frequency and percentage. The chi-square test was used as a test of significance, and for quantitative data, an unpaired t-test was used. A p-value of <0.05 was considered statistically significant.

Complete clinical response. The neoadjuvant chemotherapy clinical response was assessed using MRI and ultrasound scans, along with the RECIST version 1.1 [20].

MRI and ultrasound procedures in radiotherapy. MRI procedure. MRI scans were conducted to evaluate the response and detect the presence of any residual disease. These scans were performed using high-field MRI scanners, with magnetic field strengths ranging from 1.5 Tesla to 3 Tesla. Patients were instructed to fast or undergo bowel preparation. The scan duration ranged from 15 to 60 minutes, depending on the complexity and the number of sequences required. An intravenous gadolinium-based contrast agent was used to highlight differences between normal and abnormal tissues.

Ultrasound procedure. Ultrasound was less frequently utilised than MRI. Ultrasound imaging was carried out using a handheld transducer connected to an ultrasound machine. The frequency of the transducer varied, typically between 2 and 15 MHz, depending on the required depth and resolution. A gel was applied to the skin over the areas to be examined to facilitate the transmission of sound waves. The transducer was then moved over the skin to capture real-time images of the tissues. This procedure took 15 to 30 minutes. In some cases, contrast-enhanced ultrasound (CEUS) was employed, where microbubble contrast agents were injected to improve image quality and assess blood flow.

Pathological complete response. The absence of the primary tumour and any lymph node metastases in the surgical specimen was defined as a pathological complete response [21-23].

Histopathological analysis procedure. Samples were collected through biopsies and surgical resections, all performed under sterile conditions. Fixation was undertaken to preserve the tissue morphology, using 10% neutral buffered formalin, followed by standard fixation procedures. Staining was carried out using haematoxylin and eosin (H&E) stains, and antigen determination followed standard procedures. The analysis was conducted under a light microscope to observe cellular morphology and tissue structures.

Written informed consent was obtained from the patients before including them in the study. The study adhered strictly to the principles outlined in the Declaration of Helsinki [24], ensuring that all participants provided informed consent voluntarily and comprehensively. Confidentiality and privacy were meticulously safeguarded throughout the study, with data anonymised to prevent identification.

Results

Of the total 90 LABC patients in the study, only 78 were eligible for neoadjuvant therapy. The mean (SD) age of the patients was 47.5 (9.4) years. More than half (52.6%) were post-menopausal, while 44.8% (n = 35) were pre-menopausal. Additionally, 55% (n = 43) had right breast involvement, 57.7% (n = 45) had invasive lobular carcinoma, and 42.3% (n = 33) had invasive ductal carcinoma. In terms of staging, the most common stage was IIIB, observed in 39.7% (n = 31) of patients, followed by IIIA in 34.6% (n = 27) and IIIC in 17.9% (n = 14), with stage IV breast carcinoma being the least common, seen in only one patient (1.3%). There were 52.7% ER-positive cases (n = 30), while progesterone receptor-positive cases constituted 40.5% (n = 30), and 45.7% (n = 32) of cases were Her2neu-positive (Table 2).

Table 2. Clinical history of the patients (n = 78)

Variable	Frequency (%)
Menstrual Status	
Pre-menopausal	35 (44.8)
Peri-menopausal	02 (02.6)
Post-menopausal	41 (52.6)
Breast Involvement	
Right	43 (55.0)
Left	35 (45.0)
Morphology of Breast Carcinoma	
Invasive Ductal Carcinoma	33 (42.3)
Invasive Lobular Carcinoma	45 (57.7)
Clinical Stage	
IIa	02 (02.6)
IIb	03 (03.9)
IIIa	27 (34.6)
IIIb	31 (39.7)
IIIc	14 (17.9)
IV	01 (01.3)
ER (n = 74)*	
Positive	39 (52.70)
Negative	35 (44.87)
PR (n = 74)*	
Positive	30 (40.54)
Negative	44 (59.45)
Her2neu (n = 70)#	
Positive	32 (45.71)
Negative	38 (54.28)

Notes: * – only 74 patients were evaluated for ER-PR receptor status; # – only 70 patients Her2neu status evaluated
Source: compiled by the authors

Patients had been given various neoadjuvant chemotherapy regimens as per the four regimens described in Table 3 below. Forty-six patients were given the 4#AC-4#Tx-Sx regimen, twenty were given the 4#AC-Sx-4#Tx regimen, ten patients received the 6#FAC regimen, and two patients were treated with the 6-8#CMF – Sx regimen. A complete clinical response was observed in the 4#AC-4#Tx-Sx regimen (n = 23/46), followed by the 4#AC-Sx-4#Tx regimen (n = 11/20). A pathological complete response was also observed in similar proportions in these two regimens. There was no statistically significant difference in the clinical or pathological response concerning the different NACT regimens (Table 3).

Almost an equal proportion of pre-menopausal and post-menopausal patients exhibited a complete clinical response (n = 18/35 and n = 19/41, respectively). Less than half of the patients with invasive ductal carcinoma achieved a complete clinical response. Almost equal numbers of ER-positive and ER-negative carcinoma patients demonstrated a complete clinical response, while a greater proportion of PR-positive (17/30) patients compared to PR-negative (16/44) patients, and more Her2neu-positive (17/32) patients, achieved a complete clinical response. No statistical association was observed between the clinical characteristics of patients and their

clinical or pathological responses ($p > 0.05$) (Table 2). The clinical response was significantly associated with the type of surgery involved ($p < 0.05$); however, the different neoadjuvant chemotherapy regimens were not statistically significantly associated with complete clinical or pathological responses ($p > 0.05$). Table 4 shows the association of clinical characteristics with clinical and pathological responses.

Table 3. Clinical and pathological response to different NACT (n = 78)

Regimen	CR	NR	PD	PR	Total	p-value
Clinical response						
4#AC-Sx-4#Tx [1]	11	01	00	08	20	10.50 (0.31)
4#AC-4#Tx-Sx [2]	23	04	01	18	46	
6-8#CMF – Sx [3]	01	01	00	00	02	
6#FAC [4]	02	01	01	06	10	
Pathological response						
4#AC-Sx-4#Tx [1]	09	01	00	18	20	10.05 (0.35)
4#AC-4#Tx-Sx [2]	15	04	01	26	46	
6-8#CMF – Sx [3]	01	01	00	00	02	
6#FAC [4]	02	02	01	05	10	

Notes: CR – complete response; NR – no response; PD – progressive disease; PR – partial response; # – Chi-square test with Yate's correction; the dosages of chemotherapeutic agents are detailed in Table 1
Source: compiled by the authors

Table 4. Clinical characteristics and their association with clinical and pathological response (n = 78)

Variable	CR	NR	PD	PR	Total	p-value
Clinical response						
Menstrual status						
Pre-menopausal	18	6	1	12	35	5.45 (0.14)
Post-menopausal	19	1	1	20	41	
Morphology of breast carcinoma						
Invasive ductal carcinoma	38	7	2	38	85	0.79 (0.851)
Invasive lobular carcinoma	3	0	2	0	5	
ER status						
Positive	17	3	0	19	39	4.36 (0.628)
Negative	16	4	1	14	35	
PR status						
Positive	17	3	0	10	30	6.50 (0.369)
Negative	16	4	1	23	44	
Her2neu status						
Positive	17	3	1	11	32	5.53 (0.478)
Negative	15	4	0	19	38	
Pathological response						
Menstrual status						
Pre-menopausal	14	7	1	15	35	7.30 (0.062)
Post-menopausal	13	1	1	26	41	
Morphology of breast carcinoma						
Invasive ductal carcinoma	27	8	2	48	85	0.69 (0.874)
Invasive lobular carcinoma	2	0	0	3	5	
ER status						
Positive	9	3	0	27	39	6.30 (0.390)
Negative	14	4	1	16	35	
PR status						
Positive	8	3	0	19	30	2.87 (0.825)
Negative	15	4	1	24	44	
Her2neu status						
Positive	12	3	1	16	32	4.59 (0.596)
Negative	9	4	0	25	38	

Notes: CR – complete response; NR – no response; PD – progressive disease; PR – partial response; p-value <0.05 considered statistically significant
Source: compiled by the authors

Table 5 demonstrates the association of clinical characteristics with surgical intervention. It is important to note that clinical response had a significant impact on the choice of surgical intervention ($p < 0.05$), highlighting the importance of accurate assessment of the response to NACT for planning further treatment.

Table 5. Association of clinical characteristics with surgery (n = 78)

Drug therapy	Surgery				p-value
	BCS	MRM	Total mastectomy	Total	
Oestrogen receptor					
Positive	10	26	0	36	4.03 (0.402)
Negative	12	18	1	31	
Progesterone receptor					
Positive	8	21	0	29	3.46 (0.483)
Negative	14	23	1	38	
HER2 status					
Positive	10	19	1	30	2.96 (0.563)
Negative	12	21	0	33	
Drug therapy (n = 78)					
4#AC-Sx-4#Tx [1]	9	11	0	20	5.68 (0.460)
4#AC-4#Tx-Sx [2]	14	31	1	46	
6-8#CMF – Sx [3]	0	2	0	2	
6#FAC [4]	2	7	1	10	
Clinical response					
CR	15	22	0	37	80.47 (<0.0001)
NR	1	6	0	7	
PD	0	0	2	2	
PR	9	23	0	32	

Notes: CR – complete response; NR – no response; PD – progressive disease; PR – partial response; # – number of cycles; the dosages of chemotherapeutic agents are detailed in Table 1
Source: compiled by the authors

In a study of 90 LABC patients, 78 were eligible for neoadjuvant therapy with a mean age of 47.5 years. Clinical response rates were similar across the pre- and post-menopausal groups and varied slightly based on hormone receptor status. No significant association was found between clinical characteristics and response to treatment, but clinical response significantly influenced the choice of surgical intervention, emphasising the need for accurate assessment in treatment planning.

Discussion

Of the 90 LABC patients in this study, 78 were eligible for neoadjuvant therapy, with a mean age of 47.5 years. The majority were post-menopausal (52.6%), with right breast involvement (55%) and invasive lobular carcinoma (57.7%). Most patients were staged at IIIB (39.7%) or IIIA (34.6%). Complete clinical and pathological responses were highest in the 4#AC-4#Tx-Sx regimen and were similar across other regimens, with no significant differences observed based on therapy type. Clinical response significantly influenced surgical choice, but no strong associations were found between clinical characteristics and response outcomes.

The mean (SD) age of the patients was 47.5 (9.4) years in this study, which is comparable to the studies of P. Chidley *et al.* [25] and T. Bhattacharyya *et al.* [26]. In these studies, the majority of participants were in the post-menopausal state, which is consistent with the findings of this study. The mean age of patients in the study by C.E. DeSantis *et al.* [27] was around 50 years, with a significant proportion of post-menopausal women and right breast involvement. Invasive lobular carcinoma was less frequent compared to the findings of the Indian study. Similar to the Indian study, the one by A. van der Voort *et al.* [28] had an average age of 45 years, with a higher proportion of patients presenting with HER2-positivity.

The majority of the patients in this study received six cycles of chemotherapy, with Doxorubicin (Adriamycin) and Cyclophosphamide, also referred to as AC, used as the main regimen of treatment. Following this, AC with Taxol was administered as per institutional policy. No statistically significant difference was observed between the clinical and pathological responses to the different regimens. However, according to H.D. Bear *et al.* [12], the National Surgical Adjuvant Breast and Bowel Project (NSABP) trial, conducted by the National Cancer Institute, reported superior response rates with the sequential use of taxanes and doxorubicin, leading to superior partial and complete responses in both ER-positive and ER-negative patients.

In this study, nearly 47.43% of patients had a complete clinical response, while only 37% had a complete pathological response. This response is higher compared to the 26% complete clinical response and 13% complete pathological response in the study conducted by S.K.R. Kunnuru *et al.* [29]. A study by P. Choudhary *et al.* [30] showed

that the complete pathological response to NACT in LABC patients was 21%. Another study by A.A. Alawad [31] showed a complete clinical response of 11%. The possible reason for the differences in the response may be due to the different regimens administered to the patients in various studies. In the study by G. Curigliano *et al.* [32], NACT was shown to achieve a high rate of clinical and pathological complete responses (PCRs) in patients with HER2-positive and triple-negative breast cancers, resulting in significant downstaging and improved surgical outcomes. Meanwhile, in the study by Loibl *et al.* [33], it was highlighted that NACT led to increased rates of breast-conserving surgeries compared to pre-NACT scenarios, with better outcomes observed particularly in triple-negative and HER2-positive subtypes.

The clinical or pathological response was not dependent on other characteristics such as ER-positive status, PR-positive status, or Her2-neu status of the LABC. Conversely, the study by P. Choudhary *et al.* [30] demonstrated a positive correlation between Stage II and the absence of ER/PR expression, which showed a statistically significant correlation ($p < 0.05$) with the rate of pCR. The study conducted by O. Mermut *et al.* [34] also revealed that the T1 stage and N1 stage, along with negative oestrogen/progesterone receptor status, were significantly associated with NACT response. This limitation may be due to the use of different chemotherapy regimens.

Thirty-two per cent of the patients could undergo breast-conserving surgery (BCS) after NACT. The rates of BCS following NACT in this study are higher compared to those reported in the findings of Scientist S.K. Agrawal *et al.* [35]. These variations may be attributed to the variety of chemotherapeutic regimens and the differences in the study population. Similarly, the study by A. Soran *et al.* [36] showed increased rates of breast-conserving surgery after NACT, particularly in patients with a good clinical response. Mastectomies were still performed but were less frequent compared to pre-NACT rates. The study by M.R. Kwon *et al.* [37] found that NACT improved the feasibility of breast-conserving surgery, especially in patients who initially presented with indications for mastectomy.

Overall, patients with CR and pCR had a higher likelihood of undergoing breastconserving surgery compared to other responses. A meta-analysis by Y. Sun *et al.* [38] demonstrates similar findings. Therefore, it can be concluded that NACT resulting in complete clinical and pathological responses is associated with a greater likelihood of breast conservation. The study by L.M. Spring *et al.* [39] noted that patients with HER2-positive and triple-negative breast cancer types were more likely to achieve complete clinical responses, which correlated with a higher rate of breast-conserving surgery. Another study by A. Hennigs *et al.* [15] indicated that patients with lower clinical stages and higher response rates to NACT had a better chance of undergoing breast-conserving surgeries, while those with residual disease often required mastectomy.

Due to limited resources, BRCA status was not evaluated, which could facilitate more effective treatment strategies. Progression-free survival and overall survival after different NACT regimens were not evaluated, which could provide data regarding the long-term effects of the regimen. Therefore, this study highlights the response to NACT, wherein the clinical complete response was almost 50%, and the complete pathological response was 37%. No statistically significant associations were observed between age, menopausal status, tumour location, or tumour histology and the response; nor was there an association between the type of NACT and the response. The choice of surgical intervention significantly affected the clinical response.

Overall, the study conducted at this tertiary care centre aligns with international findings in many respects. The mean age and clinical characteristics in this Indian study are consistent with international trends, although some variations exist in histological types. The effectiveness of NACT in achieving downstaging and improving surgical options aligns with global studies, which demonstrate high rates of complete responses and increased rates of breast-conserving surgery.

The types of surgical interventions and their outcomes post-NACT are similar to those observed internationally, with an emphasis on breast-conserving surgery where possible. The correlation between clinical response to NACT and surgical options observed in the Indian study is consistent with international findings. Thus, this study contributes valuable insights into the effectiveness of NACT in a different demographic and clinical setting, reinforcing the general benefits observed globally while highlighting specific regional characteristics.

Conclusions

Of the initial 90 LABC patients considered, 78 were eligible for neoadjuvant chemotherapy. The mean age of the study patients was 47.5 years, and a significant portion of them were post-menopausal (52.6%). The majority had right breast involvement (55%) and invasive lobular carcinoma (57.7%).

The study aimed to assess both the clinical and pathological responses to neoadjuvant chemotherapy. A complete clinical response was observed in 47.43% of patients, while 37% exhibited a complete pathological response. However, no statistically significant associations were found between clinical characteristics (such as age, menopausal status, tumour location, and histological type) and either clinical or pathological response ($p > 0.05$). The findings did not reveal any statistically significant differences in clinical or pathological response based on the type of neoadjuvant chemotherapy regimen used ($p > 0.05$). This suggests that the effectiveness of different regimens in inducing tumour response was similar within the cohort studied. Although specific rates were not detailed, the study aimed to estimate breast conservation rates following neoadjuvant chemotherapy. This aspect

was likely impacted by the clinical response observed in patients, which significantly influenced the choice of surgical intervention ($p < 0.05$).

Importantly, clinical response to neoadjuvant chemotherapy significantly influenced the type of surgical intervention chosen for patients ($p < 0.05$). This underscores the critical role of accurately assessing treatment response to guide subsequent surgical decisions and optimise outcomes. In conclusion, while the study did not identify predictive clinical characteristics for treatment response to neoadjuvant chemotherapy in LABC patients, it highlighted the pivotal role of clinical response assessment in guiding surgical management decisions. Future research could further explore factors influencing response variability and refine strategies to enhance treatment outcomes in this patient population.

Acknowledgements

Acknowledgement is given to the management team of VY Institute of Medical Science Pvt Ltd for their continuous support.

Conflict of Interest

None.

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Клініко-патологічна реакція та консервація грудей у випадках місцево поширеного раку молочної залози з неоад'ювантною хіміотерапією

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Анотація. Неоад'ювантна хіміотерапія є важливою для покращення хірургічних результатів та можливості збереження грудної залози у випадках локально поширеного раку грудної залози, які в іншому випадку часто є неоперабельними. Метою цього проспективного когортного дослідження було оцінити клініко-патологічну відповідь та рівні збереження грудної залози у пацієнтів з локально поширеним раком грудної залози, які проходили неоад'ювантну хіміотерапію. Пацієнтам була проведена хіміотерапія за стандартним протоколом, і їхній стан було контролювано до отримання хірургічного результату. Із загальної кількості 90 пацієнтів з локально поширеним раком грудної залози в дослідженні лише 78 були допущені до неоад'ювантної терапії. Ці 78 пацієнтів з середнім віком 47,5 (9,4) років були включені в дослідження. Практично половина (52,6 %) пацієнтів перебували в постменопаузі; у 55 % (n = 43) було ураження правої молочної залози, а 57,7 % (n = 45) страждали на інвазивну лобулярну карциному. Практично 47,43 % пацієнтів показали повну клінічну відповідь, і лише 37 % мали повну патологічну відповідь, що не було пов'язано зі статусом естрогенових рецепторів, прогестеронових рецепторів та епідермального фактора росту людини ($p > 0,05$). Пацієнти з повною клінічною відповіддю мали вищі шанси на збереження грудної залози ($p < 0,05$). Було відзначено, що збереження грудної залози покращується завдяки неоад'ювантній хіміотерапії. Це дослідження допоможе лікарям покращити результати лікування пацієнтів з локально поширеним раком грудної залози

Ключові слова: поширений рак; інвазивний рак грудної залози; онкологічні результати; операція зі збереження грудної залози

International Journal of Medicine and Medical Research

Journal homepage: https://ijmr.com.ua/
Volume 10, No. 2. 2024

UDC 616.928.8:578.833.25]-084(599.35)
DOI: 10.61751/ijmmr/2.2024.26

Article's History:
Received: 09.07.2024; Revised: 18.09.2024; Accepted: 26.11.2024

Assessing perceived dengue risk, attitude, and prevention practices in Barangay Buhangin Proper, Davao City

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Abstract. Dengue remains a significant public health issue in the Philippines and globally. This study aimed to evaluate the risk perception, attitude, and prevention practices of Barangay Buhangin Proper residents while examining whether socio-demographic profiles significantly influenced these factors. The objective was to develop strategic community health interventions. A total of 584 residents participated in face-to-face interviews using an adapted risk perception, attitude, and practice questionnaire. The results show that residents demonstrated good risk perception (51.49, cut-off = 51.20) and attitude (43.62, cut-off = 38.40), but exhibited poor practice (47.22, cut-off = 57.60). The study also revealed that females (OR = 1.493, 95% CI = 1.016-2.194, p = 0.041), college graduates (OR = 0.653, 95% CI = 0.273-1.561, p = 0.041), and employed individuals (OR = 0.654, 95% CI = 0.441-0.970, p = 0.034) were statistically associated with good risk perception while being married (OR = 0.699, 95% CI = 0.318-1.714, p = 0.038) was associated with a positive attitude. However, no

Suggest Citation:
Bañaga J, Ang AG, Arceño EJ, Abao GJ, Exiomo RV. Assessing perceived dengue risk, attitude, and prevention practices in Barangay Buhangin Proper, Davao City. Int J Med Med Res. 2024;10(2):26–38. DOI: 10.61751/ijmmr/2.2024.26

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socio-demographic factors were linked to good practice, indicating that the issue with practices persists regardless of profile. Furthermore, weak positive correlations were observed between risk perception and attitude ($r = 0.266$, $p < 0.001$), risk perception and practice ($r = 0.092$, $p < 0.027$), and attitude and practice ($r = 0.144$, $p < 0.001$). Despite awareness of risks and having positive attitudes, the community struggles to maintain effective practices due to complacency, self-serving behaviour, and inadequately targeted interventions. This study recommends implementing tailored interventions to address the root causes and effectively combat dengue

Keywords: socio-demographic profile; development of community health interventions; modifiable factors in dengue progression; change mechanisms for dengue prevention; implementing change mechanisms

Introduction

Dengue cases are increasing rapidly worldwide and represent the most common mosquito-borne viral infection in humans. Despite continuous advancements in health interventions, it remains a significant public health concern [1, 2]. Dengue is caused by the dengue virus (DENV), which is transmitted by infected female mosquitoes, primarily *Aedes aegypti* and, to a lesser extent, *A. albopictus*. Transmission occurs from person to person through mosquito bites. Symptoms include the sudden onset of high fever, muscle and joint pain, loss of strength, pain behind the eyes, skin rashes, nosebleeds after the fever subsides, stomach pain, coffee-coloured vomit, black stools, and difficulty breathing [3].

Dengue cases are underreported globally, and there has been more than a fifteen-fold increase in cases reported to the WHO, from 505,430 in 2000 to 7.6 million in 2024 [2]. In May 2024, the Philippines recorded approximately 59,267 cases, which is higher than the 45,722 cases reported during the same period last year [4]. Locally, the Davao Region is one of the 17 dengue-endemic areas in the Philippines and recorded 9,073 cases as of June 22, 2024, an increase from 7,199 cases in the same period in 2023. Davao City accounted for 2,192 dengue cases in June 2024, which represented more than 24% of the region's total of 9,073 cases [5].

With the continuous rise in dengue infections in the Philippines, the Department of Health (DOH) developed the Dengue Prevention and Control Program to reduce the burden of dengue disease, as outlined in DILG MC No. 2012-16. This programme emphasises the surveillance of dengue cases through various government agencies, case management and diagnosis through extensive dengue clinical management training for hospitals, and the establishment of dengue confirmatory tests [6]. However, according to E.P. Ong *et al.* [7], the programme has not met its objectives, primarily due to the lack of community engagement in taking responsibility for combating dengue.

A study by S. Sulistyawati *et al.* [8] on dengue vector control through community empowerment in Yogyakarta, Indonesia, revealed that most participants adopted practices that raised awareness about the presence of larvae in water containers. These practices included cleaning and brushing containers, as well as cleaning them one to three times per week. The use of repellents, mosquito coils, or sprays in the morning and evening was also noted.

Additionally, a study by C. Aerts *et al.* [9] on the importance of disease knowledge and risk perception in shaping preventive behaviour for selected vector-borne diseases in Guyana showed that prevention strategies were employed more frequently among individuals with higher levels of education. Education significantly enhanced knowledge and understanding of dengue and malaria. Furthermore, the study highlighted the importance of raising awareness about the risks and making preventive measures more accessible to avoid decreased risk perception, which could lead to lower levels of preventive activity.

In addition, the study by V.R.D.M. Herbuela *et al.* [10] proposes two comprehensive health initiatives that will help individuals effectively apply their knowledge and attitudes to improve their practices. Health initiatives should focus on changing behaviour to translate information and perspectives into more effective strategies against dengue fever. Several initiatives continue to prioritise education and awareness campaigns over physical activity initiatives, which are more effective in influencing behaviour.

According to A.B. Siddique *et al.* [11], the findings highlight an increased perceived risk and a significant discrepancy in practices, revealing key influences such as gender, educational attainment, employment status, sufficient sleep duration, and the father's occupation. The study emphasises the urgent need for targeted public health initiatives to raise awareness and encourage proactive participation in dengue prevention, particularly among young people, who play a crucial role in shaping a community's response to viral illnesses.

E.R. Gregorio *et al.* [12] also found that, despite existing dengue programmes and policies, as well as Filipinos' high awareness of the disease, dengue remains a persistent issue with increasing case rates. To achieve a dengue-free Philippines, key themes should be prioritised, and actions should be adapted to more effectively reach and impact the target audience.

At present, no data is available on the risk perception, attitudes, and practices (RPAP) of residents in Davao City concerning dengue infection. Understanding the RPAP of residents is crucial, as it will help uncover the root causes of the problem within the community. Despite numerous initiatives and activities aimed at addressing the issue, the number of cases continues to rise due to a lack of community collaboration. Therefore, this study aimed to thoroughly assess the RPAP of residents, identify potential causes for the increasing number of dengue cases and provide valuable guidance to public health officials and policymakers involved in combating the disease.

Materials and Methods

The researchers identified the research locale based on epidemiological data obtained from the Department of Health – Davao City, following the trend in dengue cases since 2018. Barangay Buhangin Proper was selected as the locale, as it was also classified as a dengue hotspot, having exceeded the local epidemic threshold [13, 14]. The sample size was then determined using Cochran's formula:

$$\textit{Sample size for known population} = \frac{\frac{z^2 \times p(1-p)}{e^2}}{1+\left(\frac{z^2 \times p(1-p)}{e^2 N}\right)}, \quad (1)$$

where N – the population size; z – the z-score; e – the margin of error; p – the standard deviation. The initial sample size calculation was 382 individuals, based on a 5% margin of error. However, to minimise the design effect, 584 individuals were included in the study. Respondents were selected using two-stage random cluster sampling from nine (9) *puroks* in Barangay Buhangin Proper, Davao City, between April and May 2023.

The adapted RPAP questionnaire by M.I.A. Zamzuri *et al.* [15] was validated. Both the questionnaire and the Informed Consent Form (ICF) were translated by a *Bisaya* linguist to eliminate any language barriers for the respondents. The translated questionnaire then underwent content validity and reliability testing, administered to 30 randomly selected participants not included in the target population. The results were analysed using Cronbach's Alpha values, calculated through the Reliability Analysis Test in IBM® SPSS® Statistics 21 software, yielding internal consistency values ranging 0.701-0.855.

Before conducting the survey, the researchers briefed the assigned personnel on the dissemination of the questionnaires, including the purpose and agenda of the study, with particular emphasis on the correct procedure for selecting respondents. The researchers, along with the designated *barangay* personnel and BHWs, were then deployed to the nine (9) randomly selected *puroks* to conduct the survey simultaneously. The sampling criteria included allowing only one representative per household to complete the questionnaire. Eligible participants were permanent residents who had lived in Barangay Buhangin Proper, Davao City, for six (6) months or more, who could read and write, were physically and mentally fit, were not members of the indigenous community, had or had not had a history of dengue infection, and were 18 years of age or older. Excluded from the study were minors (17 years and below), transient residents who had lived in the area for less than six (6) months, those unable to read or write, individuals with compromised physical or mental health, and members of the Indigenous community.

Before distributing the questionnaires, the researchers and *barangay* personnel explained the study, its goals, and its purpose to ensure respondents fully understood their involvement and how their participation would contribute to the research. The researchers and *barangay* personnel then asked respondents to sign the ICF, indicating their voluntary participation in the study. Only those who signed the consent form were included. Once participation was confirmed, a two-part questionnaire was distributed face-to-face.

The first part of the questionnaire gathered socio-demographic information, such as age, sex, civil status, educational attainment, employment status, monthly income, type of residential building, and history of dengue. The second part consisted of a 23-item questionnaire. Questions 1-8 focused on risk perception, which assessed the dangers that pose short- or long-term risks to the community. Risk perception refers to the subjective judgment made by respondents regarding dengue as a threat to health and well-being. For example, questions on this scale included: "*Dengue fever can cause death; Fever lasting for 3 days is concerning to me. I feel that I cannot wait up to 5 days to seek treatment; I have many close friends who have recovered from dengue fever, but I am still afraid of dengue; and I need a lot of money to implement dengue prevention at home.*" etc.

Questions 9-14 addressed attitudes, which evaluate ways of thinking reflected in people's behaviour and how these directly affect their daily lives. Attitudes are shaped by respondents' past experiences with dengue and determine their actions towards the disease. For example, some questions included: "*If at least one person in the household is knowledgeable about the disease, they can help prevent it in the home; It is necessary for me to share information about dengue fever with my family members; and I become more interested in participating in dengue control/prevention when there is cooperation within the neighbourhood.*" etc. Additionally, questions 15-23 related to practices, referring to actions repeatedly performed that establish a shared identity within the community. These questions focused on how the community takes action to prevent the spread of dengue. For example, questions on this scale included: "*I use mosquito repellent (lotion/spray/coil); I always keep water containers in my house tightly sealed; and I add larvicide to water storage to kill mosquito larvae.*" etc.

The RPAP questionnaire was answered using an 8-point Likert scale, ranging from *1* = strongly disagree to *8* = strongly agree. The cumulative scores for risk perception, attitude, and practice ranged from 8 to 64, 6 to 48, and 9 to 72, respectively. To ensure the utmost anonymity and confidentiality of respondents, instead of asking for names, a reference number was assigned to each questionnaire.

All gathered information was stored in Google Drive by Google™ and organised into specific folders. Hard copies of the consent forms, questionnaires, field notes, or informal jottings were categorised and securely stored. Access to these materials was strictly limited to the study's proponents. All saved raw data and hard copies will be stored from 2023 to 2025, after which they will be destroyed to render them inaccessible. The researchers guarantee that no files or remaining data will be retained, and all information will be permanently deleted.

The data collected were encoded into Google Sheets by Google™ and were double-checked to ensure accuracy and repeatability. After this, the socio-demographic profile and

scores from the RPAP questionnaire were imported into IBM® SPSS® Statistics 21 software for descriptive and inferential statistical analyses. Descriptive statistics were used to obtain frequencies and percentages for the socio-demographic profile, and the mean was calculated for each question in the RPAP questionnaire. Total scores were calculated for each RPAP scale, and scores were classified as good or poor based on the 80% cut-off point.

The Pearson Chi-Square Test was used to interpret and compare the socio-demographic profiles with RPAP, categorising the profiles as either good or poor. In addition, the RPAP of the respondents regarding dengue infection was analysed using multivariate logistic regression to obtain the odds ratio/likelihood of each socio-demographic profile displaying good or poor RPAP. Using the Pairwise Correlation Table, cross-tabulations were calculated between respondents' risk perception, attitude, and practice to assess significant relationships. The significance of all analyses was tested at the 0.05 level.

Lastly, the development of community health interventions was guided by the pragmatic Six Steps for Quality Intervention Development (6SQuID) framework, originally developed by D. Wight *et al.* [16] and validated by L. Tirman *et al.* [17]. First, the problem and its causes, based on the risk perception, attitudes, and practices of Barangay Buhangin Proper residents concerning dengue infection, were defined and understood. The next step was to identify which of the primary or underlying factors shaping the problem had the most significant scope for change. Furthermore, the most promising modifiable causal factors were identified, and mechanisms for addressing them were formulated. With these change mechanisms in place, the fourth step involved determining the most effective ways to deliver them.

This study underwent thorough examination and approval by the San Pedro College Research Ethics Committee (Protocol No.: 2023-0056). All procedures conformed to the standards for research involving human participants, such as those outlined in the Helsinki Declaration [18]. Respondents read the ICF and provided consent prior to participating in this study.

Results

Of the 584 respondents, the majority were 38-47 years old (23.1%, n = 135), 69.2% (n = 404) were female, 51.9% (n = 303) were married, high school graduates (45.2%, n = 264), 51.2% (n = 299) were unemployed, 44.7% (n = 261) had monthly incomes less than PhP 5,000.00, lived in a single house/bungalow (65.9%, n = 385), and 88.9% (n = 519) of the respondents declared no history of dengue (Table 1).

Table 1. Socio-demographic profile of respondents in the study

Profile		No. of respondents (n = 584)	Percentage (%)
Age group	18-27 years	106	18.2
	28-37 years	113	19.3
	38-47 years	135	23.1
	48-57 years	117	20.0
	>57 years	113	19.3
Sex	Male	180	30.8
	Female	404	69.2
Civil status	Single	208	35.6
	Married	303	51.9
	Widow/Divorcee	68	11.6
	Others	5	0.9
Highest educational attainment	Elementary graduate	52	8.9
	High school graduate	264	45.2
	Senior high school graduate	43	7.4
	College graduate	180	30.8
	Graduate degree acquired	16	2.7
	Others	29	5.0
Employment status	Employed	285	48.8
	Unemployed	299	51.2
Monthly income (range in PhP)	<5,000	261	44.7
	5,000-9,999	128	21.9
	10,000-14,999	85	14.6
	15,000-24,999	66	11.3
	>25,000	44	7.5
Type of residential building	*Kubo* or *Payag*	105	18.0
	Single house/Bungalow	385	65.9
	Apartment	56	9.6
	Multi-unit building (e.g., Dormitory)	27	4.6
	Condominium	0	0.0

Continued Table 1

Profile		No. of respondents (n = 584)	Percentage (%)
	Commercial building	11	1.9
History of dengue	Yes	65	11.1
	No	519	88.9

Source: compiled by the authors

On the other hand, as seen in Table 2, it was found that age ($p < 0.001$) was a significant determinant for risk perception, where those 38 years old and above (19.7-28.7%) tend to have good risk perception regarding dengue infection. In addition, for sex ($p = 0.016$), females lean towards having better risk perception than males. Furthermore, civil status ($p < 0.001$) affects risk perception, where it was found that being married (60.0%) contributes to having good risk perception. Moreover, educational attainment ($p < 0.001$) was a significant determinant, where high school (45.0%) and college (35.7%) graduates exhibit good risk perception compared to other levels. All other variables revealed insignificant associations with risk perception. Moreover, in terms of attitude, only educational attainment ($p = 0.038$) was found to be a significant determinant, with a high proportion of respondents who are high school (44.2%) and college graduates (32.9%) exhibiting a good attitude compared to other levels of educational attainment. Lastly, it was found that all socio-demographic profiles are not significant determinants of practice.

Table 2. Distribution of participants according to risk perception, attitude, and practice (n = 584)

Variable	Risk perception			Attitude			Practice		
	Poor n (%)	Good n (%)	p-value	Poor n (%)	Good n (%)	p-value	Poor n (%)	Good n (%)	p-value
Age group			<0.001			0.315			0.096
18-27	71 (25.0)	35 (11.7)		17 (21.5)	89 (17.6)		72 (17.4)	34 (19.9)	
28-37	58 (20.4)	55 (18.3)		18 (22.8)	95 (18.8)		78 (18.9)	35 (20.5)	
38-47	49 (17.3)	86 (28.7)		12 (15.2)	123 (24.4)		88 (21.3)	47 (27.5)	
48-57	52 (18.3)	65 (21.7)		19 (24.1)	98 (19.4)		84 (20.3)	33 (19.3)	
>57	54 (19.0)	59 (19.7)		13 (16.5)	100 (19.8)		91 (22.0)	22 (12.9)	
Sex			0.016			0.223			0.354
Female	183 (64.4)	221 (73.7)		50 (63.3)	354 (70.1)		281 (68.0)	123 (71.9)	
Male	101 (35.6)	79 (26.3)		29 (36.7)	151 (29.9)		132 (32.0)	48 (28.1)	
Civil status			<0.001			0.184			0.135
Single	123 (43.3)	85 (28.3)		30 (38.0)	178 (35.2)		138 (33.4)	70 (40.9)	
Married	123 (43.3)	180 (60.0)		35 (44.3)	268 (53.1)		216 (52.3)	87 (50.9)	
Widow/Divorcee	37 (13.0)	31 (10.3)		14 (17.7)	54 (10.7)		55 (13.3)	13 (7.6)	
Others	1 (0.4)	4 (1.3)		0 (0.0)	5 (1.0)		4 (1.0)	1 (0.6)	
Highest educational attainment			<0.001			0.038			0.104
Elementary graduate	28 (9.9)	24 (8.0)		5 (6.3)	47 (9.3)		42 (10.2)	10 (5.8)	
High school graduate	129 (45.4)	135 (45.0)		41 (51.9)	223 (44.2)		188 (45.5)	76 (44.4)	
Senior high school graduate	35 (12.3)	9 (2.7)		9 (11.4)	34 (6.7)		34 (8.2)	9 (5.3)	
College graduate	73 (25.7)	107 (35.7)		14 (17.7)	166 (32.9)		115 (27.8)	65 (38.0)	
Graduate degree acquired	8 (2.8)	8 (2.7)		4 (5.1)	12 (2.4)		11 (2.7)	5 (2.9)	
Others	11 (3.9)	18 (6.0)		6 (7.6)	23 (4.6)		23 (3.5)	6 (5.6)	
Employment status			0.371			0.404			0.935
Employed	144 (50.7)	141 (47.0)		42 (53.2)	243 (48.1)		202 (48.9)	83 (48.5)	
Unemployed	159 (49.3)	140 (53.0)		37 (46.8)	262 (51.9)		211 (51.1)	88 (51.5)	
Monthly income			0.108			0.275			0.704
Less than 5,000	141 (49.6)	120 (40.0)		40 (50.6)	221 (43.8)		185 (44.8)	76 (44.4)	
5,000-9,999	62 (21.8)	66 (22.0)		18 (22.8)	110 (21.8)		91 (22.0)	37 (21.6)	
10,000-14,999	37 (13.0)	48 (16.0)		13 (16.5)	72 (14.3)		63 (15.3)	22 (12.9)	
15,000-24,999	28 (9.9)	38 (12.7)		6 (7.6)	60 (11.9)		42 (10.2)	24 (14.0)	
More than 25,000	16 (5.6)	28 (9.3)		2 (2.5)	42 (8.3)		32 (7.7)	12 (7.0)	
Type of residential building			0.426			0.482			0.471
Kubo or *Payag*	53 (18.7)	52 (17.3)		20 (25.3)	85 (16.8)		74 (17.9)	31 (18.1)	
Single house/Bungalow	186 (65.5)	199 (66.3)		48 (60.8)	337 (66.7)		278 (67.3)	107 (62.6)	
Apartment	31 (10.9)	25 (8.3)		7 (8.9)	49 (9.7)		39 (9.4)	17 (9.9)	

Continued Table 2

Variable	Risk perception			Attitude			Practice		
	Poor n (%)	Good n (%)	p-value	Poor n (%)	Good n (%)	p-value	Poor n (%)	Good n (%)	p-value
Multi-unit building	11 (3.9)	16 (5.3)		3 (3.8)	24 (4.8)		16 (3.9)	11 (6.4)	
Commercial building	3 (1.1)	8 (2.7)		1 (1.3)	10 (2.0)		6 (1.5)	5 (2.9)	
History of dengue			0.372			0.642			0.244
Yes	35 (12.3)	30 (10.0)		10 (12.7)	55 (10.9)		50 (12.1)	15 (8.8)	
No	349 (87.7)	270 (90.0)		69 (87.3)	450 (89.1)		363 (87.9)	156 (91.2)	

Source: compiled by the authors

Based on the 80% cut-off point, the respondents of Barangay Buhangin Proper had a good risk perception, with a total score of 51.49. It was found that there is a strong emphasis among respondents that dengue can cause death ($\bar{x}$ = 7.48). Furthermore, seeking immediate medical intervention when fever is persistent ($\bar{x}$ = 6.69), investing money to live in a dengue-free household ($\bar{x}$ = 6.51), and ensuring there are no breeding spots around the house are all considered highly important ($\bar{x}$ = 7.46) (Table 3). Additionally, it was found that respondents agree that intrapersonal, interpersonal, and community factors play a role in developing a good risk perception, such as learning from the experiences of recovered individuals and being involved in community efforts for dengue prevention, both having a mean of 6.63. In addition, the respondents display a slight agreement that there is a need for financial stability when it comes to the success of the implementation against dengue ($\bar{x}$ = 5.14). However, respondents also slightly agree that prioritising other responsibilities contributes to having less time for dengue prevention practices ($\bar{x}$ = 4.95).

Table 3. Details of the mean scores for risk perception questions

Question No.	Mean ($\bar{x}$)	Interpretation
1	7.48	Agree
2	6.69	Agree
3	6.63	Agree
4	6.51	Agree
5	7.46	Agree
6	6.63	Agree
7	5.14**	Slightly Agree
8	4.95**	Slightly Agree
Total score	**51.49***	**Good**

Notes: * – total score interpretation as "Good" or "Poor" is based on an 80% cut-off point [15] (Total mark = 64, 80% cut-off point = 51.2); ** – negatively-worded questions use reverse scoring [19]
Source: compiled by the authors

In Table 4, it can be observed that the respondents of Barangay Buhangin Proper had a good attitude with a total score of 43.62, based on an 80% cut-off point. Generally, the residents' attitudes indicate that the risk of contracting dengue is lessened when at least one person is knowledgeable about the disease in the household ($\bar{x}$ = 6.93). Furthermore, it was found that it is necessary to convey information about dengue fever to family members, ensure that old and unused containers are kept closed, and that drainage or water flow systems are properly maintained, with means of 7.44, 7.44, and 7.31, respectively. Moreover, the respondents agree that interest in performing dengue control or prevention interventions depends on the cooperation of other households ($\bar{x}$ = 6.98). Additionally, the respondents place a strong emphasis on the necessity of disposing of rubbish in designated locations, with a mean of 7.52.

Table 4. Details of the mean scores for attitude questions

Question No.	Mean ($\bar{x}$)	Interpretation
9	6.93	Agree
10	7.44	Agree
11	6.98	Agree
12	7.44	Agree
13	7.31	Agree
14	7.52	Strongly Agree
Total score	**43.62***	**Good**

Notes: * – total score interpretation as "Good" or "Poor" is based on an 80% cut-off point [15] (Total mark = 48; 80% cut-off point = 38.40)
Source: compiled by the authors

Based on an 80% cut-off point, as seen in Table 5, the respondents exhibit poor practice with a total score of 47.22. It was discovered that the respondents moderately agreed with the use of mosquito repellent in the form of lotion, spray, or coil to prevent dengue infection ($\bar{x}$ = 6.35). Furthermore, the respondents agreed that it is essential to keep water containers in their houses tightly closed ($\bar{x}$ = 7.33) and to ensure that proper drainage systems are maintained ($\bar{x}$ = 6.92). The respondents also expressed slight agreement regarding the importance of checking potential mosquito breeding sites around their neighbourhood ($\bar{x}$ = 5.54); therefore, according to the respondents, it is essential to report illegal dumping sites, which could serve as possible mosquito breeding grounds, to the appropriate authorities ($\bar{x}$ = 5.30).

On the other hand, the respondents moderately disagreed with the practice of killing mosquito larvae by adding larvicide to the water storage ($\bar{x}$ = 3.45). In addition, the respondents slightly disagreed with the practice of

reporting abandoned and damaged vehicles that are idling in the neighbourhood ($\bar{x}$ = 4.49), the presence of illegal gardens ($\bar{x}$ = 3.87), and illegal building structures to the appropriate authorities ($\bar{x}$ = 3.97).

Table 5. Details of the mean scores for practice questions

Question No.	Mean ($\bar{x}$)	Interpretation
15	6.35	Moderately Agree
16	7.33	Agree
17	3.45	Moderately Disagree
18	6.92	Agree
19	5.30	Slightly Agree
20	4.49	Slightly Disagree
21	5.54	Slightly Agree
22	3.87	Slightly Disagree
23	3.97	Slightly Disagree
Total score	**47.22***	**Poor**

Notes: * – total score interpretation as "Good" or "Poor" is based on an 80% cut-off point [15] (Total mark = 72; 80% cut-off point = 57.60)
Source: compiled by the authors

Table 6 presents the determination of strong predictors of good RPAP based on the multivariate regression analysis of the association between socio-demographic profiles and risk perception, attitude, and practice. It was found that sex (p = 0.041), educational attainment (p = 0.041), and employment status (p = 0.034) were factors associated with good risk perception. The findings revealed that females (OR = 1.493, 95% CI = 1.016-2.190) were statistically associated with higher odds of having good risk perception than males. In addition, college graduates (95% CI = 0.273-1.561) are 0.653 times more likely to exhibit a good risk perception of dengue fever than respondents with other educational attainments. Furthermore, unemployed individuals were statistically associated with lower odds of having a good risk perception of dengue fever than those employed (OR = 0.654, 95% CI = 0.441-0.970). With those factors in play, there is a need to reject the null hypothesis (H_{01} = p < 0.05); therefore, a significant relationship exists between being female, being a college graduate, being employed about having good risk perception.

Table 6. Multivariate logistic regression analysis between socio-demographic profile and risk perception, attitude, and practice, respectively

Characteristic	Risk perception*		Attitude*		Practice*	
	OR (95% CI)[a]	p-value	OR (95% CI)[a]	p-value	OR (95% CI)[a]	p-value
Age group		0.231		0.410		0.523
18-27 years	0.748 (0.357-1.567)		0.678 (0.216-2.126)		1.770 (0.791-3.963)	
28-37 years	0.800 (0.428-1.495)		0.546 (0.212-1.404)		1.591 (0.787-3.217)	
38-47 years	1.427 (0.802-2.536)		0.987 (0.385-2.532)		1.737 (0.915-3.299)	
48-57 years	1.025 (0.584-1.800)		0.549 (0.235-1.285)		1.468 (0.767-2.811)	
>57 years	0**		0**		0**	
Sex		0.041		0.297		0.583
Male	0**		0**		0**	
Female	1.493 (1.016-2.194)		1.331 (0.781-2.269)		1.123 (0.741-1.701)	
Civil status		0.056		0.038		0.431
Single	0.180 (0.018-1.822)		0.662 (0.255-1.714)		2.651 (0.257-26.268)	
Married	0.254 (0.026-2.520)		0.699 (0.318-1.714)		2.094 (0.216-20.264)	
Widow/Divorcee	0.141 (0.013-1.476)		0.250 (0.250-2.504)		1.451 (0.139-15.167)	
Others	0**		0**		0**	
Highest educational attainment		0.041		0.078		0.059
Elementary graduate	0.542 (0.205-1.430)		2.745 (0.724-10.401)		1.093 (0.340-3.517)	
High school graduate	0.546 (0.241-1.241)		1.231 (0.454-3.342)		1.619 (0.615-4.256)	
Senior high school graduate	0.171 (0.051-0.567)		0.701 (0.175-2.799)		0.746 (0.206-2.696)	
College graduate	0.653 (0.273-1.561)		1.948 (0.625-6.070)		2.405 (0.874-6.614)	
Graduate degree acquired	0.362 (0.093-1.410)		0.371 (0.072-1.924)		2.060 (0.469-9.055)	
Others	0**		0**		0**	
Employment status		0.034		0.111		0.566
Employed	0.654 (0.441-0.970)		0.638 (0.367-1.110)		0.884 (0.581-1.346)	
Unemployed	0**		0**		0**	
Monthly income (range in PhP)		0.155		0.234		0.579
<5,000	0.449 (0.208-0.968)		0.221 (0.046-1.070)		1.386 (0.617-3.110)	
5,000-9,999	0.588 (0.271-1.273)		0.274 (0.056-1.334)		1.193 (0.529-2.693)	
10,000-14,999	0.798 (0.358-1.780)		0.261 (0.053-1.283)		0.971 (0.412-2.289)	
15,000-24,999	0.743 (0.320-1.725)		0.421 (0.076-2.334)		1.653 (0.695-3.931)	

Continued Table 6

Characteristic	Risk perception*		Attitude*		Practice*	
	OR (95% CI)[a]	p-value	OR (95% CI)[a]	p-value	OR (95% CI)[a]	p-value
>25,000	0**		0**		0**	
Type of residential building		0.596		0.577		0.402
Kubo or *Payag*	0.358 (0.081-1.580)		0.377 (0.040-3.553)		0.517 (0.134-1.997)	
Single house/Bungalow	0.366 (0.088-1.520)		0.559 (0.063-4.939)		0.414 (0.116-1.481)	
Apartment	0.374 (0.082-1.698)		0.763 (0.076-7.630)		0.492 (0.124-1.951)	
Multi-unit building	0.528 (0.103-2.712)		0.063 (0.063-9.139)		0.772 (0.175-3.399)	
Condominium	0**		0**		0**	
Commercial building	0**		0**		0**	
History of dengue		0.457		0.709		0.194
Yes	0.809 (0.462-1.415)		0.863 (0.400-1.860)		0.666 (0.355-1.249)	
No	0**		0**		0**	

Notes: a – OR, Odds ratio (at 95% confidence interval); * – the reference category is: Poor; ** – this parameter is set to zero because it is redundant

Source: compiled by the authors

Moreover, it was noted that individuals who are 38-47 years old (95% CI = 0.802-2.536) are 1.427 times more likely to have a good risk perception of dengue fever compared to other age groups. In addition, married individuals (OR = 0.254, 95% CI = 0.026-2.520) were statistically associated with higher odds of having good risk perception than those with other civil statuses. Furthermore, individuals who have monthly incomes of less than PhP 5,000, live in *kubo* or *payag* and have no history of dengue were associated with lower odds of having good risk perception. It can be noted that individuals earning PhP 10,000-14,999 monthly (OR = 0.798, 95% CI = 0.358-1.780), living in multi-unit buildings like dormitories (OR = 0.508, 95% CI = 0.103-2.712), and having a history of dengue (OR = 0.809, 95% CI = 0.462-1.415) were more likely to have good risk perception of dengue fever.

However, it is worth noting that the null hypothesis (H_{01}) should not be rejected because the discussed sociodemographic profiles indicate $p \geq 0.05$; therefore, age (p = 0.231), civil status (p = 0.056), monthly income (p = 0.155), type of residential building (p = 0.596), and history of dengue (p = 0.457) were factors that do not have a significant relationship with good risk perception.

Furthermore, married (OR = 0.699, 95% CI = 0.318-1.714) individuals are statistically associated with higher odds of having a good attitude, and only civil status (p = 0.038) allows the null hypothesis ($H_{02} = p < 0.05$) to be rejected, which indicates that there is a significant relationship between being married and having a good attitude towards dengue prevention.

On the other hand, those who are 38-47 years old (95% CI = 0.385-2.532) are 0.987 times more likely to exhibit a good attitude than the other age groups. Aside from that, females (OR = 1.331, 95% CI = 0.781-2.269), elementary graduates (OR = 2.745, 95% CI = 0.724-10.401), employed individuals (OR = 0.638, 95% CI = 0.367-1.110), those earning PhP 15,000-24,999 (OR = 0.421, 95% CI = 0.076-2.334), individuals living in apartments (OR = 0.763, 95% CI = 0.076-7.630), and those with a history of dengue (OR = 0.863, 95% CI = 0.400-1.860) were associated with higher odds of having a good attitude towards dengue prevention.

However, the rest of the socio-demographic profiles fail to reject the null hypothesis ($H_{02} = p \geq 0.05$); therefore, age (p = 0.410), sex (p = 0.297), educational attainment (p = 0.078), employment status (p = 0.111), monthly income (p = 0.234), type of residential building (p = 0.577), and having a history of dengue (p = 0.709) were factors that did not have a significant relationship with having a good attitude towards dengue prevention.

Lastly, it was found that individuals who are 38-47 years old (OR = 1.737, 95% CI = 0.915-3.299), females (OR = 1.123, 95% CI = 0.741-1.702), single individuals (OR = 2.651, 95% CI = 0.257-26.268), college graduates (OR = 2.405, 95% CI = 0.874-6.614), employed individuals (OR = 0.884, 95% CI = 0.581-1.346), those earning PhP 15,000-24,999 monthly (OR = 1.653, 95% CI = 0.695-3.931), individuals living in multi-unit buildings like dormitories (OR = 0.772, 95% CI = 0.175-3.399), and those with a history of dengue (OR = 0.666, 95% CI = 0.355-1.249) were associated with higher odds of having good practices for dengue prevention. However, the null hypothesis (H_{03}) cannot be rejected ($H_{03} = p \geq 0.05$); therefore, age (p = 0.523), sex (p = 0.583), civil status (p = 0.431), educational attainment (p = 0.059), employment status (p = 0.566), monthly income (p = 0.579), type of residential building (p = 0.402), and history of dengue (p = 0.194) were factors that did not have a significant relationship with having good practices for dengue prevention.

The findings show that risk perception and attitude (r = 0.266, p < 0.001), risk perception and practice (r = 0.092, p < 0.027), and attitude and practice (r = 0.144, p < 0.001) show a positive linear relationship despite the weak correlation, and all indicate the rejection of the null hypothesis (H_{04}: $x1 \neq x2 \neq x3$). Therefore, there exists enough evidence that there is a significant relationship between the risk perception, attitude, and practice of Barangay Buhangin Proper residents regarding dengue infection (Table 7).

Table 7. Pairwise correlation matrix for RPAP

RPAP-area R	r	p-value	Relationship	Size of correlation
Risk perception vs. Attitude	0.266**	<0.001	Positive	Weak
Risk perception vs. Practice	0.092*	0.027	Positive	Weak
Attitude vs. Practice	0.144**	<0.001	Positive	Weak

Notes: r – pearson correlation coefficient, interpretation: -1.0 (perfect negative), -0.8>r>-1.0 (strong negative), -0.4>r>-0.8 (moderate negative), 0>r>-0.4 (weak negative), 0 (no linear relationship), 0<r<0.4 (weak positive), 0.4<r<0.8 (moderate positive), 0.8<r<1.0 (strong positive), 1.0 (perfect positive); ** – correlation is significant at the 0.01 level (2-tailed); * – correlation is significant at the 0.05 level (2-tailed)
Source: compiled by the authors

With this information considered, it is now important to develop more personalised interventions and to adapt practical approaches that could prove beneficial in addressing deep-rooted problems. To effect a behaviour change, health interventions may find it challenging to discourage ingrained negative habits through conventional knowledge sharing alone; however, the existence of the problem will only be resolved when all members of the community act in solidarity – with one goal: to eliminate dengue once and for all.

Utilising the Pragmatic 6SQuID Framework, community health interventions were developed by first defining and understanding the problem. It is known that Barangay Buhangin Proper has still recorded a significant increase in dengue cases despite the implementation of numerous interventions, as supported by the residents' poor level of practice. Generally, it affects all individuals in Barangay Buhangin Proper, and the problem exists at every socio-demographic level. The causes of the problem were identified, as shown in Figure 1, with complacency, self-serving behaviour, and interventions that are not targeted and personalised being the most significant. So far, the reproduction and dissemination of dengue information materials, regular clean-up drives, and the *"5S Laban sa Dengue"* programme have been effective in addressing the problem. However, the immediate consequence of poor practices leads to individuals acquiring dengue, and the long-term continuation of poor practices without appropriate intervention will inculcate a sense of normalcy regarding that habit within the members of the community, leading to rising dengue cases, affecting the general population's health and diminishing the health security of the residents.

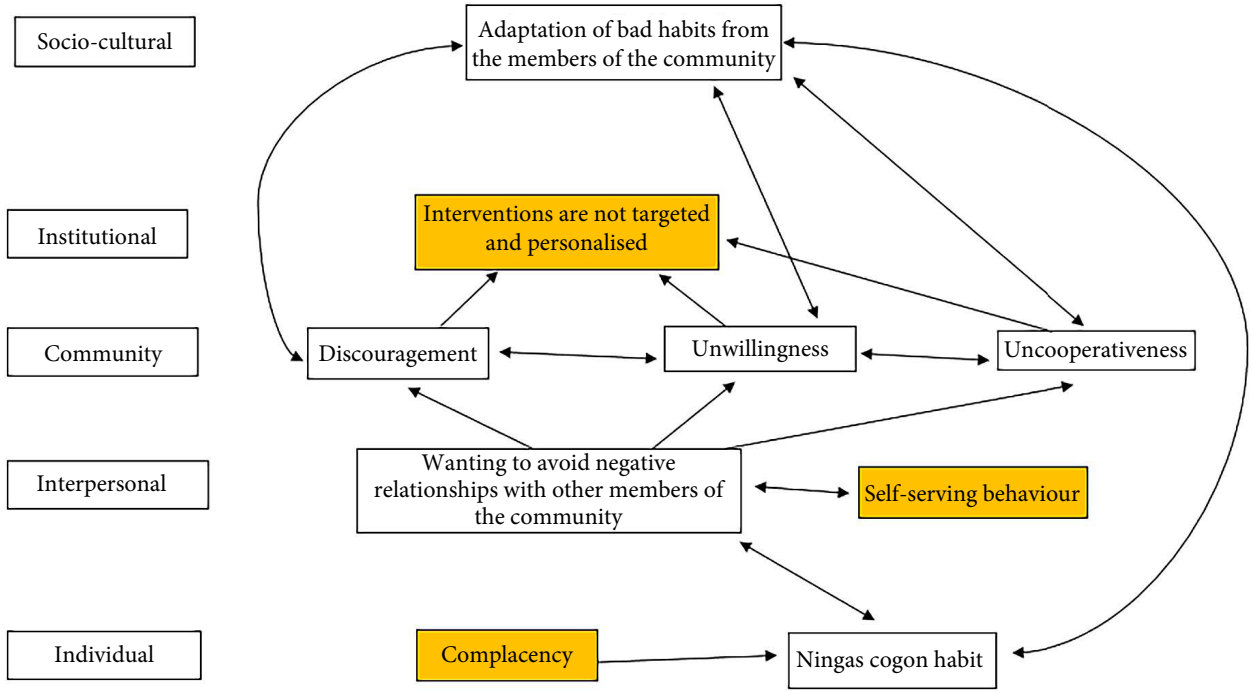

Figure 1. Causal pathways worsening the state of dengue in the community
Source: developed by the authors of this study

Accordingly, complacency is a factor that is malleable and has the greatest scope for change by reinforcing risk perception and attitude towards dengue and building on practising long-term commitment to the interventions.

The change mechanism that could aid this is to develop interventions that would promote action against dengue even with or without the risk, and this is sufficient to reduce the problem if the factor is truly eliminated. Additionally, self-serving behaviour is another factor that could be mediated by promoting a sense of community and strengthening the shared responsibilities of each member of the community through the development of prevention programmes that promote collaboration. Lastly, the lack of targeted and personalised interventions is a factor with great potential for change and it can be sufficient in reducing the problem by utilising existing policies and interventions but requires a reconceptualization of the goals and objectives that target the significant determinants of poor RPAP.

With the significant determinants and malleable factors identified, it is important to deliver the change mechanisms by implementing the following developed community health interventions: (1) involving youth organisations in effective actions by spearheading long-term campaigns for the young population that commit to practising good prevention practices and reinforcing perceived risk and attitude towards dengue in the community; (2) engaging unemployed residents to fill pivotal roles in the dengue task forces in each purok so that risk perception, attitude, and practice regarding dengue infection are simultaneously addressed. At the same time, the population will have opportunities to earn money; (3) reinforcing knowledge on the risk and assessing the need for behavioural change for the male population by facilitating seminars regarding dengue, disseminating appropriate information, and exercising good dengue prevention practices; (4) continuing to facilitate weekly clean-up drives and inspections in residences to ensure appropriate practices are followed by all households to prevent potential breeding sites within their houses and surroundings; (5) taking advantage of social media by boosting the use of the Barangay Information Page as an avenue for the authorities to learn about the problems in the community and for the residents to share appropriate information on dengue, promoting community engagement by encouraging shared responsibilities and ensuring healthy discussions take place to address the problems related to dengue; (6) establishing dengue-specific support programme booths in the local barangay hall to alleviate the stigma associated with locals being reluctant to deal with and treat dengue, such as booths that re-evaluate the need for financial and blood banking assistance during admissions; and (7) utilising monitoring and assessment strategies by including routine operational evaluations to ascertain the state of the project and the actual inputs that the programme has received, along with epidemiological evaluations to determine the effectiveness or failure of the health interventions implemented in the community.

In summary, this study offers important insights into the factors associated with perceived dengue risk, attitude towards it, and practices among the residents in Barangay Buhangin Proper. The results are consistent with past research and provide novel insights into the association between risk perception, attitude, and practices. The findings may provide direction for community health interventions that enhance understanding and propel individuals to actively participate in dengue prevention – the ultimate objective.

Discussion

Generally, the residents of Barangay Buhangin Proper exhibit good risk perception and attitude towards dengue infection, based on the 80% cutoff point; however, it was noted that they have poor practices for dengue prevention. The study's results on RPAP were comparable to a previous study by M.I.A. Zamzuri *et al.* [19], which found that the perceived risk of dengue infection exerts a positive effect on health behaviour change, although a lack of preventive practices is a problem identified in the study. However, it was found that RPAPs are significantly correlated with each other because risk perception was known to be a mediating variable between attitude and dengue prevention practice, and vice versa.

In the study, it can be observed that age can be a determinant of having good risk perception, as older individuals display better risk perception of dengue than those who are younger. This is supported by E.M. Bonem *et al.* [20], wherein older adults are less likely to engage in risky health behaviours than younger adults.

Moreover, the results of this study show that females are generally more cautious regarding dengue than males, and this has been recognised as a socio-demographic profile that has a significant association with good risk perception. This is reinforced by the study of A.N. Rakhmani *et al.* [21], which found that female respondents demonstrate better risk perception due to women's social role of caring for their households and families.

Furthermore, it was found that married individuals tend to have a heightened perceived risk and a better attitude towards dengue because, as elaborated by S. Selvarajoo *et al.* [22] and further supported by M. Hamed [23], married couples have a greater sense of obligation to protect their family members than those who are single, who are more dependent on their families. This results in a better attitude towards preventing dengue infection since families possess more resources for the comfort of their households and surroundings and the safety of their children.

In addition, the study reveals that college graduates notably have enhanced risk perception and a better attitude towards dengue. However, being a college graduate is only statistically associated with having good risk perception. Even though the majority of college graduates responded positively and were identified as having a good attitude, this is statistically not directly associated. This is supported by F.A. Diaz-Quijano *et al.* [24], who found that the higher an individual's education level, the more likely they are to recognise the risk of dengue and seek medical help in a timely manner. However, regardless of the level of education, people understand dengue as a disease and their attitude towards it is equally valid.

Aside from this, it can be noted that employed individuals have a stronger perceived risk than those who are unemployed. As determined by M. Nur Ain *et al.* [25], employed individuals possess better knowledge of dengue fever because of their exposure to workplace health campaigns, which increases their perception of dengue as a risk. Moreover, this socio-demographic profile has a direct association with good risk perception; therefore, being unemployed increases, the likelihood of an individual having poor risk perception. S. Selvarajoo *et al.* [22] state that the unemployed have a lesser outlook on risk, which results in higher-risk behaviours.

Additionally, the study reveals that there is a lack of evidence indicating that the socio-demographic profiles of the respondents are significantly associated with good practices. Therefore, the problem of practice exists among all respondents, regardless of their socio-demographic profile. Despite having good perceived risk and attitude towards dengue, it is noteworthy that although they are known to be correlated with each other, adequate knowledge of the risks and good behaviour towards dengue does not necessarily reflect good dengue prevention practices when complacency is at play, especially when the perceived risk diminishes [22].

M.I. Hossain *et al.* [26] have discovered that poor execution of preventive practices, despite having basic knowledge of dengue, leads to misunderstandings that increase in dengue cases. The majority of the causes of poor practices stem from bad habits and complacency in adhering to anti-dengue campaigns and measures [14, 27]. It was also highlighted that the residents' behaviour towards dengue is hindered by a lack of community cooperation and support, which remains an obstacle; only a few residents implement the necessary practices, and the majority tend to act only when cases are reported in their respective areas [28].

In the case of the residents of Barangay Buhangin Proper, it can be observed that significant dengue prevention practices are still performed within households. This could explain their acknowledgement of the importance of seeking immediate medical intervention when symptoms arise and ensuring that breeding sites for mosquitoes around their homes are eliminated, to name just a few. Moreover, the local government unit implements numerous interventions to combat the problem, such as regular clean-up drives. However, when the perceived risk diminishes, residents tend to become complacent and discontinue good prevention practices, leading to a rise in dengue cases. Furthermore, interpersonal and community factors may also be major contributors to the problem. It can be observed that residents address dengue issues independently and are reluctant to intervene in the affairs of their neighbours to avoid conflicts. This attitude may explain their reluctance to report the negative behaviours of other community members, which are known to pose significant risk factors contributing to the escalating dengue cases in the community. The shared responsibility among community members is lost, rendering it ineffective for a household to maintain good prevention practices when those around them do not reciprocate this behaviour.

Conclusions

The goal was to assess the level of RPAP among residents in Barangay Buhangin Proper, Davao City, and to determine the relationship between RPAP and socio-demographic profiles. Overall, the study has provided a comprehensive analysis of the RPAP of residents in Barangay Buhangin Proper, Davao City, and has identified significant determinants of good or poor RPAP. It was found that the residents display good risk perception and attitude but have poor practices. Significant determinants such as age, sex, civil status, and educational attainment were revealed for risk perception, and educational attainment was noted for attitude; however, there were no significant determinants for practice. Furthermore, females, college graduates, and those who are employed have higher odds of exhibiting good risk perception, while married individuals are more likely to display a good attitude towards dengue. The results also indicate that there is a lack of evidence to suggest that the socio-demographic profile has a significant association with good practice, which means that the problem exists regardless of the profile.

The study also emphasises the need to develop prevention programmes that promote collaboration among community members, create interventions that encourage action against dengue, regardless of the perceived risk, and reinforce existing policies that target significant determinants contributing to poor RPAP. The problems, their causes, mechanisms for change, and methods of delivery have been identified. Therefore, the next step is to implement the developed community health interventions in the target community and evaluate their effectiveness. To effect change, the existence of the problem must be addressed by ensuring that community members act in solidarity – with a unified goal – to eliminate dengue once and for all.

Acknowledgements

Gratitude is extended to co-researchers (Kylle Aguilar, Nikki Berico, Cristian Buena, Lou Denzo, Byen Estimada, Thobby Gamosa, Jezel Macaranas, Emely Odtojan, Kian Oredina, Angelyka Sanchez, and Roshane Ukkong), mentors, the *barangay* office of Buhangin Proper, the respondents, the personnel who assisted authors, and supportive friends and parents.

Conflict of Interest

None.

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Оцінка сприйняття ризику, ставлення та методів профілактики лихоманки денге у адміністративному регіоні Buhangin Proper міста Давао

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Анотація. Лихоманка денге продовжує бути проблемою громадського здоров'я на Філіппінах та у світі. Це дослідження мало на меті оцінити сприйняття ризику, ставлення та практику жителів Barangay Buhangin Proper та встановити, чи мають соціально-демографічні профілі значний вплив на сприйняття ризику, ставлення та практику запобігання, щоб розробити стратегічні заходи у сфері охорони здоров'я громади. Загалом 584 жителя відповіли на очне опитування з використанням адаптованого опитувальника сприйняття ризику, ставлення та практики. Згідно з балами сприйняття ризику, ставлення та практики, жителі мають добре сприйняття ризику (51,49, поріг = 51,20) та ставлення (43,62, поріг = 38,40), але мають погану практику (47,22, поріг = 57,60). Дослідження також виявило, що жінки (ВШ = 1,493, 95 % ДІ = 1,016-2,194, p = 0,041), випускники коледжу (ВШ = 0,653, 95 % ДІ = 0,273-1,561, p = 0,041) та працевлаштовані особи (ВШ = 0,654, 95 % ДІ = 0,441-0,970, p = 0,034) статистично пов'язані з добрим сприйняттям ризику, а одружені особи (ВШ = 0,699, 95 % ДІ = 0,318-1,714, p = 0,038) – з добрим ставленням, але жоден з факторів не був пов'язаний з доброю практикою. Тому проблема з практикою існує незалежно від профілю. Крім того, сприйняття ризику та ставлення (r = 0,266, p < 0,001), сприйняття ризику та практика (r = 0,092, p = 0,027), та ставлення та практика (r = 0,144, p < 0,001) показують слабкий позитивний лінійний зв'язок. Незважаючи на усвідомлення ризику та посилене ставлення, громада стикається з викликами у підтриманні добрих практик через самозаспокоєння, корисливу поведінку та неадекватні втручання. Дослідження пропонує впровадження розроблених заходів для вирішення корінних причин та ефективного усунення лихоманки денге

Ключові слова: соціально-демографічний профіль; розробка заходів у сфері охорони здоров'я громади; модифіковані фактори, що погіршують стан денге; механізми змін для запобігання денге; впровадження механізмів змін

International Journal of Medicine and Medical Research

Journal homepage: https://ijmr.com.ua/
Volume 10, No. 2. 2024

UDC 616.9:578.834.1:616-074(547/548)
DOI: 10.61751/ijmmr/2.2024.39

Article's History:
Received: 28.07.2024; Revised: 06.10.2024; Accepted: 26.11.2024

Clinical correlation of acute phase reactants and vitamin D in patients with coronavirus disease in the north-eastern part of India

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Abstract. COVID-19 is associated with pre-existing co-morbid conditions and vitamin D insufficiency or deficiency as risk factors. Inflammatory biomarkers like acute phase reactants are widely used for monitoring treatment and outcome of the disease. A prospective and observational study was conducted with a purpose to analyse any clinical association of COVID-19 severity with levels of vitamin D, ferritin, lactate dehydrogenase, C-reactive protein, and D-dimer in 100 patients of COVID-19 at a zonal hospital in Tezpur, Assam, India in 2021. All relevant data including age, gender, or co-morbid conditions were retrieved from medical case sheets and laboratory test results. Serum samples of vitamin D and acute phase reactants were collected in COVID wards within 24 hours of admission. Prevalence of 71% of vitamin D deficiency was observed in the current study with mean ±2 SD of vitamin D of 16.6 ± 6.9 ng/mL in Group 1 and 17.1 ± 7.4 ng/mL in Group 2. No significant correlation of COVID-19 with deficiency of vitamin D was observed

Suggest Citation:
Singh TS, Bhalla S, Chakrabarty BK, Boruah D, Meetei ST. Clinical correlation of acute phase reactants and vitamin D in patients with coronavirus disease in the north-eastern part of India. Int J Med Med Res. 2024;10(2):39–48. DOI: 10.61751/ijmmr/2.2024.39

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(p = 0.8107). Serum C-reactive protein levels varied substantially between Group 1 (24.46 ± 34.4 mg/L) and Group 2 (38.5 ± 32.5 mg/L) and were significantly associated with disease severity (p = 0.0397). Levels of ferritin, LDH, and D-dimer were also significantly elevated in Group 2 as compared to Group 1 (p < 0.05). It is therefore concluded that low vitamin D levels do not predict severity and outcomes in COVID-19. C-reactive protein, ferritin, lactate dehydrogenase and D-dimer levels are significantly associated and raised in patients with moderate to severe COVID-19

Keywords: COVID-19; observational; C-reactive protein; inflammation; association

Introduction

Coronavirus disease, also known as 2019-nCoV was discovered from Group of people working in seafood market in China who had some unknown pneumonia [1]. The disease swiftly evolved from an epidemic to a pandemic after it spread quickly from China to other parts of the world. As of April 21, 2024, 775,364,261 COVID-19 cases had been confirmed globally, and over 7,046,320 cases of death had been recorded [2].

Many researchers claimed that vitamin D levels can determine severity of COVID-19 infection and its outcomes. S. Singh *et al.* [3] in their cross-sectional study found that COVID-19 is associated with deficiency of vitamin D and older age, presence of diabetes and hypertension as risk factors. D.O. Meltzer *et al.* [4] in their cohort study discovered that deficiency of this vitamin makes people more prone to COVID-19. M. Sobczak & R. Pawliczak [5] in their meta-analysis on the subject found positive effect of vitamin D supplements in decreasing admissions in intensive care units and deaths related to COVID-19 which indirectly supported the aforementioned fact. Some researchers claimed that deficiency of this vitamin is not linked with proneness or negative outcomes of the infection. P. Sanamandra *et al.* [6] in their prospective observational study involving 200 COVID-19 patients concluded that vitamin D deficiency is not linked with severity of infection in Indian population. Similarly, meta- analysis by G. Butler-Laporte *et al.* [7] on 443,734 participants from 11 countries found no correlation between COVID-19 susceptibility, severity, or hospitalisation and vitamin D level. Older age and presence of co-morbid conditions are thought to increase the risk of infection. S. Yastremska *et al.* [8] studied the influence of chronic diseases on COVID-19 manifestation by taking sample of patients from 14 states of United States of America. They found that case fatality rate below 60 years is much lesser (1.5%) than those above 60 years (4.5%) and concluded that chronic diseases are the main risk of life-threatening complications and poor prognosis for patients. It is hence cleared during literature search that difference of opinions among authors exist with regards to vitamin D levels, however all seems to agree with associated severity with older age and presence of co-morbidities.

Variation in the levels of acute phase proteins is bound to happen during the infection and associated inflammatory response. It is known that levels of lactate dehydrogenase (LDH), ferritin, and D-dimers are increased with severity of infection being positive acute phase reactants [9, 10] However, there is a conflicting consensus on C-reactive protein (CRP) level with severity of COVID-19 infections. Meta-analysis conducted by F. Zeng *et al.* [11] on 3,962 COVID-19 patients found that milder form of infection has less elevation in serum CRP. However, a retrospective study conducted by H.C. Luo *et al.* [12] involving 85 patients found no relation of CRP levels with disease severity. Based on above facts, it is observed that there is still a lack of consensus on relation of deficiency of vitamin D and C-reactive protein with the severity of COVID-19 infection. Therefore, it was necessary to conduct a study with the aim of analysing relations of vitamin D levels, acute phase reactants, age group, and comorbidities against the severity of COVID-19 patients. Additionally, the role of COVID vaccination in relation to severity was also investigated.

Materials and Methods

Prospective, cross-sectional, and observational study was undertaken at a zonal hospital in Tezpur, Assam, India, from May to August 2021. Patients who were admitted in the COVID wards during the above period were targeted for the study. Total of 100 patients (22 female, 78 male) were included and divided into 2 groups: Group 1 with mild disease (n = 50) and Group 2 with moderate to severe disease (n = 50) based on the case definition as described below. Moderate and severe cases were put together in Group 2 as there were very few severe COVID-19 patients during the study period. Details of age, gender, and any presence of co-morbid conditions were collected from medical case sheets.

All confirmed cases of COVID-19 diagnosed by RT-PCR test performed on Real-Time PCR Thermal Cyclers by Analytik Jena using Meril COVID-19 RT-PCR kits in a period from 01 May to 31 August 2021. Case definitions for COVID-19 severity were categorised as follows: Mild COVID-19 was characterised by upper respiratory tract symptoms and/or fever without the presence of shortness of breath or hypoxia. Moderate COVID-19 presented with breathlessness, a respiratory rate of 24 or more respirations per minute, and aSpO_2 level ranging from 90 to 93% on room air, accompanied by radiological signs of pneumonia. Severe COVID-19 involved significant breathlessness, a respiratory rate exceeding 30 respirations per minute, and a SpO_2 level below 90% on room air, along with radiological evidence of pneumonia. Chronic debilitating diseases such as chronic obstructive pulmonary diseases, chronic kidney diseases, cancers.

All patients who were newly diagnosed and admitted in the COVID wards were sampled within 24 hours of their admission for all the routine blood tests including CRP and D-dimers as per established protocol by laboratory

technician wearing adequate PPE. Patients were well informed and taken consent for doing additional tests such as vitamin D, LDH, and ferritin for the current study. No special preparation of patients was needed for sample collection. CRP and LDH tests were estimated using a fully automated biochemistry analyser, Dimension EXL-200 manufactured by M/s Siemens healthcare diagnostics (USA). Ferritin, D-dimer, and vitamin D levels were measured using a fully automated immunoassay (Vidas) developed by M/s BioMerieux (France).

Serum samples were refrigerated at 4-5 degrees if these tests were not conducted within 2-3 hours. Reference values differed from one laboratory to another. Values used in the present study are given in Table 1.

Table 1. References values

Serum vitamin D	Serum CRP	Serum D-dimer	Serum Ferritin	LDH
Deficient <20 ng/mL Insufficient <30 ng/mL Sufficient 30-100 ng/mL	Normal <5 mg/L	Normal <500 ng/mL	Males 70-435 ng/mL Females 10-160 ng/mL	Normal 100-190 IU/L

Source: compiled by the authors

An excel worksheet was prepared and data analysed through SPSS software suite. Unpaired t-test and z-test were performed for statistical results. p-value of <0.05 was regarded significant. Data were presented in mean ±SD and in percentages. The prevalence of vitamin D deficiency among patients was assessed using a conventional equation as below:

$$P = X \div N \cdot 100, \quad (1)$$

where P – prevalence of vitamin D deficiency; X – number of patients with vitamin D less than 20 ng/mL; N – total of patients studied.

The study was in compliance with the principles of Declaration of Helsinki [13]. There was no risk or burdens to the patients but would only benefit in understanding the relation of studied parameters in blood in COVID-19 patients. All patients were well informed and given written consent. The study was approved by Hospital Ethics Committee vide application number 155BH/ 01/IEC/2021.

Results

100 confirmed COVID-19 cases were evaluated for their severity and several blood tests were conducted. They were divided into Group 1 with milder severity and Group 2 with moderate to severe COVID-19. 22 (22%) out of the 100 patients were females, whereas 78 (78%) were males. Age ranged from 19 to 71 years however, 82% of patients were under 50 years. 18% of patients were over 50 years with majority (14 cases) belonged to Group 2. Most patients (82%) were under the age of 50. Only one person had all the three co-morbid conditions i.e., obesity, diabetes, and hypertension. Obesity affected 11%, diabetes – 7%, and hypertension – 10% of the study population. During the study, three people succumbed due to complications related to severe COVID-19. Later, it was found that they had one of the co-morbid conditions and were found unvaccinated. 35% of patients received 2 doses of COVID vaccines (Covishield/Covaxin) at the time of admission, 65% had received one dose, and 5% had no history of vaccination. Table 2 summarises the demographic details of patients.

Table 2. Demographic details of patients as per their groups

No.	Parameters	Group 1 (n = 50)	Group 2 (n = 50)
1.	Sex Male Female	 43 7	 35 15
2.	Age <50 years >50 years	 46 4	 36 14
3.	Co-morbid conditions (i) Obesity (ii) Diabetes (iii) Hypertension	 1 1 1	 10 6 9
4.	Vaccination Complete (2 doses) Incomplete (1 dose) Non vaccinated	 28 22 0	 7 38 5
5.	Fatality	0	3

Source: compiled by the authors

Correlation studies were conducted to evaluate any clinical correlation of acute phase reactants, (APRs), deficiency of vitamin D with severity of COVID-19. Prevalence of vitamin D deficiency <20 ng/mL was observed in 71% of all patients (70 and 72% in Group 1 and 2 respectively). Overall, more than 60% of patients had elevated APRs, except for ferritin which showed elevated levels in only 12-19% of patients. The demographic characteristics included in Table 1 were found to differ significantly with respect to disease severity in terms of age, gender, co-morbid conditions, and vaccination status. Younger patients appeared to have a milder form of COVID-19. Co-morbid conditions are less common in Group 1 than in Group 2 patients. Box plot analysis of the parameters for the two groups is also given in Figure 1 (A-F) with p-values.

Mean vitamin D level was 16.6 ± 6.9 ng/mL in Group 1 and 17.1 ± 7.4 ng/mL in Group 2. When data were analysed, no concrete relationship was found regarding severity of COVID-19 infection. Figure 2 demonstrates the percentage of vitamin D deficiency amongst study groups.

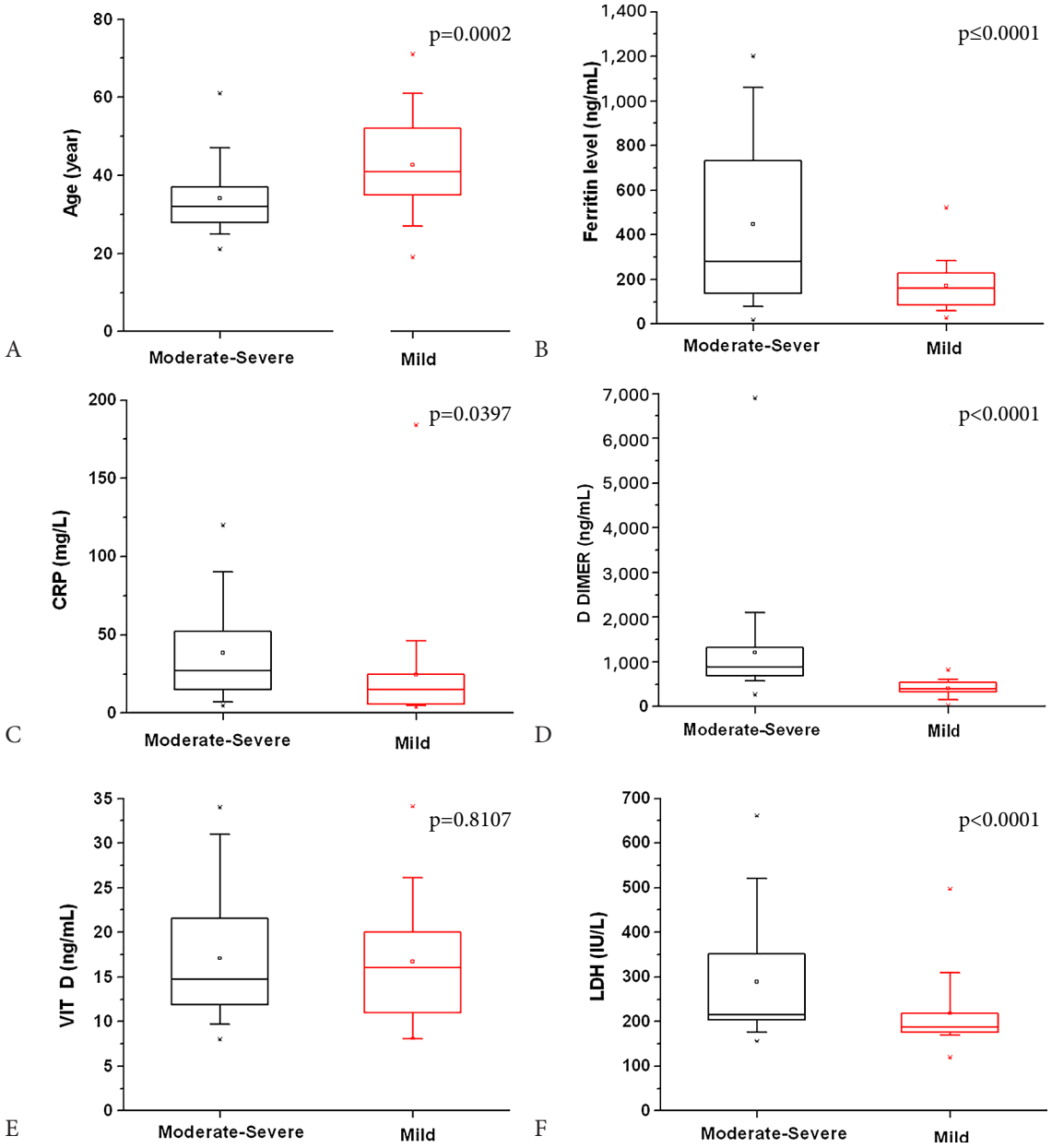

Figure 1. Box plot of the parameters for the two groups (A-F)

Source: compiled by the authors

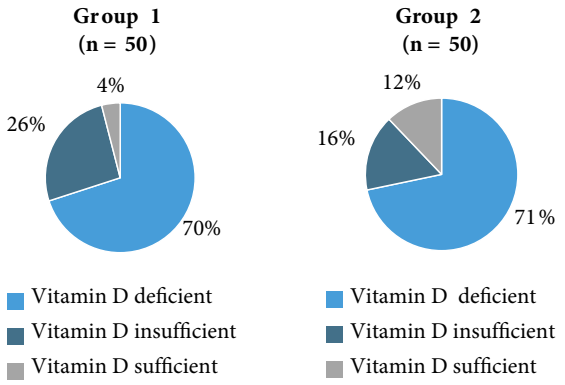

Figure 2. Vitamin D status in Group 1 and Group 2
Source: compiled by the authors

Serum CRP levels also shown significant association between Group 1 (24.46 ± 34.4 mg/L) and Group 2 (38.5 ± 32.5 mg/L), and were linked with COVID-19 severity ($p = 0.0397$). D-dimers, ferritin, and LDH were substantially higher and significant in moderate to severe COVID-19. Group 1 had a mean D-dimer level of 410 ± 171 ng/mL while Group 2 had 1,209 ± 1,050 ng/mL ($p < 0.0001$). Similarly mean ferritin level in Group 1 was 172 ± 111 ng/mL while in Group 2 it was 448 ± 377 ng/mL ($p < 0.001$). Mean level of LDH in Group 1 was 219 ± 82 IU/L while in Group 2 it was 289 ± 136 IU/L ($p = 0.0024$). There was significant finding that patients without proper vaccination had higher risk of moderate to severe COVID-19 infection ($p < 0.0001$). Table 3 summarises analysis of all the above parameters with extent of their significant association with severity of COVID-19.

Table 3. Mean value with standard deviations and range of the studied parameters

Parameters (unit)	Mild (n = 50, male = 43, female = 7)	Moderate/Severe (n = 50, male = 35, female = 15)	p-value
1. Age (years)	34.1 ± 8.8 (21-61)	42.6 ± 12.8 (19-71)	0.0002 (t-test)
2. FERRITIN (ng/mL)	172 ± 111 (27-521)	448 ± 377 (17-1,200)	<0.0001 (t-test)
3. CRP (mg/L)	24.46 ± 34.4 (4-184)	38.5 ± 32.5 (5-120)	0.0397 (t-test)
4. D-dimer (ng/mL)	410 ± 171 (24-823)	1,209 ± 1,050 (270-6,900)	<0.0001 (t-test)
5. Vitamin D (ng/mL)	16.6 ± 6.9 (8.1-34.1)	17.1 ± 7.4 (8-34)	0.8107 (t-test)
6. LDH (IU/L)	219 ± 82 (119-497)	289 ± 136 (156-661)	0.0024 (t-test)
7. Vaccination score (non-vaccinated = 0, incomplete = 1, complete = 2)	1.56 ± 0.50 (1-2)	1.04 ± 0.49 (0-2)	<0.0001 (t-test)
8. Obesity	2%	20%	0.0039 (z-test)
9. Diabetes	2%	12%	0.0510 (z-test)
10. Hypertension	2%	18%	0.0076 (z-test)
11. Survival	100%	94%	-

Source: compiled by the authors

In the current study, overall prevalence of 71% in vitamin D deficiency amongst study participants with mean of 16.6 ± 6.9 and 17.1 ± 7.4 ng/mL were found in Group 1 and Group 2 respectively. In this study age of patients ranged from 19 to 71 years with mean of 34.1 ± 8.8 and 42.6 ± 12.8 years in the Group 1 and Group 2 separately. It was also significantly associated. However, there was no significant relation of COVID-19 severity with deficiency of vitamin D even though there were definite significant association with all acute phase reactants studied. Out of 100 patients only 11% were obese, 7% – diabetic, and 10% – hypertension; hence no significant analysis of co-morbid conditions could be done. 3 patients who died had either one of the co-morbid conditions and were unvaccinated. 35% of patients vaccinated, 65% – partially vaccinated and 5% – had no history of immunisation. Definite positive association was also observed between vaccination and COVID-19 severity.

Discussion

WHO named coronavirus disease 2019 (COVID-19) in February 2020 and declared pandemic on March 11, 2020 [2, 14]. It can spread through droplets, aerosols, and fomites. It can enter the lungs through the nose or mouth, causing respiratory problems. It acts by inhibiting ACE2 function thereby increasing Angiotensin-2 production which causes tissue injury in COVID-19 patients, notably, in the lungs and heart [14, 15]. Cytokine storms is the primary mechanism for injury to these organs [16, 17]. Complications like ARDS, sepsis, and multiorgan failure are major causes of death [18]. Vitamin D exerts its beneficiary effects through reduction of cytokine storms by increasing proinflammatory and decreasing inhibitory cytokines [19]. Several literatures have advocated its role in prevention of infections including COVID-19 by influencing innate & adaptive immunity [20, 21]. Normal level of vitamin D ranges from 30-100 ng/mL. Value of less than 20 ng/mL was considered deficient in this study.

Current study was approached with the initial assumption that deficient vitamin D status is linked with increased severity and complications associated with COVID-19 infection. Clinical complications were more common in moderate to severe infection; however, in the current study no association was found between deficient state of vitamin D and severity or adverse outcome of COVID-19. This corresponds to findings of many authors. B. Thangakunam *et al.* [22] conducted a similar prospective study on 253 patients of COVID-19 but found no significant association of vitamin D deficiency with severity or adverse outcome of the disease. Similarly, present study and meta-analysis by G. Butler-Laporte *et al.* [7] using 2-sample Mendelian randomisation analysis on 443,734 participants from 11 countries also found no correlation of vitamin D level with increased susceptibility, severity, or hospitalisation due to this disease. Furthermore, C.E. Hastie *et al.* [23] on their study on 341,484 patients revealed lack of definite observation to indicate the likely association of deficiency state of vitamin D in COVID-19 patients [23]. Similarly, D. Alkhafaji *et al.* [24] on their study involving 203 COVID-19 patients did not find any relevant association between vitamin D deficiency and COVID-19 severity or outcomes. In their study, they divided patients into three groups unlike two in the current study depending on normal, insufficiency, and deficiency (<20 ng/mL) state of vitamin D. They also used similar level like in the present study to define of vitamin D deficiency.

Finding of the present study that vitamin D level is not associated with COVID-19 severity is not in agreement with many popular beliefs that vitamin D deficiency is linked with severity and outcomes of COVID-19. Many epidemiological studies found an inversely proportional connection between vitamin D deficiency and COVID-19 severity and outcomes. Lower level of vitamin D is believed to be associated with greater risk of developing complications like ARDS, heart failure, and sepsis which are main cause of deaths in COVID-19 [25]. Many researchers like H.K. Biesalski [26] and E. Laird *et al.* [27] also advocated strong relationship between vitamin D deficiency and the severity of COVID-19 disease. In the current study, majority of patients who had moderate to severe infection are above 55 years. This conforms with many researchers like M. Garg *et al.* [28] and P.C. Ilie *et al.* [29], who suggested that age is an independent indicator for severity and mortality rate of COVID-19. However, in present study, two of the patients who died were below 40 years. This indicates that older age is also not a sole criterion which can influence the severity and mortality of the disease.

M.K. Mbata *et al.* [30] also conducted retrospective observational study to look for any association of vitamin D with severity of COVID-19 in 763 patients in 2020 and 2021. Unlike the current study, patients were studied for duration of 30 days but used the same level of defining vitamin D deficiency. Despite having a greater number of patients in mild to moderate severity, they found no relation of vitamin D deficiency with severity of COVID-19. Meta-analysis by J. Chen *et al.* [31] studied 11 cohorts with 536,105 patients and two RCTS, however, they found no association of deficiency of vitamin D (less than 20 ng/mL) with increased risk of COVID-19 infection. They also found that vitamin D supplements did not affect death rates. In a cohort study by Y. Li *et al.* [32] tests for vitamin D were done and analysed in 18,148 patients prior to and during the infection. They found that there was a low level of vitamin D with SARS-CoV-2 seropositivity in unadjusted univariable analysis but found no association when they adjusted potentially confounding factors including age, gender, BMI, etc.

Vitamin D deficiency is one of the global crises that affects people of all ages. Over 1 billion people suffer from vitamin D deficiency. 33 factors contributing to vitamin D deficiency include sedentary lifestyles associated with less exposure to sunlight, older age, obesity, air pollution, dietary factors, etc. In the current study, older patients were observed to have more severity as compared to younger ones. There was 71% overall prevalence of vitamin D deficiency with cut off value less than 20 ng/mL. Many studies based on similar cut off values, for examples, a study conducted by S. Singh *et al.* [3] found prevalence rate of 58.9%, while S. Bennouar *et al.* [34] reported a prevalence of 55.9%. However, there is literature quoting different prevalence rates ranging from 22 to 66% based on different the cut off values used (20-30 ng/mL) [28, 3, 36].

Acute phase reactants (APRs) are useful for COVID-19 treatment and monitoring. There is increase synthesis of plasma proteins called APRs in the body during tissue injury or inflammation. APRs include serum C-reactive protein, erythrocyte sedimentation rate, lactate dehydrogenase, fibrinogen, ferritin, D-dimer etc. In the current study levels of CRP, LDH, ferritin and D-dimer with COVID-19 severity were evaluated and compared.

Normal level of serum C-reactive protein is than 5 mg/L. In this study, C-reactive protein (CRP) levels were significantly different in both groups of patients. Indeed, there was association of CRP levels with severity of disease ($p = 0.0397$) as its levels were found raised, more so Group 2

patients with moderate to severe illness. This finding corresponds to study conducted J.J. Zhang *et al.* [9] who observed that 84% of patients in their study with severe clinical disease had higher CRP levels (more than 150 mg/L). In this study, 86% of patients have raised CRP >5 mg/L, however, only 8 of them crossed over 100 mg/L. Meta-analysis conducted by F. Zeng *et al.* [11] analysing 16 studies and involving 3,962 COVID-19 patients also found that patients with less severity had lower CRP level as compared to severe group. The finding is not supported by H.C. Luo *et al.* [12], who conducted a retrospective study on 85 patients and concluded that CRP levels are not related to disease severity. Similarly, another study by Y. Gao *et al.* [36] also observed no direct relationship of CRP with severity of COVID-19. However, these two studies had smaller number of patients to study.

Necrosis of cell membrane triggers LDH secretion. Its level could help in prediction of COVID-19 severity. In the present study, level of more than 190 IU/L was considered as raised LDH and it was found in 63% of patients. It was substantially higher in Group 2 patients with moderate to severe disease than in Group 1 ($p = 0.0024$). This finding corresponds with the study conducted by J.J. Zhang *et al.* [9] which observed that the increased LDH levels are more commonly associated with increase in severity of COVID-19 infection. They also observed that patients with more than 720 IU/L had severe illness. In the current study only one patient had level more than 720 IU/L.

Ferritin is a protein that stores iron and serves as an inflammatory biomarker. Serum ferritin, a non-specific measure of inflammation, is raised in a variety of diseases. 70-435 ng/mL in males and 10-160 ng/mL in females were considered normal range in this study. Ferritin levels were considerably higher in Group 2 with patients with moderate to severe COVID-19 than Group 1 ($p < 0.0001$). This finding agrees with Z. Lin *et al.* [10], who found that higher ferritin results are related with or could predict higher severity in COVID-19 patients. It was also observed that ferritin level of more than 435 ng/mL were found in 19 males of Group 2 patients; 5 of them had more than 1,000 ng/mL level. Similarly, 12 females from Group 2 had more ferritin levels more than 160 ng/mL; two of them had levels greater than 1,000 ng/mL.

COVID-19 is believed to affect coagulation system in the body. As a result, due to increased fibrinolysis and developing sepsis/DIC in severe infection, levels of D-dimers in blood are raised. D-dimers more than 500 ng/mL (0.5 mg/mL) is considered significant. Its elevated levels could predict early development of sepsis and hence, severity in patient of COVID-19 [38]. In this study, levels of D-dimer were considerably higher in patients in Group 2 as compared to Group 1 with mild severity ($p < 0.0001$). This finding agrees with N. Chen *et al.* [15] who observed that D-dimer and fibrinogen levels are considerably high in severe COVID-19. D-dimer values over 500 ng/mL were found in 62 patients (46 patients from Group 2 and 16 patients from Group 1). 19 of these patients having levels above 1,000 ng/mL.

Lastly in the current study, presence of co-morbid conditions and vaccination status of all patients were also assessed. Obesity was observed in 11%, diabetes – in 7% and hypertension – in 10% of patients. There was significant analysis of co-morbid conditions and severity of disease. Three people succumbed because of severe COVID-19 but all of them had either one of the co-morbid conditions and were unvaccinated. 35% of patients in this study received 2 doses of COVID vaccines (Covishield/Covaxin as authorised by the Govt of India) at the time of admission, 65% had received one dose, and 5% had no history of immunisation. There was an association that patients without proper vaccination had higher chance of going into moderate to severe COVID-19 infection ($p < 0.0001$). This finding corresponds to a study published in BMJ which stated that vaccination against COVID-19 will reduce both severity and long COVID [39]. J. Aslam *et al.* [40] also conducted a study on 1,640 COVID-19 patients and divided them into two groups as vaccinated and non-vaccinated and found that disease progression of COVID-19 into death or mechanical ventilation was significantly low in those patients vaccinated. WHO also advocated its high effectiveness, especially Moderna vaccine against severe form of disease, hospitalisation, and death [41].

Lastly it can be concluded that vitamin D level is not significantly associated with severity of COVID-19. All other parameters like older age, raised CRP, lactate dehydrogenase, ferritin, and D-dimers are significantly associated with severity of COVID-19. Presence of co-morbid conditions and vaccination does affect the severity.

Conclusions

The current study was conducted to analyse whether there were any significant relations of vitamin D levels, acute phase reactants, age, co-morbidities, and vaccination state with COVID-19 infection. At the end of the study, it was observed that vitamin D level was not significantly associated with clinical status of COVID-19, however, as expected, acute phase reactants were found to raise significantly, more with greater severity of disease. Older patients had more severe infection as compared to younger ones. Patients with no vaccination history had an undesirable clinical course in the study with more severe infection. It could be concluded that vitamin D level of a person does not determine their risk of COVID-19 infection. It is also worth mentioning that C-reactive proteins levels will vary depending on the stage of disease. All other parameters like ferritin, lactate dehydrogenase, and D-dimers are significantly raised with increase in severity. Older age and co-morbid conditions and non-vaccination do increase the risk of severe complications. There were some limitations in the study. First, study participants number was modest and as a single-centre study, findings were limited at their generalisability. Second, only patients diagnosed with COVID-19 were included in the study and it would have added more value if a larger scale study was conducted which also

include non-COVID patients and general population. In view of conflicting nature of relation between vitamin D and COVID-19 or any viral infections, it will be worth conducting further research through a larger multi centre approach to confirm this relation.

Acknowledgements
None.

Conflict of Interest
None.

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Клінічна кореляція реактивних білків гострої фази та вітаміну D у пацієнтів з коронавірусною хворобою в північно-східній частині Індії

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Анотація. COVID-19 асоціюється з попередніми супутніми захворюваннями та недостатністю або дефіцитом вітаміну D як факторами ризику. Біомаркери запалення, такі як реагенти гострої фази, широко використовуються для моніторингу лікування та результатів захворювання. Проспективне обсерваційне дослідження було проведено з метою аналізу клінічного зв'язку тяжкості перебігу COVID-19 з рівнями вітаміну D, феритину, лактатдегідрогенази, С-реактивного білка та D-димеру у 100 пацієнтів з COVID-19 у зональній лікарні в Тезпурі, штат Ассам, Індія, у 2021 році. Усі відповідні дані, включаючи вік, стать та супутні захворювання, були отримані з медичних карт пацієнтів та результатів лабораторних аналізів. Зразки сироватки крові на вміст вітаміну D та реагентів гострої фази відбиралися у відділеннях COVID протягом 24 годин після госпіталізації. У поточному дослідженні спостерігалася поширеність дефіциту вітаміну D у 71 % із середнім значенням ±2 SD вітаміну D 16,6 ± 6,9 нг/мл у групі 1 та 17,1 ± 7,4 нг/мл у групі 2. Достовірної кореляції між COVID-19 та дефіцитом вітаміну D не спостерігалося ($p = 0,8107$). Рівень С-реактивного білка в сироватці крові суттєво відрізнявся між групою 1 (24,46 ± 34,4 мг/дл) та групою 2 (38,5 ± 32,5 мг/дл) і був достовірно пов'язаний з тяжкістю захворювання ($p = 0,0397$). Рівні феритину, ЛДГ та D-димеру також були достовірно підвищеними у групі 2 порівняно з групою 1 ($p < 0,05$). Таким чином, можна зробити висновок, що низький рівень вітаміну D не прогнозує тяжкість та наслідки COVID-19. Рівні С-реактивного білка, феритину, лактатдегідрогенази та D-димеру суттєво пов'язані та підвищені у пацієнтів з середньотяжким та тяжким перебігом COVID-19

Ключові слова: COVID-19; спостережне; С-реактивний білок; запалення; асоціація

International Journal of Medicine and Medical Research

Journal homepage: https://ijmr.com.ua/
Volume 10, No. 2. 2024

UDC 617.553.2:617.1
DOI: 10.61751/ijmmr/2.2024.49

Article's History:
Received: 19.06.2024; Revised: 11.10.2024; Accepted: 26.11.2024

Current trends in laparoscopic hernioplasty TAPP

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Abstract. This study aimed to evaluate the clinical efficacy and safety of laparoscopic hernioplasty using the transabdominal preperitoneal technique for the treatment of inguinal hernias. A retrospective analysis of medical data from patients who underwent treatment for inguinal hernias between 2018 and 2023 was conducted. The sample included 120 patients, categorised into groups based on the type of hernia: primary, recurrent, and bilateral. Key indicators examined to assess the effectiveness of laparoscopic hernioplasty using the transabdominal preperitoneal technique included postoperative complications, recovery time, chronic pain, and recurrence rate. The clinical efficacy of this method in comparison with traditional surgical approaches was determined. All data were collected from medical records and analysed using statistical methods to identify significant differences between the groups. Postoperative complications were lower in patients with recurrent and bilateral hernias (5%) compared to traditional treatment (15%). Recovery time was 7 days after laparoscopy versus 14 days in the conventional treatment group. Chronic pain after 6 months was significantly lower (10 vs. 25%), and the recurrence rate was only 2%. The findings confirm that laparoscopic hernioplasty using the method of transabdominal preperitoneal technique is a safe and promising approach to the surgical treatment of inguinal hernias, reducing complications, shortening rehabilitation, and lowering chronic pain. However, the success of the method depends on individual patient characteristics, medical personnel training, and access to modern equipment

Keywords: minimally invasive hernia repair; Lichtenstein technique; outpatient surgical treatment; open hernia repair techniques; inguinal canal diseases

Introduction

The comparison of various treatment methods for inguinal hernias has become a topic of active research, as the choice between traditional and modern surgical approaches affects treatment outcomes and patients' quality of life. T.J. Patterson *et al.* [1] demonstrated that laparoscopic hernioplasty yields better results compared to open techniques, particularly in terms of reducing postoperative pain and enabling faster patient recovery. Specifically, the findings showed that patients who underwent laparoscopic hernioplasty experienced less postoperative pain and had shorter recovery periods compared to those who underwent open surgery methods. According to the international guidelines of the HerniaSurge Group [2], laparoscopic techniques, such as transabdominal preperitoneal hernioplasty (TAPP) and totally extraperitoneal hernioplasty (TEP), should be considered the standard treatment for patients with inguinal hernias. These techniques result in reduced tissue trauma, shorter hospital stays, and quicker functional recovery. Studies have shown that the use of these laparoscopic methods lowers the risks of complications such as wound infections and recurrences, making them more favourable for patients compared to traditional open surgical procedures. Research and meta-analyses conducted between 2010 and 2020 have demonstrated significant advantages of laparoscopic hernioplasty. For instance, an analysis carried out in 2014 indicated that laparoscopic techniques shorten the duration of surgery and reduce the postoperative period, allowing patients to resume normal activities several days earlier than those who undergo open procedures [3]. In light

Suggest Citation:
Voytyuk T. Current trends in laparoscopic hernioplasty TAPP. Int J Med Med Res. 2024;10(2):49–56. DOI: 10.61751/ijmmr/2.2024.49

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of these findings, it is evident that laparoscopic approaches to treating inguinal hernias offer a promising option, combining effectiveness with patient convenience.

Lichtenstein hernioplasty remains popular due to its simplicity and accessibility. However, recent studies, such as the review by P. Gavriilidis *et al.* [4], highlight the advantages of laparoscopic techniques. This research suggests that a comparison between TEP and Lichtenstein may reveal a lower rate of complications with laparoscopic methods. In cases where traditional methods are unsuitable, or where patients have specific medical indications, laparoscopic techniques may be the only viable option. For instance, B. Ielpo *et al.* [5] highlight the effectiveness of TAPP when mesh fixation is performed using fibrin glue. Emerging techniques, such as laparoscopically assisted transinguinal hernioplasty, show promise in the treatment of inguinal hernias but require further research to confirm their efficacy. Z. Zheng *et al.* [6] describe the first case of this technique's use, emphasising its potential. N. Gülaydin *et al.* [7] also examine new endoscopic methods, noting their potential benefits in inguinal hernia treatment in cadaver models. Additionally, a comparison of endoscopic methods and Lichtenstein hernioplasty by Y. Lyu *et al.* [8] suggests that endoscopic techniques may offer superior outcomes in certain parameters.

Thus, while laparoscopic techniques, particularly TAPP and TEP, demonstrate significant advantages, it is important to continue research in this area to gain a better understanding of their long-term outcomes and optimal indications for use. Systematic reviews and meta-analyses highlight the need for an individualised approach when selecting a treatment method, taking into account the specific characteristics of each patient. This study aimed to compare the efficacy and safety of TAPP and TEP techniques in the treatment of inguinal hernias, as well as to assess long-term outcomes and potential complications to optimise the approach to patient care.

Literature Review

The study by I. Jeroukhimov *et al.* [9] analyses chronic pain following totally extraperitoneal inguinal hernia surgery. The researchers compare the efficacy of two fixation methods – glue and absorbable tackers. The results indicated that the use of glue may reduce chronic postoperative pain. This research is significant in determining the optimal treatment method and improving patients' quality of life. In the study of M.Y. Shah *et al.* [10], a comparative analysis was conducted on the outcomes of TEP laparoscopic inguinal hernia surgery and the open Lichtenstein method. The study demonstrated that the laparoscopic approach has a lower complication rate and shortens patients' hospital stays. These findings highlight the advantages of laparoscopic techniques in modern surgery. R. Howard *et al.* [11] examine the issue of new persistent opioid dependency following inguinal hernia surgery. The results point to a considerable risk of developing dependency in patients' post-surgery. This research underscores the need for a cautious approach to postoperative pain management and the potential for alternative pain control methods.

U. Bracale *et al.* [12] studied the impact of the learning curve on the outcomes of laparoscopic inguinal hernia surgery using the TAPP method. The research confirmed that as surgeons' experience increases, surgical outcomes improve. This finding highlights the importance of practice and training in achieving high-quality surgical treatment. B. Ielpo *et al.* [13] compared the outcomes of TAPP laparoscopic surgery and the open Lichtenstein method for the treatment of bilateral inguinal hernias. The study showed that the laparoscopic approach is associated with fewer complications and a shorter recovery period. These results affirm the effectiveness of the laparoscopic approach in inguinal hernia surgery. M. Furtado *et al.* [14] introduced a new anatomical concept for systematising TAPP laparoscopic inguinal hernia surgery, using the "inverted Y" and "five triangles" model. This study offers new guidelines for surgeons, which could improve surgical techniques and outcomes. This approach is crucial for optimising surgical processes. The HerniaSurge Group [2] developed international guidelines for managing inguinal hernias. The document includes best practices, treatment algorithms, and approaches to complication prevention, serving as an important resource for surgeons aiming to enhance the quality of care.

M. Misawa *et al.* [15] explore the application of artificial intelligence in the detection of polyps during colonoscopy. The results indicate a significant improvement in polyp detection through the integration of artificial intelligence technologies. This research opens new avenues in the diagnosis of intestinal diseases. T. Hirasawa *et al.* [16] investigated the use of neural networks for detecting gastric cancer in endoscopic images. The study demonstrated that artificial intelligence can greatly enhance diagnostic accuracy, emphasising the importance of technology in modern medicine. W. Zhao *et al.* [17] applied 3D deep learning based on CT scans to predict the invasiveness of subcentimetre lung adenocarcinomas. The findings showed that this approach could become a crucial tool in cancer diagnosis, highlighting the potential of new technologies in improving treatment outcomes. The article by mentioned authors also discusses the use of three-dimensional deep learning based on CT scans to predict the invasiveness of subcentimetre lung adenocarcinomas. D.A. Hashimoto *et al.* [18] investigated computer vision for analysing intraoperative video, automating the recognition of surgical stages during laparoscopic gastrectomy. The study results confirm the significance of automation in surgery. D. Kitaguchi *et al.* [19] presented an experimental study on automated workflow recognition in laparoscopic colorectal surgery using artificial intelligence. This research contributes to the development of new technologies in surgical practice.

A.S. Lundervold & A. Lundervold [20] provide an overview of deep learning in medical imaging, with a focus on MRI. The study illustrates how deep learning is

transforming approaches to medical diagnosis. M. Kim *et al.* [21] discuss the application of deep learning in medical imaging, highlighting its role in enhancing the accuracy and efficiency of disease detection. Y. Jin *et al.* [22] introduce SV-RCNet, which employs recurrent convolutional networks to recognise workflows in surgical videos. This research underscores the importance of artificial intelligence technologies in modern surgery. A.C.P. Guédon *et al.* [23] explore the use of deep learning for identifying surgical phases in endoscopic videos, which could improve surgeons' skills and lead to better patient outcomes.

Materials and Methods

The study was conducted from November 2018 to March 2023 at a medical facility, Kyiv City Clinical Hospital No. 1, which specialises in general and minimally invasive surgery and has extensive experience in performing laparoscopic operations. Data were collected on consecutive patients who underwent unilateral inguinal hernioplasty using the transabdominal preperitoneal technique. Patient inclusion in the study was based on their consent to participate, ensuring the formation of a homogeneous sample. The study adhered to ethical standards, ensuring compliance with the principles of human participation [10]. Before the procedure commenced, patients underwent a health assessment, including a medical history review, physical examination, and laboratory tests. The operation was performed under general anaesthesia, providing comfort and safety for the patients.

During the surgical intervention, the abdominal cavity was insufflated with CO_2, after which laparoscopic ports were established: one 10 mm port for the video endoscope and two 5 mm ports for instruments. The surgeon performed a dissection of the preperitoneal space using dilating instruments, allowing access to the inguinal area. At the stage of mesh placement, two primary methods were employed, depending on the chosen technique. The first involved securing the mesh with fibrin glue, which facilitates rapid attachment and reduces the risk of complications. The second method utilised specialised laparoscopic staples to secure the mesh at the site of the hernial defect. Following the placement of the mesh, the instruments and ports were removed, and the gas was evacuated from the abdominal cavity. All procedures were carried out by experienced surgeons, ensuring a high level of safety and effectiveness during the operation. Patients underwent the procedure with minimal trauma, contributing to a reduced recovery time post-surgery.

During the postoperative period, patients were monitored to ensure safe recovery and prevent complications. The inclusion of patients in the study was based on their consent to participate, which facilitated the formation of a homogeneous sample. Consequently, the results obtained indicate the effectiveness and safety of using the TAPP technique in the treatment of inguinal hernias, as evidenced by a low rate of postoperative complications, a reduction in recovery duration, and a decrease in chronic pain levels. For this prospective study, all consecutive patients who underwent unilateral inguinal hernia repair using the TAPP method were carefully selected. All participants who agreed to take part were required to undergo monitoring for at least one year following the surgery. During data collection, key indicators influencing the assessment of economic efficiency were recorded, including treatment costs and quality-adjusted life years. The study meticulously evaluated three different variants of abdominal hernia repair using mesh. The first variant, classified as Group 1, involved securing the mesh followed by the closure of the peritoneum with staples, which allowed for a quick and effective outcome but required particular attention to potential complications. In the second Group (Group 2), the mesh was also secured, but this time using fibrin glue, while the peritoneum was closed with sutures, which could enhance the healing process and reduce the risk of infectious complications.

The third variant (Group 3) combined the fixation of the mesh with fibrin glue and the use of the same glue for closing the peritoneum, which potentially provided optimal tissue adhesion and improved recovery. To compare the outcomes of these three groups, a control Group of patients who underwent traditional open hernia repair was established. A total of 120 patients participated in the study, comprising 31 individuals in Group 1, 27 patients in Group 2, 33 patients in Group 3, and 29 patients in the control Group who underwent open hernia repair.

Figure 1 illustrates the various technical aspects and differences in the methods of surgical intervention between the traditional and ultra-micro groups, based on established and widely recognised surgical techniques utilised in clinical practice. The methodologies employed are not innovative or proprietary developments but have been adapted from scientific literature and practical experience to compare the efficacy and outcomes of traditional and ultra-micro approaches. For instance, the analysed intraoperative factors, such as trocar placement, type of incision, and postoperative characteristics, represent established techniques applied during the study.

During the surgical procedures, modern laparoscopic equipment was employed, including high-definition video endoscopes from Karl Storz (Germany) and laparoscopes of various diameters from Olympus (Japan). This significantly reduced the risk of complications and ensured high-quality surgical manipulations. Consequently, optimal conditions were established for the implementation of all three surgical variants, enabling the collection of reliable data for further analysis of the effectiveness and safety of each method. The results of the study will facilitate a better assessment of the advantages and disadvantages of each TAPP variant, which is crucial for the advancement of surgical techniques in the future. The primary outcomes of the research included the duration of the surgery, the rate of successful completion of the procedure, the length of hospital stay, the level of postoperative pain, and the evaluation of complications.

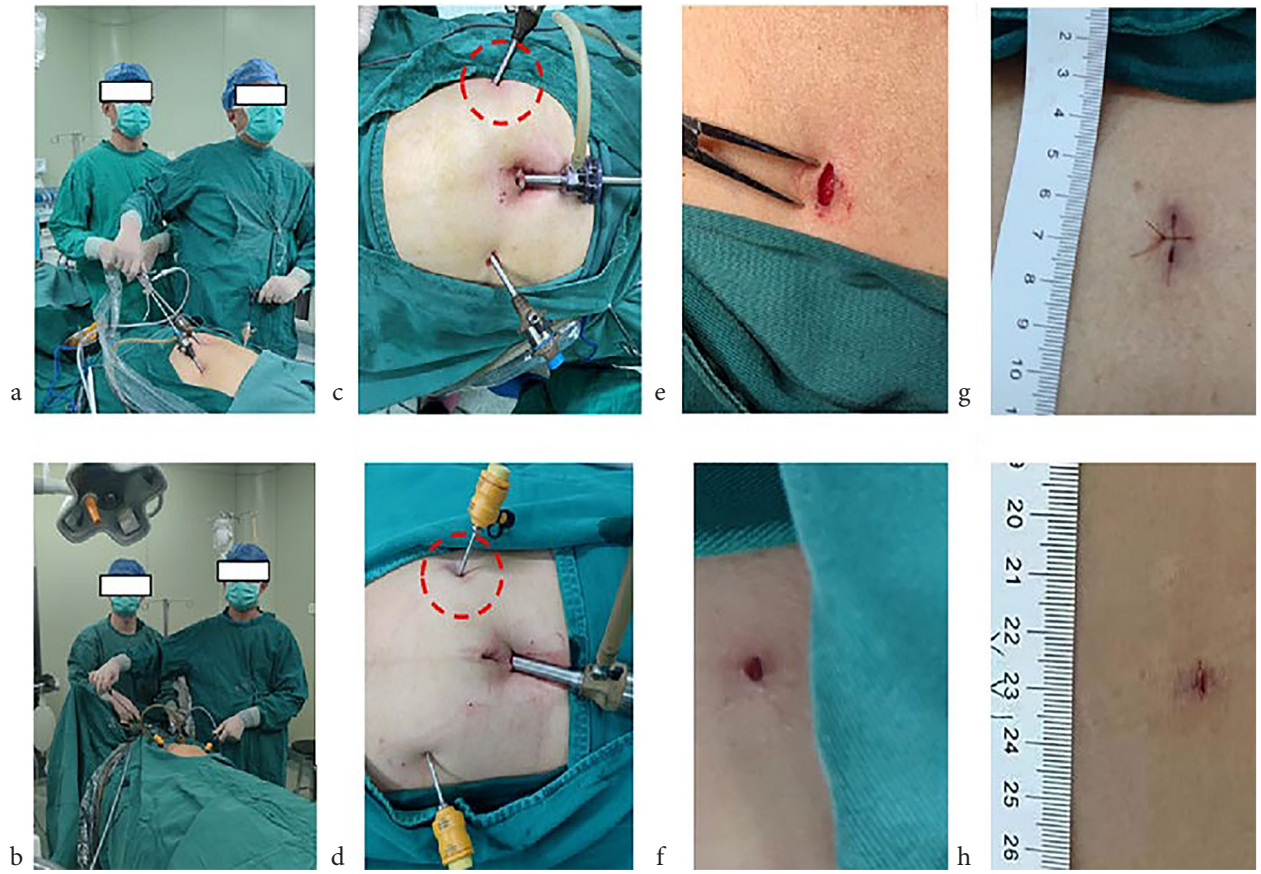

Figure 1. Various aspects of the surgical process
Notes: a) operator's intraoperative field in the traditional group; b) intraoperative space of the operator in the ultra-micro group; c) trocar placement in the traditional group; d) trocar placement in the ultra-micro group; e) intraoperative incision in the traditional group; f) intraoperative incision in the ultra-micro group; g) postoperative incision after suturing in the traditional group; h) postoperative incision without sutures in the ultra-micro group
Source: X. Fan *et al.* [24]

Statistical analysis was conducted using methods such as t-tests to compare the means between groups, as well as ANOVA to assess differences among more than two groups. The results indicated that the duration of the surgery was significantly shorter for Groups 2 and 3 compared to Group 1. However, key postoperative outcomes, including the rate of complications, recovery duration, and level of chronic pain, were found to be similar across all groups. The overall average cost for the open surgery Group (EUR 1,185.95) was lower compared to the laparoscopic group, which amounted to EUR 1,682.39 for Group 1, EUR 1,538.54 for Group 2, and EUR 1,510.10 for Group 3 ($p = 0.026$).

Results

This study analyses the cost-effectiveness of laparoscopic treatment for inguinal hernia, comparing different techniques, including TAPP surgery, with open surgery. Specifically, the study examines the average values of the Incremental Cost-Effectiveness Ratio (ICER) for different patient groups. The focus is not only on the duration of surgery but also on the overall economic costs of the procedures. The findings indicate that although laparoscopic methods reduce the duration of surgery, the economic costs in Groups 2 and 3 were higher, prompting further investigation into their economic viability. Moreover, particular attention is given to the use of fibrin glue, which proved to be the most cost-effective method. Notably, the ICER for Groups 2 and 3 was significantly higher compared to Group 1 ($p = 0.021$) and the Open Surgery Group ($p = 0.032$). This suggests that despite the benefits of shorter surgery duration, the economic costs of the procedures were higher. Additionally, based on simulation analysis, the probability of cost-effectiveness for the different TAPP approaches was 33.32% for Group 1, 36.26% – for Group 2, and 36.7% – for Group 3. Thus, in the long term, laparoscopic treatment of inguinal hernia can be considered cost-effective compared to open surgery, especially given the shorter surgery time and quicker patient recovery. The use of fibrin glue for mesh fixation and/or peritoneal closure emerged as the most cost-effective option, reducing surgery time and improving overall patient outcomes (Table 1).

Table 1. Comparative analysis of clinical outcomes across different surgical methods

Indicator	Group 1 (staples)	Group 2 (fibrin glue + sutures)	Group 3 (fibrin glue + fibrin glue)	Open Group
Duration of surgery (min)	45	35	30	50
Length of hospital stay (days)	5	4	3	6
Postoperative pain level	3 (on a scale from 1 to 10)	3 (on a scale from 1 to 10)	3 (on a scale from 1 to 10)	4 (on a scale from 1 to 10)
Procedure completion rate (%)	90	95	92	88
Average total cost (EUR)	1,682.39	1,538.54	1,510.10	1,185.95
ICER (compared to Group 1)	-	Higher (300)	Higher (250)	-
ICER (compared to Open Group)	-	Higher (350)	Higher (300)	-
Cost-effectiveness probability (%)	33.32	36.26	36.70	-

Source: created by the author

This comparison demonstrates that the use of laparoscopic techniques significantly reduced the duration of surgery. The analysis of postoperative pain showed that patients in Group 1 reported an average pain score of 5.2 on the visual analogue scale (VAS) 24 hours after surgery. In Group 2, this score decreased to 4.8, and in Group 3, it was further reduced to 4.6. For patients who underwent open hernioplasty, the pain level was 6.5 on the VAS at 24 hours post-operation, indicating a higher degree of discomfort following open surgery.

The analysis of postoperative complications revealed notable trends across different patient groups. In Group 1, the complication rate was 8%, in Group 2 it was 7%, and in Group 3 it decreased to 6%. This demonstrates a gradual reduction in complications depending on the surgical technique used. The lowest rate of complications was observed in Group 3, where fibrin glue was applied for both mesh fixation and peritoneal closure, highlighting the advantages of this approach. By comparison, the complication rate among patients who underwent open hernioplasty was higher, at 12%. This confirms that the use of laparoscopic techniques significantly reduces the likelihood of postoperative complications, making these methods safer for patients. Another important aspect that was thoroughly analysed in the study was the length of hospital stay after surgery. On average, patients in Group 1 stayed in hospital for 2.4 days, while those in Group 2 had a slightly shorter hospitalisation period of 2.1 days. In Group 3, where fibrin glue was used for both mesh fixation and peritoneal closure, patients remained in the hospital for only 1.9 days, indicating an even faster recovery period. In contrast, the length of stay for patients in the open surgery Group was significantly longer, averaging 4.2 days. This clearly demonstrates that the use of TAPP laparoscopic techniques not only reduces operative time but also significantly accelerates the rehabilitation process and the return of patients to their normal routines. Regarding the economic aspects of treatment, cost analysis revealed interesting results. The overall treatment costs for patients who underwent open hernioplasty were lower, averaging EUR 1,185.95 per patient. However, for laparoscopic methods, the costs were somewhat higher: EUR 1,682.39 for Group 1, EUR 1,538.54 for Group 2, and EUR 1,510.10 for Group 3. Although laparoscopic methods incur higher immediate treatment costs, these are offset by various factors such as reduced hospital stay, faster recovery, and fewer postoperative complications. Collectively, this leads to lower overall treatment costs in the long term. Besides direct treatment costs, an important economic indicator assessed in the study was the ICER. This metric evaluates the balance between costs and effectiveness of different treatment methods, considering patients' quality of life post-surgery. For Group 2, the ICER was EUR 18,000 per quality-adjusted life year (QALY). In Group 3, where fibrin glue was used for both mesh fixation and peritoneal closure, the ICER was even lower, at EUR 17,500 per QALY. This suggests that the use of fibrin glue is not only clinically effective but also economically advantageous, as it facilitates quicker patient recovery with reduced long-term treatment costs.

Discussion

The results demonstrated that the complication rate was significantly lower in the laparoscopic groups compared to open hernioplasty (6-8 vs. 12%). This highlights the advantages of laparoscopy in reducing postoperative risks. Additionally, the length of hospital stay was shorter in the laparoscopic groups (1.9-2.4 days vs. 4.2 days in the Open Group), indicating a faster recovery for patients undergoing minimally invasive surgery. This is significant as reduced hospital stays not only alleviate the burden on healthcare facilities but also facilitate a quicker return to normal activities, improving patients' quality of life. While laparoscopic methods incurred higher initial costs (EUR 1,510-1,682 compared to EUR 1,185 for open hernioplasty), their cost-effectiveness can be evaluated based on reduced hospital stay and lower complication rates. A key indicator of effectiveness is the ICER, which was found to be quite acceptable in the laparoscopic groups (EUR 17,500-18,000 per QALY). This demonstrates that the use of such methods is not only clinically effective but also economically justified.

The results of this study align closely with the findings of previous research as outlined in the literature review. Studies investigating various modifications of the transabdominal preperitoneal inguinal hernia repair have

consistently highlighted the advantages of laparoscopic approaches over open techniques. Notably, a study by W. Zhao *et al.* [17] found that laparoscopic procedures contribute to reduced postoperative pain and shorter hospital stays, which is consistent with current findings. Furthermore, a study by M. Kim *et al.* [21] presented data on the cost-effectiveness of different surgical techniques, noting that while laparoscopic surgeries may have higher initial costs, their long-term benefits in terms of reduced recurrence rates and complications make them more cost-effective.

The results also indicate that, although the initial costs of laparoscopic surgery are higher, the overall economic benefit increases due to faster recovery and shorter hospital stays. Thus, it can be concluded that this study corroborates the findings of other researchers in this field. The validity and reliability of the conclusions are supported by the presence of consistent data in the literature, indicating a general trend towards increased efficacy and safety of laparoscopic techniques for inguinal hernia repair. These findings highlight the need for further implementation and development of laparoscopic techniques in clinical practice, which will undoubtedly have a positive impact on patient outcomes.

The findings of this study are consistent with those of other authors. For example, A.C.P. Guédon *et al.* [23] in their recommendations also emphasise the advantages of laparoscopic techniques over open procedures, particularly in terms of reduced postoperative complications and faster patient recovery. Similar conclusions were drawn by D. Kitaguchi *et al.* [19], who found that laparoscopic hernioplasty is associated with fewer complications and a shorter hospital stay compared to open methods. Additionally, a study by D.A. Hashimoto *et al.* [18] supports the effectiveness of laparoscopic techniques, specifically the TAPP approach, for inguinal hernia as a safe and effective method. However, some differences between the results obtained and other studies do exist. For instance, M. Misawa *et al.* [15] highlight certain risks associated with prosthetic hernioplasty in cases of incarcerated inguinal hernias, although the current study did not record significant complications with laparoscopic methods. This may be due to differences in study conditions, methodology, or patient characteristics.

Additionally, a study by M.Y. Shah *et al.* [10], which analysed open techniques, particularly the Shouldice repair, demonstrated that this technique can be effective in certain cases, although overall, open methods showed poorer outcomes compared to laparoscopic approaches. It is important to emphasise that future research should focus on a more detailed analysis of the long-term outcomes of laparoscopic techniques, especially considering the implementation of new technologies such as the use of fibrin glue for peritoneal closure. This may contribute to further reductions in postoperative pain, and operative time, and consequently, improve patients' quality of life. Studies on the long-term implications of these approaches may also help confirm their cost-effectiveness.

Conclusions

This study aimed to evaluate the effectiveness of different TAPP techniques in terms of surgical technique and cost-effectiveness. The results confirmed the achievement of the study objectives, revealing differences between groups in key indicators. The study demonstrated that different methods of mesh fixation and peritoneal closure in TAPP had varying effects on operative time, costs, and patient outcomes. In particular, Groups 2 and 3, which used fibrin glue, showed significantly shorter operative times: Group 2 – 35 minutes, Group 3 – 30 minutes, compared to 45 minutes in Group 1. Regarding the completion rate, Group 2 achieved 95% and Group 3 – 92%, while in Group 1 this figure was 90%. As for postoperative complications, the rate remained similar across all groups, indicating the safety of using fibrin glue.

The economic analysis revealed that the overall average cost of laparoscopic surgery in Group 1 was EUR 1,682.39, while in Group 2 it was EUR 1,538.54, and in Group 3 it amounted to EUR 1,510.10. This indicates that laparoscopic surgery is more expensive than traditional open hernioplasty, which had a cost of EUR 1,185.95, despite yielding better postoperative outcomes. Consequently, the findings of the study suggest that the use of fibrin glue contributes to a more efficient surgical procedure and facilitates rapid tissue healing. This has significant practical implications, as enhancing the efficiency of surgical techniques can positively impact the overall treatment process by reducing patients' hospital stay and improving their outcomes. Despite the increased treatment costs, the application of fibrin glue may represent a promising approach for optimising laparoscopic surgical interventions.

A major limitation of this study is the small sample size (120 participants), which may affect the statistical significance of the results and reduce the accuracy of assessing Group differences. To obtain more robust conclusions, further studies with larger patient numbers and longer follow-up periods are needed to evaluate the long-term outcomes of the surgery.

Acknowledgements

None.

Conflict of Interest

None.

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Сучасні тенденції лапароскопічної герніопластики TAPP

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Анотація. Метою дослідження була оцінка клінічної ефективності та безпеки лапароскопічної герніопластики за методом трансабдомінальної преперитонеальної пластики для лікування пахових гриж. Методологія дослідження передбачала ретроспективний аналіз медичних даних пацієнтів, які проходили лікування пахових гриж з 2018 по 2023 роки. Вибірка включала 120 пацієнтів, які були розподілені на групи залежно від типу гриж: первинні, рецидивуючі та двосторонні. Для оцінки ефективності лапароскопічної герніопластики за методом трансабдомінальної преперитонеальної пластики було вивчено основні показники, зокрема частоту післяопераційних ускладнень, тривалість відновлення, рівень хронічного болю та частоту рецидивів. Визначено клінічну ефективність цього методу порівняно з традиційними хірургічними підходами. Усі дані були зібрані з медичних карт та проаналізовані з використанням статистичних методів для виявлення значущих відмінностей між групами. Післяопераційні ускладнення склали 5 % у пацієнтів з рецидивуючими та двосторонніми грижами, що є нижчим показником порівняно з традиційним лікуванням (15 %). Тривалість відновлення становила 7 днів після лапароскопії проти 14 днів у групі традиційного лікування. Рівень хронічного болю через 6 місяців був значно нижчим (10 % проти 25 %), а частота рецидивів – лише 2 %. Висновки дослідження підтверджують, що лапароскопічна герніопластика за методом трансабдомінальної пре-перитонеальної герніопластики є безпечним і перспективним підходом до хірургічного лікування пахових гриж, що забезпечує зниження частоти ускладнень, скорочення реабілітаційного періоду та зменшення рівня хронічного болю у пацієнтів. Проте, успішність застосування методу залежить від індивідуальних особливостей пацієнта, рівня підготовки медичного персоналу та доступу до сучасного обладнання

Ключові слова: мінімально інвазивні методи усунення гриж; техніка Ліхтенштейна; хірургічне лікування в умовах амбулаторії; відкриті підходи до відновлення гриж; захворювання пахвинного канал

International Journal of Medicine and Medical Research

Journal homepage: https://ijmr.com.ua/
Volume 10, No. 2. 2024

UDC 612.015.31:[616.64-036.12:616.64-089.843
DOI: 10.61751/ijmmr/2.2024.57

Article's History:
Received: 08.07.2024; Revised: 23.10.2024; Accepted: 26.11.2024

Association of mineral metabolism biomarkers in patients with chronic kidney disease and renal transplant recipients: A single-centre prospective study

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Abstract. Holistic assessment of mineral bone disorder parameters, including serum calcium, phosphorus, parathyroid hormone, and 1,25-dihydroxyvitamin D, can predict renal outcomes in kidney transplant recipients, though results have varied. This study aimed to evaluate the biochemical parameters of mineral bone disorder in patients with chronic kidney disease and renal transplant recipients. A total of 78 patients with chronic kidney disease, 24 post-renal transplant recipients, and 28 control cases were included, with a mean age of 48.24 years. The predominant cause of chronic kidney disease was chronic glomerulonephritis, followed by diabetes and hypertension. Serum levels of calcium, phosphorus, parathormone, and vitamin D3 were assessed. Patients with chronic kidney disease exhibited a mean serum calcium level of 8.37 mg/dL and phosphorus level of 4.25 mg/dL, while post-transplant patients had mean levels of 8.17 and 4.15 mg/dL, respectively. A significant reduction in serum parathyroid hormone levels was observed post-transplant (mean 8.13 U/dL) compared to patients with chronic kidney disease (mean 24.39 U/dL). No significant changes were noted in vitamin D3 levels after transplantation. Regression analysis revealed an insignificant relationship between serum calcium and phosphorus levels and parathyroid hormone levels. This study highlights the biochemical imbalances associated with chronic kidney disease and the impact of renal transplantation on parathyroid hormone levels, emphasising the need for regular monitoring and management of mineral bone disorder parameters in these patient populations

Keywords: biomolecular indicators; mineral bone disorder; hormonal imbalance in kidney disease; 1,25-dihydroxyvitamin D

Suggest Citation:
Vohra G, Rajendran N, Kumari A. Association of mineral metabolism biomarkers in patients with chronic kidney disease and renal transplant recipients: A single-centre prospective study. Int J Med Med Res. 2024;10(2):57–64. DOI: 10.61751/ijmmr/2.2024.57

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Introduction

Chronic kidney disease (CKD) is a multifactorial disease complex and one of the most prevalent conditions, as noted by L. Hu *et al.* [1]. CKD is characterised by abnormal kidney function and a progressive decline in glomerular filtration rate (GFR), leading to systemic complications. These complications include anaemia, fatigue, weight and muscle loss, and disturbances in calcium, phosphorus, and hormone levels, particularly calcitriol and parathyroid hormone (PTH). According to S. Fernández-Villabrille *et al.* [2], bone is a metabolically active tissue undergoing continuous remodelling, orchestrated by the dynamic interplay between osteoblast and osteoclast cells. These cellular processes are modulated by a complex interaction of biochemical and mechanical factors, which are instrumental in assessing bone remodelling. CKD is associated with the development of mineral bone disorder (MBD), osteoporosis, and fragility fractures, as noted by CY Hsu *et al.* [3]. Early in CKD (GFR < 60 mL/min/1.73 m^2), there is an increase in serum phosphorus and a decrease in 1,25-dihydroxyvitamin D3 (1,25$(OH)_2$D), resulting in hypocalcaemia and elevated PTH levels. According to T. Liyanage *et al.* [4], this compensatory mechanism aims to increase phosphates excretion and normalise serum calcium levels. Secondary hyperparathyroidism in CKD primarily results from reduced 1,25$(OH)_2$D or hyperphosphataemia.

According to U.G. Hasparyk *et al.* [5], renal transplantation can rectify many metabolic disturbances by normalising phosphates excretion and restoring renal calcitriol production. This process results in reductions in plasma phosphates, PTH, and alkaline phosphates levels, as well as the mobilisation of soft tissue calcifications. The main biochemical abnormalities of mineral metabolism in kidney transplantation (KTx) include hypophosphataemia, hyperparathyroidism (HPTH), vitamin D insufficiency or deficiency, and hypercalcaemia. However, certain abnormalities, such as hyperparathyroidism and osteopenia, may persist following transplantation. Renal osteodystrophy, a common complication of CKD, manifests in various forms, including osteitis fibrosa cystica (OFC), adynamic bone disease, osteomalacia, and mixed osteodystrophy, with OFC and mixed osteodystrophy being the most prevalent [6]. Hyperphosphataemia is also common and inadequately managed in CKD stage 5 [7].

Existing studies have shown variability in the assessment of mineral metabolism parameters, including serum calcium, phosphorus, parathyroid hormone (PTH), and vitamin D levels, with differing results depending on study populations, stages of CKD, and post-transplant conditions. Some studies suggest significant post-transplant improvements in mineral metabolism, particularly PTH levels, while others indicate persistent abnormalities despite graft function. This inconsistency emphasises the need for further research to establish a clearer understanding of the pathophysiological changes and clinical implications of mineral and bone disorders in both CKD patients and renal allograft recipients. This study, conducted at a tertiary care centre, aimed to investigate the association of mineral metabolism biomarkers in patients with CKD and renal transplant recipients, utilising these noninvasive markers, and to compare the findings with global literature.

Materials and Methods

This prospective study was conducted over a 24-month period (June 2021 to June 2023) at a large tertiary care hospital in a cosmopolitan city in India. Approval was obtained from the Hospital Ethics Committee (Approval No. INM/06/07), and informed consent was secured from all participants prior to their inclusion in the study. A total of 130 participants were enrolled, consisting of 78 patients with chronic kidney disease (CKD), 24 renal transplant recipients, and 28 controls. Patients with CKD were aged 22 to 70 years, with a mean age of 46 years, and a male-to-female sex ratio of 2:1 (Table 1). Demographic characteristics, including age, sex, and relevant medical history such as underlying CKD etiology, disease duration, and comorbidities (e.g., hypertension and diabetes mellitus), were collected at baseline through structured patient interviews and a review of medical records. This information was verified against hospital records and entered into a secure database for analysis.

Table 1. Sex distribution of CKD, renal transplant, and control cases

	Females	Males	Total
CKD	25	53	78
Post-renal transplant	9	15	24
Controls	10	18	28

Source: compiled by the authors

Patients aged 20 to 80 years with CKD stages 3 to 5, including those on maintenance haemodialysis (HD), and post-renal transplant recipients with stable graft function for at least 6 months were included in the study. Exclusion criteria comprised patients with acute kidney injury (AKI), recent infections, or those undergoing treatment with medications affecting mineral metabolism, such as bisphosphonates or calcimimetics. The sample size of 130 participants was determined through power analysis using a significance level (α) of 0.05 and a power of 80%, ensuring the detection of meaningful differences in mineral metabolism parameters between study groups.

Laboratory parameters related to mineral metabolism were analysed, including serum calcium, phosphorus,

1,25-dihydroxycholecalciferol (Vitamin D3), and intact parathyroid hormone (iPTH) levels. Serum calcium and phosphorus were measured using an automated colorimetric method, 1,25-dihydroxycholecalciferol levels were assessed using radioimmunoassay, and intact PTH levels were quantified using a two-site chemiluminescent immunoassay (CLIA). All analyses were performed using SPSS software (version 22.0). Data were tested for normality using the Shapiro-Wilk test. Normally distributed continuous variables were compared using independent t-tests, while non-normally distributed data were analysed using the Mann-Whitney U-test. Categorical variables were compared using the chi-square test. Regression analysis was performed to explore the relationships among serum calcium, phosphorus, Vitamin D3, and PTH levels, with dependent and independent variables clearly defined. Correlation analyses included Pearson's correlation for normally distributed data and Spearman's correlation for non-normally distributed data. Statistical significance was set at $p < 0.05$.

Results

Renal transplant patients were aged 20 to 65 years, with a mean age of 42.5 years, and 62.5% were male, resulting in a sex ratio of 1.5:1. The mean age of all patients studied was 48.24 years, with a standard deviation of 14.13 years. For CKD patients, the mean age was 51.59 years (SD 12.82), and for transplant patients, it was 37.33 years (SD 12.82) (Table 2; Fig. 1).

Table 2. Age distribution of CKD, renal transplant, and control cases

Age (Years)	CKD	Renal transplant	Controls	Total
20-29	06	06	02	14
30-39	09	10	15	34
40-49	15	04	08	27
50-59	19	02	03	24
60-69	28	02	00	30
70-80	01	00	00	01
Grand total	78	24	28	130

Source: compiled by the authors

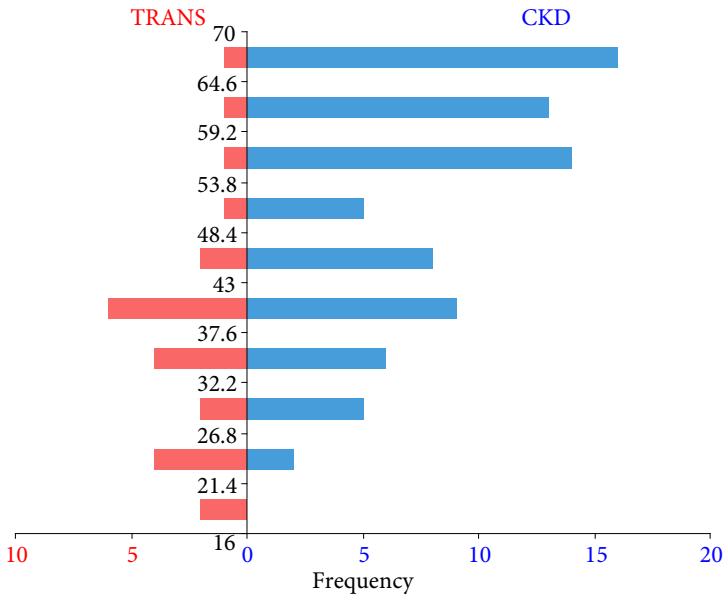

Figure 1. Frequency of CKD and renal transplant patients

Source: compiled by the authors

Chronic glomerulonephritis was the leading cause of CKD, accounting for 43.5% of cases, followed by diabetes mellitus, hypertension, and other diseases. The average duration of CKD from diagnosis was 2.5 years, ranging from 1 month to 5 years. Comorbidities in CKD patients included diabetes mellitus, hypertension, ischaemic heart disease, and chronic viral hepatitis caused by Hepatitis B and C viruses (Fig. 2).

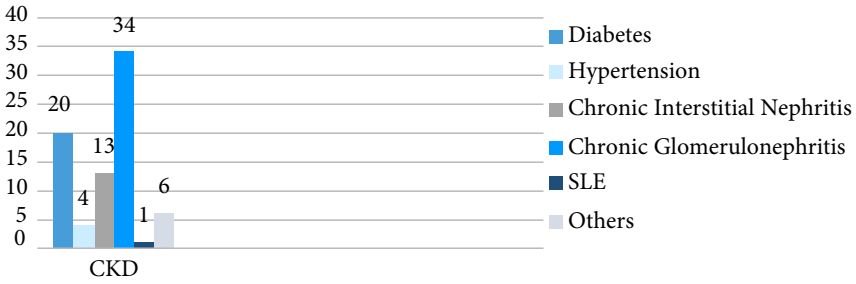

Figure 2. Leading causes of CKD

Source: compiled by the authors

Various investigations were conducted in CKD and post-renal transplant patients, including serum calcium, phosphates, parathormone, and vitamin D3 levels. The mean serum calcium level in CKD cases was 8.37 mg/dL (SD 0.92), ranging from 1.3 to 9.4 mg/dL, while post-transplant cases had a mean serum calcium level of 8.17 mg/dL (SD 1.09), ranging from 3.6 to 9.0 mg/dL (Fig. 3), which depicts minimal reduction in mean calcium levels post transplantation. For serum phosphorus, CKD cases had a mean level of 4.25 mg/dL (SD 0.47), with a range of 3.4 to 5.6 mg/dL, compared to post-transplant cases, which had a mean of 4.15 mg/dL (SD 0.51), ranging from 3.4 to 5.8 mg/dL (Fig. 4). The mean serum parathormone level in CKD cases was 24.39 U/dLL (SD 41.8), ranging from 1.6 to 260 U/dL, whereas post-transplant cases had a mean level of 8.13 U/dLL (SD 7.13), with a range of 2 to 24 U/dLL. A significant reduction in serum parathormone levels after transplantation was observed (p = 0.001, CI 0.05) (Fig. 5).

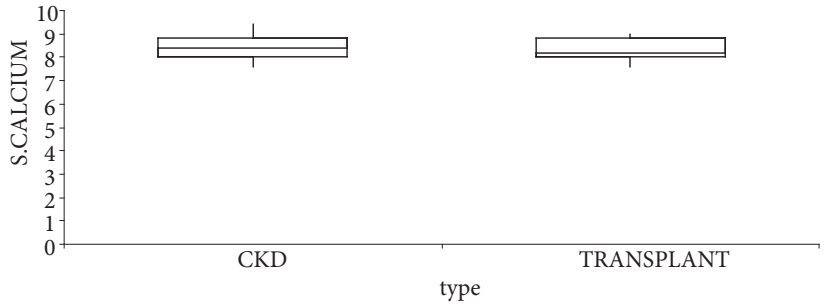

Figure 3. Serum calcium levels

Source: compiled by the authors

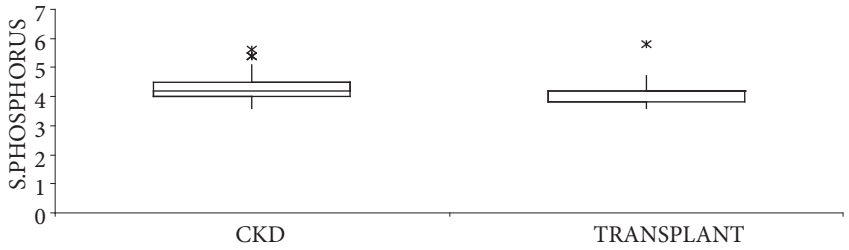

Figure 4. Serum phosphorus levels

Source: compiled by the authors

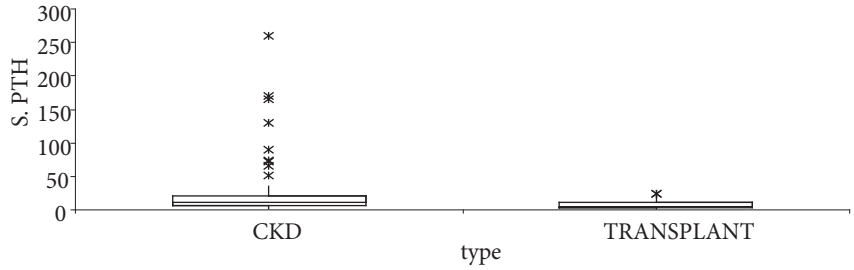

Figure 5. Serum parathormone levels

Source: compiled by the authors

Regression analysis was conducted to examine the relationships between various variables. The relationship between serum calcium and phosphorus was found to be insignificant ($p > 0.05$), as was the relationship between serum calcium and PTH levels ($p = 0.63$). Similarly, the associations between serum vitamin D3 and PTH levels ($p = 0.11$) and between serum vitamin D3 and calcium levels ($p = 0.11$) were also insignificant. The comparison between CKD and post-transplant patients revealed a significant decline in parathyroid hormone (PTH) levels in the post-transplant group (mean PTH: 24.39 U/dLL in CKD vs. 8.13 U/dLL in post-transplant; $p = 0.001$), suggesting improved mineral metabolism after transplantation. There were no significant differences in serum calcium and phosphorus levels between the two groups, although the post-transplant patients exhibited more stable control of these parameters.

The observed reduction in PTH post-transplant is clinically relevant, as it indicates effective reversal of secondary hyperparathyroidism and better bone-mineral regulation. This finding has important implications for managing bone health in CKD patients, suggesting that post-transplant care should focus on maintaining this improvement to reduce the risk of renal osteodystrophy and associated fractures. These results enhance understanding of mineral metabolism in CKD and posttransplant patients and support the need for tailored interventions to address disruption of mineral and bone metabolism in CKD.

Discussion

This study included a cohort of 78 patients with CKD, 24 post-renal transplant patients, and 28 control cases. The analysis of various biochemical parameters related to MBD revealed significant insights, particularly in the context of serum calcium, phosphates, vitamin D3, and PTH levels. Each of these parameters plays a crucial role in the management of CKD and the outcomes following renal transplantation. In this study, the mean serum calcium level among CKD patients was 8.37 mg/dL (SD 0.92), while for post-transplant patients, it was 8.17 mg/dL (SD 1.09). Both levels are below the normal range, indicating potential disturbances in calcium metabolism. In contrast, A.K. Yadav *et al.* [8] reported a mean calcium level of 9.5 ± 0.6 mg/dL in their cohort, which was higher than the levels observed in this study. The differences could be attributed to variations in dietary intake, geographic factors, or differences in the patient population. The study by A.K. Yadav *et al.* [8] also demonstrated significant associations between vitamin D deficiency and calcium levels, reinforcing the notion that vitamin D status is a critical factor influencing calcium homeostasis in CKD patients.

The mean serum phosphorus levels in this study were 4.25 mg/dL (SD 0.47) for CKD patients and 4.15 mg/dL (SD 0.51) for post-transplant patients, both of which are above the normal range. This is consistent with the findings of C.P. Kovesdy *et al.* [9], who reported an association between elevated serum phosphorus levels and anaemia in kidney transplant recipients. In their analysis, every 1 standard deviation increase in phosphorus was linked to a significant decrease in haemoglobin levels. This correlation highlights the importance of managing phosphates levels to prevent complications such as anaemia, a common issue in CKD patients. Furthermore, the findings of Y. Doi *et al.* [10] indicate that high phosphates levels, along with parathormone levels predict poor renal outcomes in kidney transplant patients. Their study underscores the need for careful monitoring of phosphorus levels in this cohort, as elevated phosphorus could lead to increased cardiovascular risk and graft dysfunction. A. Rastogi *et al.* [11] suggest an integrated approach to phosphorus control in CKD patients on dialysis by incorporating measurements of calcium, phosphorus, and PTH along with correlations between dietary adjustments and CKD-MBD drugs, which facilitate improved patient management. A study by M. Conley *et al.* [12] emphasises the role of dietary phosphates intake in renal disease patients. The study concluded that levels of total dietary phosphates intake showed no statistically significant relationship with biochemical markers of bone and mineral metabolism or intermediate cardiovascular markers. This finding mandates further extensive research into this group of patients.

This study reported the mean serum vitamin D3 levels of CKD patients as 45.21 U/dLL (SD 36.74), with a range of 1.8 to 149 U/dLL, while post-transplant patients had a mean vitamin D3 level of 30.14 U/dLL (SD 33.06), ranging from 1.2 to 122 U/dLL. Notably, the comparison of serum vitamin D3 levels between CKD and post-transplant patients showed no significant change after transplantation ($p = 0.06$, CI 0.05). This finding suggests that, despite successful transplantation, vitamin D3 levels may not improve significantly, which contrasts with expectations of enhanced vitamin D metabolism following renal transplantation. In the study by C.S. Kim *et al.* [13], vitamin D deficiency was prevalent among CKD patients, with 76.7% of stage 1 CKD patients exhibiting deficiency. This indicates that vitamin D status may remain a concern even after transplantation, potentially due to ongoing metabolic alterations or insufficient dietary intake. Additionally, the observations of A.K. Yadav *et al.* [8] reinforce the significance of vitamin D in maintaining calcium and phosphorus balance. Their findings revealed that lower vitamin D levels were associated with reduced serum calcium levels, which could explain the suboptimal vitamin D3 levels in both CKD and post-transplant patients in this study. Furthermore, the impact of vitamin D on bone health and its correlation with PTH levels in this study suggest that interventions aimed at correcting vitamin D deficiency could improve PTH levels and overall mineral metabolism in CKD patients. Regular assessment and supplementation of vitamin D may, therefore, be necessary to manage MBD in this population effectively.

The mean serum PTH level in this study for CKD patients was 24.39 U/dLL (SD 41.8), which decreased significantly to 8.13 U/dLL (SD 7.13) post-transplant ($p = 0.001$). This finding is consistent with Y. Doi *et al.* [10], who found

that high iPTH levels are associated with poor renal outcomes. In their study, they reported that elevated PTH levels can indicate underlying metabolic disturbances that may impact graft survival. In comparison, H. Komaba *et al.* [14] reported a mean iPTH level of 58.4 ± 32.9 ng/mL, significantly higher than the levels observed in this CKD cohort. This discrepancy may suggest that the patients in this study might have been at an earlier stage of MBD or that the treatment and dietary management of CKD patients differed between the studies. It also emphasises the importance of regular monitoring and management of PTH levels to prevent complications associated with secondary hyperparathyroidism. A study conducted in Japan recommends parathyroid interventions, such as parathyroidectomy and percutaneous ethanol injection therapy, for mineral disorders that are not managed by pharmacological means.

F. Li *et al.* [15] supported the correlation between iPTH levels and anaemia severity, suggesting that interventions targeting PTH levels may improve haemoglobin levels. In the findings of the current study, the significant reduction in PTH levels post-transplant could potentially lead to similar improvements in anaemia, highlighting the interconnectedness of these parameters. J. Malyzko *et al.* [16] discussed the challenges in assessing calcium and phosphates levels in kidney transplant recipients, indicating a potential gap in monitoring bone metabolism parameters post-transplant. This underscores the importance of regular evaluation of PTH and other mineral metabolism markers, as highlighted in this study, to prevent complications such as secondary hyperparathyroidism.

M. Fusaro *et al.* [17] surveyed 106 Italian nephrologists, providing valuable insights into the clinical practices surrounding the management of CKD and secondary hyperparathyroidism (sHPT). Their findings present a stark contrast to those of the current study. In this research, which involved CKD and post-renal transplant patients, significant variations were observed in calcium, phosphorus, and PTH levels, as well as in the relationships among these parameters. Notably, the current study reported a mean serum calcium level of 8.37 mg/dL in CKD patients, which is relatively lower than the normal range, indicating potential hypocalcaemia. This contrasts with the findings from the Italian nephrologists' survey, which highlighted high accessibility to ionised calcium and PTH measurements, suggesting that nephrologists in Italy have the resources to closely monitor these critical parameters.

K. Nakai *et al.* [18] emphasised the role of persistent hyperparathyroidism concerning graft function. The findings of this study, particularly the significant reduction in PTH levels posttransplant, support the view that effective management of PTH levels is crucial for optimising graft function and improving patient outcomes. While biochemical markers are essential in monitoring bone health, bone histomorphometry remains the gold standard for assessing bone turnover. However, it is rarely performed in routine clinical practice. Bone turnover markers (BTMs) could serve as a much-needed, non-invasive diagnostic tool to bridge the therapeutic gap for patients with advanced CKD and bone fragility [19]. Future research comparing the efficacy of bone histomorphometry and BTMs will likely yield useful insights. Other indicators of bone turnover, such as dual-energy X-ray absorptiometry (DEXA), have not been found to be reliable markers in this context [20].

This study demonstrated that abnormalities in mineral metabolism, particularly elevated PTH levels, in CKD patients significantly improve following renal transplantation. The stabilisation of calcium and phosphorus levels post-transplant highlights the effectiveness of restored kidney function in mitigating CKD-MBD. These findings underscore the importance of monitoring and managing mineral imbalances to enhance long-term bone health outcomes in both CKD and post-transplant patients.

Conclusions

This study contributes to the understanding of MBD parameters in CKD and post-renal transplant patients. It highlights significant differences in key markers of mineral metabolism between these groups. In CKD patients, the mean serum PTH level was markedly elevated at 24.39 U/dLL (SD 41.8), while post-transplant patients exhibited a significantly lower mean PTH level of 8.13 U/dLL (SD 7.13), indicating a statistically significant reduction ($p = 0.001$). Similarly, the mean serum calcium levels were slightly higher in CKD patients (8.37 mg/dL, SD 0.92) compared to post-transplant patients (8.17 mg/dL, SD 1.09), although this difference was not statistically significant. Serum phosphorus levels followed a similar trend, with CKD patients recording a mean of 4.25 mg/dL (SD 0.47), while post-transplant patients had a mean of 4.15 mg/dL (SD 0.51). Despite the minor differences in calcium and phosphorus levels between the groups, the most notable improvement post-transplant was the significant decline in PTH levels, reflecting enhanced control of bone-mineral metabolism. These results underscore the critical impact of renal transplantation on normalising mineral metabolism, particularly the reversal of secondary hyperparathyroidism, which plays a key role in managing CKDMBD and reducing the risk of complications such as renal osteodystrophy. Regular assessment of vitamin D status should also be integrated into management plans, given its pivotal role in calcium and phosphorus homeostasis. As understanding of the complex interactions among these parameters improves, it will be essential to implement targeted interventions aimed at optimising mineral metabolism in CKD and transplant populations to enhance patient outcomes and quality of life. Future studies should focus on longitudinal data to evaluate the long-term effects of managing these parameters on clinical outcomes.

Acknowledgements

None.

Conflict of Interest

None.

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Асоціація біомаркерів мінерального обміну у пацієнтів з хронічною хворобою нирок та реципієнтів ниркового трансплантата: одноцентрове проспективне дослідження

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Анотація. Цілісна оцінка параметрів мінеральних порушень кісткової тканини, включаючи сироватковий кальцій, фосфор, паратгормон і 1,25-дигідроксивітамін D, може прогнозувати ниркові наслідки у реципієнтів ниркового трансплантата, хоча результати варіюють. Метою цього дослідження було оцінити біохімічні параметри мінеральних порушень кісткової тканини у пацієнтів з хронічною хворобою нирок та реципієнтів ниркового трансплантата. Всього було включено 78 пацієнтів з хронічною хворобою нирок, 24 реципієнти після трансплантації нирки та 28 осіб контрольної групи, середній вік яких становив 48,24. Переважною причиною хронічної хвороби нирок був хронічний гломерулонефрит, за яким слідували діабет і гіпертонія. Було оцінено рівні кальцію, фосфору, паратгормону та вітаміну D3 у сироватці крові. У пацієнтів з хронічними захворюваннями нирок середній рівень кальцію в сироватці крові становив 8,37 мг/дл, а фосфору – 4,25 мг/дл, тоді як у пацієнтів після трансплантації середній рівень становив 8,17 і 4,15 мг/дл відповідно. Значне зниження рівня паратгормону в сироватці крові спостерігалося у пацієнтів після трансплантації (в середньому 8,13 Од/дл) порівняно з пацієнтами з хронічною хворобою нирок (в середньому 24,39 Од/дл). Після трансплантації не було відмічено суттєвих змін у рівні вітаміну D3. Регресійний аналіз виявив незначний зв'язок між рівнями кальцію і фосфору в сироватці крові та рівнем паратгормону. Це дослідження висвітлює біохімічний дисбаланс, пов'язаний з хронічними захворюваннями нирок, та вплив трансплантації нирки на рівень паратгормону, підкреслюючи необхідність регулярного моніторингу та управління параметрами мінеральних порушень кісткової тканини у цих популяціях пацієнтів

Ключові слова: біомолекулярні показники; мінеральні порушення кісткової тканини; гормональний дисбаланс при захворюваннях нирок; 1,25-дигідроксивітамін D

International Journal of Medicine and Medical Research

Journal homepage: https://ijmr.com.ua/
Volume 10, No. 2. 2024

UDC 615.31:599.323.4
DOI: 10.61751/ijmmr/2.2024.65

Article's History:
Received: 19.06.2024; Revised: 22.09.2024; Accepted: 26.11.2024

Effect of antioxidants on oocyte morphology and function in rats in systemic inflammatory process

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Abstract. The study aimed to evaluate the impact of antioxidants on the morphological and functional properties of oocytes in rats under conditions of systemic inflammation, focusing on their potential to reduce oxidative stress and improve reproductive health. A female rats at three weeks of age were used as experimental models and divided into three groups: control, systemic inflammation-induced, and antioxidant-treated groups. Human chorionic gonadotropin was administered to stimulate superovulation, and oocytes were isolated for analysis. Morphological changes in oocytes were assessed using immunofluorescence staining, while mitochondrial function and oxidative stress were evaluated using JC-1 dye detection kit. Fertilisation rates and oocyte maturation stages were compared among the groups. All experiments adhered to international ethical standards for animal research. Systemic inflammation led to ovarian atrophy, decreasing ovarian weight to 0.3 g, which reflects a reduction in follicle number and oocyte quality. This resulted in a 30% reduction in fertilisation rates. Antioxidant therapy helped restore ovarian weight to 0.4 g, improving oocyte morphology and increasing the proportion of oocytes achieving metaphase to 75%, demonstrating the protective effect of antioxidants. The fertilisation rate improved to 65% in the antioxidant-treated group, indicating a protective effect against oxidative damage and inflammation. The findings highlight the potential of antioxidants in modulating apoptosis pathways, reducing oxidative stress, and enhancing mitochondrial function, which collectively improved oocyte quality and reproductive outcomes. Antioxidant therapy demonstrated substantial benefits in mitigating the effects of oxidative stress and inflammation on oocytes, promoting normal maturation and increasing fertilisation success. These results underscore the therapeutic potential of antioxidants for treating infertility associated with inflammatory conditions

Keywords: post-ovulatory ageing; egg; antioxidant; oxidative imbalance; citrus nutrients

Introduction

The need to investigate the effect of antioxidants on oocyte quality is due to the growing problem of infertility in modern society. Among the factors that negatively affect reproductive function, special attention is drawn to postovulatory ageing of oocytes which is caused by a decrease in their quality after ovulation. Recent trends show that women are increasingly delaying the time when they will become mothers. This has led to an increase in the number of women whose oocytes undergo quality changes due to age factors. The ageing of oocytes after ovulation is an important factor limiting the success of assisted reproductive technologies. The quality of an oocyte directly affects its ability to fertilise and further develop the embryo. A study by B. Bibak *et al.* [1] showed that long-term storage of oocytes without fertilisation leads to their progressive deterioration, which is described as postovulatory ageing. This phenomenon is accompanied by a number of pathological changes that negatively affect fertility. These changes include mitotic spindle abnormalities, chromosome integrity disorders, mitochondrial dysfunction, and changes in ion concentration that affect normal oocyte function. Oxidative stress is one of the key causes of reduced oocyte quality

Suggest Citation:
Stewart A. Effect of antioxidants on oocyte morphology and function in rats in systemic inflammatory process. Int J Med Med Res. 2024;10(2):65–72. DOI: 10.61751/ijmmr/2.2024.65

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during ageing. In particular, as pointed out by B. Bibak *et al.* [1], the accumulation of reactive oxygen species that negatively affect the structure and function of oocytes causes an oxidative imbalance, which substantially reduces female fertility. A paper of V.R. Askari *et al.* [2] show that reactive oxygen species cause oxidative damage, which leads to the destruction of cell membranes, a decrease in the energy potential of mitochondria, and changes in the genetic material of oocytes. The discrepancy between the production of reactive oxygen species and the mechanisms of antioxidant protection is an important factor leading to the development of oxidative stress. This can cause various defects in germ cells, such as mitotic spindle abnormalities and chromosomal aberrations, as stated by V.R. Askari *et al.* [3]. Postovulatory ageing of oocytes is an inevitable process associated with the duration of their stay in culture after ovulation. A study by S. Fiorito [4] demonstrates that the duration of oocyte storage without fertilisation substantially affects their quality and potential for fertilisation.

Recent studies confirm that oxidative stress negatively affects the metabolic processes that affect the functioning of oocytes, and further research is needed to protect them [5]. As noted by Z. Tayarani-Najaran *et al.* [6], in assisted reproductive technologies actively used for infertility treatment, oocyte manipulation is critical, as the technical aspects of the procedure can expose oocytes to additional stress and increase oxidative damage. This highlights the importance of using antioxidants that reduce reactive oxygen species levels, protect oocytes from oxidative stress, and improve their survival and quality, as indicated by H. Kobayashi *et al.* [7]. The results of such studies can substantially affect clinical practice in the field of reproductive medicine and open up new prospects for improving the quality of germ cells and the prognosis of reproduction, as emphasised by M. Igase *et al.* [8]. S. Galluzzi *et al.* [9] demonstrated that the examination of the effects of aurapten is an important step in the search for new therapeutic approaches to improve the success of assisted reproductive technologies.

Assisted reproductive technologies *in vitro* can lead to an increase in oxidative stress due to the technical features of manipulations and the lack of endogenous protection systems. Oxidative damage can cause oocyte apoptosis, which negatively affects the fertilisation process and embryo development. In this context, the use of antioxidants can be a safe and effective strategy for slowing or preventing oocyte ageing by reducing oxidative stress. Understanding the mechanisms by which antioxidants act may provide new insights into their potential to improve germ cell quality. Among the many antioxidants examined for their effects on oocytes, aurapten (7-geranyloxycoumarin) stands out as a promising candidate. This naturally occurring biologically active compound, derived from citrus fruits, has a wide range of pharmacological properties, including antioxidant, anti-inflammatory, antitumour and neuroprotective effects. Aurapten can protect cells from oxidative stress by reducing reactive oxygen species and restoring normal mitochondrial function. However, despite these promising data, there are not enough studies evaluating the effect of aurapten on postovulatory ageing of oocytes and embryo development *in vitro*. The study aimed to investigate the effect of antioxidants on the morphological and functional properties of oocytes in rats under conditions of systemic inflammation.

Literature Review

Oxidative stress is an important aspect in reproductive medicine research because it directly affects the quality of germ cells. V. Di Nisio *et al.* [10] note that postovulatory ageing of oocytes can impair their quality, making it difficult to fertilise and develop embryos both *in vivo* and *in vitro*. C. Yu & J.-H. Xiao [11] draw attention to the Keap1-Nrf2 system, which may be a target for therapy that increases antioxidant protection in older women. K.-H. Kim *et al.* [12] established that GAS6 improves mitochondrial function in old oocytes by reducing meiotic defects, potentially improving germ cell quality. A. Almansa-Ordonez *et al.* [13] focus on the effect of oxidative stress on the energy metabolism of oocytes, which negatively affects their viability. M. Khazaei & F. Aghaz [14] showed that antioxidants during *in vitro* oocyte maturation reduce oxidative stress, improving their quality, which is critical for the success of reproductive technologies. A. Cecchele *et al.* [15] consider the cellular and molecular nature of fragmentation of human embryos. Their study highlights that fragmentation may be the result of oxidative stress affecting both oocytes and embryos, suggesting the need to develop new approaches to improve their quality.

Oxidative stress substantially affects the quality of oocytes and, in general, the reproductive health of women, especially in the context of polycystic ovary syndrome. M. Abizadeh *et al.* [16] investigated the possibility of using aurapten to improve oocyte maturation, increase fertilisation, and reduce inflammatory processes in a mouse model of polycystic ovary syndrome. The results of their study suggest the potential use of aurapten as a therapeutic agent to improve reproductive function. Y. Jang *et al.* [17] analysed the effect of aurapten on behavioural aspects in experimental models of Parkinson's disease, emphasising its ability to protect mitochondrial respiration from inhibition and reduce the level of reactive oxygen species. This opens up prospects for the use of aurapten not only in neurological disorders but also in the field of reproductive medicine because mitochondrial stress substantially affects the quality of oocytes. M.J. Lee *et al.* [18] established that aurapten promotes the activation of antioxidant enzymes and improves connectivity in brain endothelial cells, which helps reduce mitochondrial stress and enhance cellular function. This may be an important mechanism for improving oocyte maturity and functionality in the context of ageing. E.H. Hassanein *et al.* [19] examined the role of coumarins, such as aurapten, in regulating the Keap1/Nrf2/ARE pathway, emphasising the ability to stimulate antioxidant protection in cells, which can positively affect oocyte quality.

N. Akino *et al*. [20] investigate the effect of dimethyl fumarate on the activation of the Nrf2/Keap1 pathway, evaluating its ability to reduce oxidative stress and delay the development of age-related infertility in the ovaries of mice, emphasising the importance of antioxidant mechanisms for maintaining reproductive function with age. E. Fonseca *et al*. [21] investigate the effect of urolithin A on slowing the ageing of bovine oocytes *in vitro*, which can improve the quality of oocytes and increase reproductive efficiency in animal husbandry. Y. Furukawa *et al*. [22] show that aurapten stimulates the expression of brain neurotrophic factor in Neuro2a cells, indicating a possible neuroprotective effect and protection of oocytes from oxidative stress. G. Shimoi *et al*. [23] state that spindle destabilisation can cause aneuploidy in senescent oocytes, which is important for ensuring the quality of germ cells. D. Zhang *et al*. [24] emphasise the role of mitochondrial changes in oocyte ageing, which requires further research to understand these processes.

J. van der Reest *et al*. [25] emphasise the important role of mitochondria in oocyte ageing, noting that maintaining their activity is key to preventing age-related defects in oocytes. Oxidative stress substantially affects women's reproductive function. J. Lu *et al*. [26] consider the mechanisms of its negative effects, focusing on the violation of the balance between reactive oxygen species and antioxidant protection, which can lead to a deterioration in the quality of oocytes. A.T. Perkins *et al*. [27] investigate the role of superoxide dismutase in reducing meiotic errors in senescent oocytes, which highlights the prospects for antioxidant therapy for genetic stability. S. Armstrong *et al*. [28] discuss the importance of technologies such as time laps systems for monitoring embryos in reproductive procedures, which can increase the chances of successful fertilisation. C. Lagalla *et al*. [29] highlight the importance of atypical patterns of partial embryo compression, paying attention to their prevalence and possible influence on the results of reproduction. This study highlights the importance of analysing the morphokinetic features of embryo development in improving assisted reproductive technology protocols. The results of these studies confirm the importance of antioxidant therapy and the latest technologies in assisted reproductive procedures for improving the quality of oocytes and the overall effectiveness of infertility treatment. Given the role of oxidative stress, such approaches can be substantial components in preventing age-related changes in the female reproductive system.

Materials and Methods

The study was conducted by an author affiliated with Ukraine, with experimental work performed in collaboration with facilities in the Republic of Korea. The study used the female rats at three weeks of age purchased from Koatech, which is located in Phentek, Gyeonggi Province, Republic of Korea. The animals were kept in appropriate conditions at the Cha University Laboratory Animal Research Centre, where a standard temperature regime and humidity level were maintained. The rats were in conditions that mimic the natural rhythm, in particular, the 12-hour cycle of light and dark, which contributes to the normalisation of physiological processes in the animals. 10 IU of human chorionic gonadotropin was administered to stimulate superovulation, which was purchased from Sigma-Aldrich, St. Louis, Missouri, USA. After the preparatory stage, which included anaesthesia, an abdominal autopsy was performed and oocytes were removed from the ovaries and isolated from follicular cells using the hyaluronidase enzyme (Sigma-Aldrich). Isolation ensured the purity of the samples for further analysis, eliminating follicular cells that could affect the results of the study.

Immunofluorescence staining was used for detailed analysis of morphological changes in oocytes. Oocytes were treated with a phosphate buffer with the addition of 0.1% polyvinyl alcohol, which prevented precipitation and ensured the uniform application of the solution to the samples. The samples were then fixed in a 4% paraformaldehyde solution, which stabilised the cell structures for further processing. For the purpose of permeability, a solution of Triton X-100 (0.2%) was used, which facilitated the penetration of antibodies into intracellular structures. Incubation with the primary antibody against α-tubulin (Santa Cruz Biotechnology) was conducted at a concentration of 1:100 at a temperature of 4°C overnight, which provided high specificity and sensitivity of staining. After thorough washing of the samples, they were treated with a secondary antibody obtained from goat anti-mouse IgG, labelled with Alexa Fluor 488 fluorescent dye at a concentration of 1:100. The dye 4',6-diamidine-2-phenylindole (Sigma-Aldrich) was used to visualise the nuclear material, which clearly stained the nuclei. The image was obtained using a Leica TCS SP5 II confocal microscope (Germany), which allowed obtaining high-quality images with high resolution for further analysis.

The functional state of mitochondria was evaluated using JC-1 dye, which allowed determining the membrane potential of mitochondria (ΔΨm). Oocytes were incubated in M16 medium with the addition of JC-1 (1 μg/ml) at 37°C for 30 minutes, which contributed to the maximum accumulation of dye in mitochondria. After incubation, fluorescent signals in the red and green channels were analysed to determine the relative membrane potential. Reactive oxygen and glutathione levels were determined using the OxiSelect™ kit (STA-342, Cell Biolabs, San Diego, USA) and CELLTRACKER™ blue CMF2HC dye (Invitrogen), which provided high sensitivity and specificity in the measurements. Oocytes were cultured in M16 medium with the addition of dichlorodihydrofluorescein diacetate for 30 minutes, after which detailed analysis was performed using confocal microscopy to assess the fluorescent signal indicating the level of oxidative stress in the samples.

All experiments were conducted in accordance with international ethical standards, including the animal research guidelines: Reporting of *In Vivo* Experiments [30]

and Recommendations of the Cha University Ethics Committee [31] aimed at the humane treatment of laboratory animals and minimising their suffering.

Results and Discussion

Antioxidants were identified to substantially improve reproductive health by reducing the harmful effects of oxidative stress. Oxidative stress occurs due to an imbalance between the production of free radicals and antioxidant protection, which causes damage to cells, including oocytes, and can negatively affect their quality and ability to fertilise. For example, vitamins C and E, and substances such as coenzyme Q10, can neutralise free radicals, improving the functioning of cell membranes and maintaining their integrity. This is extremely important for oocytes, which are vulnerable to oxidative damage because they contain a substantial amount of unsaturated fatty acids and other components that are subject to oxidation. In addition, this study examined the effect of antioxidant therapy on oocyte apoptosis processes. Inflammation can stimulate signalling pathways that trigger the cell death programme, leading to the loss of functional oocytes. The use of antioxidants can have a protective effect, preventing the activation of these pathways and maintaining cell viability.

Figure 1 shows a comparison of ovarian weight in three groups: the control group, the group with induced systemic inflammation, and the group receiving antioxidants. In the control group, the ovarian mass was 0.45 g, which indicates a normal state of the reproductive system. In the group with induced inflammation, the ovarian mass decreased to 0.3 g, which indicates ovarian atrophy under the influence of an inflammatory process that disrupts endocrine regulation, which is critical for the proper functioning of the ovaries. This weight loss reflects a decrease in the number of follicles and a deterioration in their morphology and functionality, which can substantially affect the quality of oocytes. In the group that received antioxidant therapy, ovarian weight increased to 0.4 g, which demonstrates the protective effect of antioxidants. Restoring ovarian weight is vital to maintaining reproductive function, as positive dynamics indicate the ability of antioxidants to prevent atrophic changes in the ovaries, maintaining their morphological integrity and functional state.

Figure 2 describes in detail the process of nuclear maturation of oocytes, including the stages of metaphase and anaphase. In the control group, normal organisation of chromosomes was observed in oocytes that were at the metaphase stage, indicating readiness for division. The chromosomes were correctly aligned on the equatorial plate, which is crucial for the successful division and further development of embryos. A substantial number of oocytes with abnormal maturation were found in the group with induced inflammation. They had an uneven distribution of chromosomes and a large number of oocytes with disorders in the organisation of the division spindle. This is a serious indicator of the negative impact of the inflammatory process on the quality of oocytes, which can lead to a decrease in their ability to fertilise.

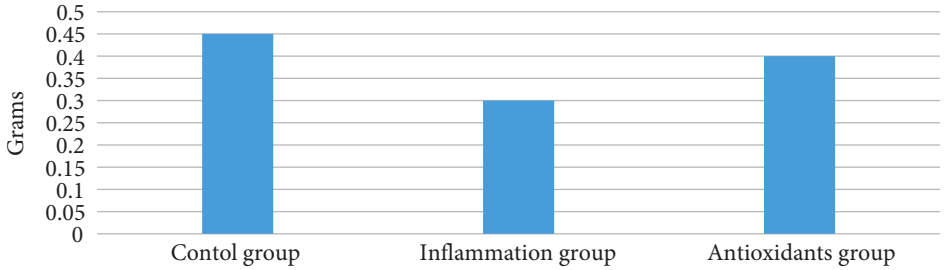

Figure 1. Comparison of rat ovarian mass by group

Source: compiled by the author

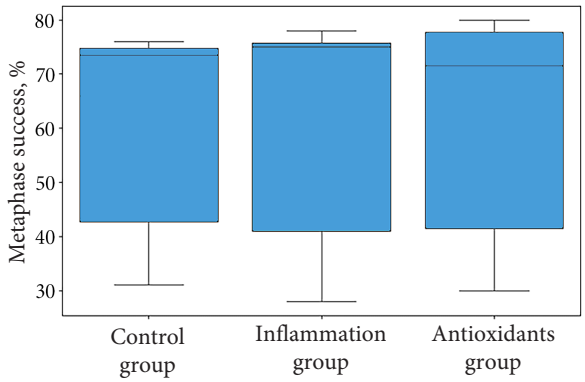

Figure 2. Comparison of the proportion of oocytes in metaphase between groups

Source: compiled by the author

In the antioxidant-treated group, there was a substantial improvement in the morphological and functional parameters of oocytes. The proportion of oocytes that successfully achieved the correct metaphase increased to 75%. This indicates a positive effect of antioxidants on the maturation process of oocytes, which is important for ensuring their ability to fertilise. Reducing the frequency of abnormal oocytes in this group highlights the effectiveness of antioxidant therapy in maintaining normal oocyte maturation, which contributes to the preservation of their reproductive function. The quality of oocytes that mature is an important factor for successful fertilisation and further development of embryos, so the results of the study are of great scientific and practical importance. In addition, a detailed analysis of the ability of oocytes to fertilise and further develop embryos was conducted as part of the study. As shown in Figure 3, in the control group of untreated rats, the fertilisation rate was relatively high, with the median rate approaching 80%. This indicates a favourable oocyte quality that supported successful fertilisation. However, in the group of rats with systemic inflammation, the fertilisation rate was significantly reduced, with the median rate around 30%, reflecting a marked decrease in oocyte quality likely due to inflammation-induced oxidative stress. In the group receiving antioxidant therapy, the fertilisation rate improved, with a median rate of approximately 65%, indicating a protective effect of antioxidants in enhancing oocyte quality and fertilisation success.

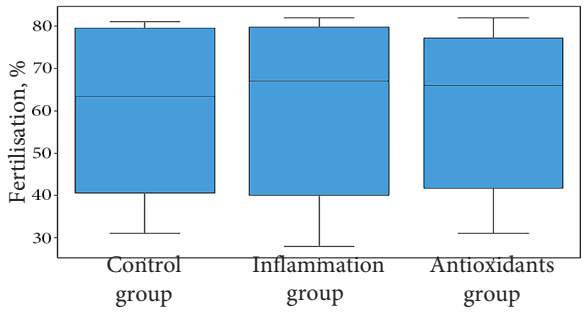

Figure 3. Comparison of fertilisation success between groups
Source: compiled by the author

In the group that received antioxidant therapy, the fertilisation rate increased to 65%. These results indicate that antioxidants have a pronounced positive effect on reproductive performance, as they contribute to improving the quality of oocytes and their ability to fertilise. The improvement in this indicator can be explained by an improvement in the morphological characteristics of oocytes, as well as an increase in their resistance to stressful conditions caused by the inflammatory process. It is important to emphasise that antioxidants not only protect oocytes from oxidative damage but can also modulate the mechanisms that regulate apoptosis and inflammation, thereby reducing the risk of loss of viable oocytes. Thus, the results of the study point to the important role of antioxidants in maintaining reproductive health. Antioxidant therapy has demonstrated the ability not only to improve the quality of oocytes but also to increase their reproductive properties. This is of great clinical importance, as it opens up opportunities for creating new therapeutic strategies aimed at treating infertility associated with inflammatory conditions. The study highlights the role of antioxidants as possible protectors of oocytes against damage caused by oxidative stress and inflammatory processes, which is crucial for maintaining their structure and functionality.

The study by M. Khazaei & F. Aghaz [14] emphasises the importance of antioxidants during pregnancy, which correlates with the results obtained, which indicate the ability of antioxidants to reduce the risk of inflammatory processes that can negatively affect reproductive health. The collected data indicate that the use of antioxidants may have a positive effect on pregnancy outcomes, reducing the risk of complications, which is confirmed by a decrease in inflammatory markers in animals treated with antioxidant therapy. A paper of C. Lagalla *et al.* [29] indicates that the parallels with the effects of antioxidants at the cellular level demonstrate their ability to reduce oxidative stress and improve the overall health of organisms. This confirms the effectiveness of antioxidant strategies in improving reproductive health and preventing the negative effects of inflammatory processes in various biological systems. A study by S. Galluzzi *et al.* [9] shows that citrus phytonutrients have a positive effect on cognitive and biological processes, especially in the context of age-related cognitive decline, which confirms the importance of natural antioxidants in reducing oxidative stress. K.-H. Kim *et al.* [12] established that the GAS6 protein is able to improve mitochondrial function and prevent meiosis defects in oocytes in older mice, which indicates the prospects for using antioxidant therapies to improve oocyte quality in the age group. A paper of Y. Jang *et al.* [17] emphasises the protective properties of aurapten, which reduces oxidative stress and prevents inhibition of mitochondrial respiration, which also confirms the positive effect of antioxidants in various biological systems. Lastly, N. Akino *et al.* [20] indicate activation of the Nrf2/Keap1 pathway, which reduces oxidative stress and may delay age-related infertility in mice after the

administration of dimethylfumarate. This supports the conclusion that antioxidant therapy can have a positive effect on reproductive health by reducing oxidative stress and improving oocyte quality.

The results of this study highlight the potential of antioxidants as therapeutic agents to improve oocyte quality and women's reproductive health. The use of antioxidants can be an important step in creating new approaches to infertility treatment, especially in women with concomitant inflammatory diseases. The key aspect is the determination of optimal doses and methods of administration of antioxidants, which requires further study. Thus, the results of the study not only deepen understanding of the role of antioxidants in reproductive medicine but also open up new opportunities for clinical applications in improving women's reproductive health.

Conclusions

The results of the study showed that the use of antioxidants helps to reduce the effects of oxidative stress, which is key to normalising the process of oocyte maturation, in particular, in conditions when this process is disrupted due to inflammation. Under conditions of inflammation, oocyte maturation is substantially disrupted, particularly the abnormal division of chromosomes and the violation of the organisation of the division spindle. Antioxidants, in turn, can help reduce the frequency of abnormal oocytes and improve their ability to fertilise. The use of antioxidant therapy helped restore ovarian mass, which decreased in the group with induced inflammation, which indicates its protective effect. In the group that received antioxidants, there was an improvement in the morphological and functional parameters of oocytes – an increase in the proportion of oocytes that successfully achieved the correct metaphase (up to 75%).

Antioxidant therapy improved the ability of oocytes to fertilise, which was demonstrated by an increase in the proportion of successful fertilisation to 65%. Prospects for further research should focus on investigating the mechanisms of action of antioxidants at the cellular level, which will allow for a better understanding of their effect on reproductive function. Future studies can also analyse the effectiveness of various antioxidant compounds, including natural and synthetic ones, and their combinations, which may contribute to the development of new therapeutic strategies for preventing reproductive disorders. The use of additional models and experimental conditions will allow evaluating a wide range of antioxidant effects, which will expand knowledge in this important area and open up new opportunities for the clinical use of antioxidant therapies in medicine.

Acknowledgements

None.

Conflict of Interest

None.

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Вплив антиоксидантів на морфологію та функцію ооцитів у щурів при системному запальному процесі

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Анотація. Дослідження мало на меті оцінити вплив антиоксидантів на морфологічні та функціональні властивості ооцитів у щурів в умовах системного запалення, зосереджуючись на їхньому потенціалі зменшувати окислювальний стрес і покращувати репродуктивне здоров'я. Як експериментальні моделі було використано тритижневих самок щурів, яких розділили на три групи: контрольну; групу, викликану системним запаленням; групу, що отримувала антиоксиданти. Для стимуляції суперовуляції вводився хоріонічний гонадотропін людини, потім було виділено ооцити для аналізу. Морфологічні зміни в ооцитах оцінювалися за допомогою імунофлуоресцентного фарбування, тоді як функцію мітохондрій та окислювальний стрес оцінювалися за допомогою набору для виявлення барвника JC-1. У групах порівнювалися рівень запліднення та стадії дозрівання ооцитів. Усі експерименти відповідали міжнародним етичним стандартам досліджень на тваринах. Системне запалення призвело до атрофії яєчників, зменшення маси яєчника до 0,3 г, що відображає зменшення кількості фолікулів і якості ооцитів. Це призвело до зниження норми внесення добрив на 30 %. Антиоксидантна терапія допомогла відновити масу яєчників до 0,4 г, покращивши морфологію ооцитів і збільшивши частку ооцитів, які досягли метафази, до 75 %, демонструючи захисну дію антиоксидантів. Коефіцієнт запліднення підвищився до 65 % у групі, яка отримувала антиоксиданти, що вказує на захисний ефект проти окисного пошкодження та запалення. Отримані дані підкреслюють потенціал антиоксидантів у модулюванні шляхів апоптозу, зниженні окисного стресу та посиленні функції мітохондрій, що разом покращує якість ооцитів і репродуктивні результати. Антиоксидантна терапія продемонструвала значні переваги в пом'якшенні впливу окислювального стресу та запалення на ооцити, сприянні нормальному дозріванню та збільшенню успіху запліднення. Ці результати підкреслюють терапевтичний потенціал антиоксидантів для лікування безпліддя, пов'язаного із запальними процесами

Ключові слова: післяовуляторне старіння; яйцеклітина; антиоксидативна речовина; окислювальний дисбаланс; цитрусові нутрієнти

International Journal of Medicine and Medical Research

Journal homepage: https://ijmr.com.ua/
Volume 10, No. 2. 2024

UDC 615.825:616-009.7
DOI: 10.61751/ijmmr/2.2024.73

Article's History:
Received: 30.07.2024; Revised: 07.10.2024; Accepted: 26.11.2024

Effectiveness of treatment of myofascial pain syndrome with the help of physical therapy and massage

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Abstract. The aim of the study was to determine the effectiveness of physical therapy and massage methods for reducing pain symptoms in patients with chronic myofascial pain syndrome, the duration of which was at least 12 weeks. The main focus was on eliminating pain trigger points and long-term muscle relaxation around the affected areas. The study involved 20 patients who complained of chronic pain accompanied by limited joint mobility, in particular temporomandibular joints, discomfort while chewing and episodic attacks of acute pain. The patients were prescribed a 12-week course of treatment, which consisted of weekly physical therapy and massage sessions aimed at reducing muscle tension and improving joint mobility. During the course, three sessions were held per week, each lasting 45 to 60 minutes. Physical therapy included exercises aimed at strengthening muscles and stretching myofascial tissues to facilitate movement. Massage techniques included deep tissue massage, myofascial release, and manual therapy aimed at relaxing tight muscles around the temporomandibular joint and reducing pain trigger points. The results of the study showed a significant reduction in pain intensity after treatment: the average pain score on the visual analogue scale decreased from 7.8 ± 0.9 to 2.3 ± 0.7 at rest and from 8.1 ± 1.1 to 3.1 ± 0.8 during exercise ($p < 0.01$). In addition, the amplitude of mouth opening increased from 27 ± 3 to 39 ± 4 mm ($p < 0.01$), indicating improved joint mobility, and the functional activity score on the Helkimo scale improved from 3.5 to 1.2 ($p < 0.01$), indicating an overall reduction in symptoms and improvement in joint function. Six months after completion of the treatment, 85% of patients maintained a stable therapeutic effect: 12% of patients had complete pain relief, and 5% had low-level pain. Also, the restoration of normal muscle function was recorded in 80% of patients, improved joint flexibility and correction of postural position in 75% of cases

Keywords: trigger points; temporomandibular joint; muscle relaxation; physical activity; restoration of joint mobility

Introduction

Myofascial discomfort, also known as myofascial pain syndrome, is an important problem in modern medicine. It is characterised by chronic muscle pain and trigger points that, when pressed, cause acute pain and muscle spasms. Patients often report decreased joint mobility, which can significantly affect their quality of life. Despite numerous studies, the issues of diagnosis and treatment of this syndrome remain relevant. Myofascial therapy, including

Suggest Citation:
Yur V, Lebediev O. Effectiveness of treatment of myofascial pain syndrome with the help of physical therapy and massage. Int J Med Med Res. 2024;10(2):73–80. DOI: 10.61751/ijmmr/2.2024.73

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release and manual therapy, is widely used to relieve pain, but the effectiveness of these methods is not fully understood.

Studies looking at the treatment of myofascial pain have shown that transcutaneous electrical nerve stimulation can be an effective technique for reducing chronic pain. W. Gibson *et al.* [1] conducted a review of Cochrane reviews confirming the potential of transcutaneous electrical nerve stimulation in this area. A. Galasso *et al.* [2] provided a comprehensive review of the treatment and management of myofascial pain syndrome, emphasising the importance of an individual approach to each patient. Furthermore, L. Manchikanti *et al.* [3] conducted a comparative analysis of the use of epidural procedures in patients with chronic pain in the Medicare population, which notes the need to optimise treatment in this group. M. Kashif *et al.* [4] pointed out the connection between triggers of myofascial pain in the neck and shoulders and psychological factors such as depression, anxiety, and stress among university students.

In this regard, J.L. Dieleman *et al.* [5] analysed healthcare costs in the United States, emphasising the economic aspects of chronic pain treatment. P. Valiente-Castrillo *et al.* [6] studied the effects of education in neurophysiology of pain and dry needling on patients with chronic neck pain, noting their positive impact on treatment outcomes. M. Paoletta *et al.* [7] conducted a scoping review of the effectiveness of extracorporeal shock wave therapy for patients with myofascial pain or fibromyalgia, which indicates the prospects of this method. I. Urits *et al.* [8] emphasise the importance of a systematic approach to the treatment and management of myofascial pain syndrome, including various therapeutic modalities. The study of N. Yılmaz *et al.* [9] compare the effectiveness of dry needle therapy and kinesiotaping in the treatment of myofascial pain, which shows a difference in the results of these approaches. Finally, A. Pignatelli Vilajeliu *et al.* [10] conducted a systematic review of invasive and non-invasive methods of treating cervical myofascial pain, pointing out the importance of individualising therapy.

Despite the positive results of numerous studies, the effect of myofascial release on different tissue types remains poorly understood. This applies to both short-term and long-term effects of this therapy in different groups of patients. There are many studies that emphasise the importance of an individual approach to each patient, in particular with regard to the choice of intensity and duration of therapy sessions. However, the overall problem is that there are no standardised treatment protocols that could ensure maximum therapy effectiveness for each patient, taking into account their individual physiological and anatomical characteristics. Thus, the purpose of this article was to study in detail the effect of myofascial therapy on improving joint mobility and reducing pain in patients with chronic myofascial pain syndrome. Particular attention is paid to the analysis of the effectiveness of various myofascial release techniques, as well as their impact on the general condition of patients.

Literature Review

The study by T.P. Do *et al.* [11] examines the relationship between myofascial trigger points and various types of headache, including migraine and tension headache. The results indicate that myofascial trigger points can play a significant role in the pathophysiology of headache. The authors recommend integrating myofascial pain management approaches into standard headache treatment protocols to improve therapeutic outcomes. B. Peral-Cagigal *et al.* [12] emphasise the clinical significance of transient headache and jaw clenching in the diagnosis of large cell arteritis. They highlight that these symptoms should be considered as critical markers for early diagnosis, as timely intervention can prevent serious complications. The study calls for increased awareness of these indicators among healthcare professionals and promotes the need for prompt diagnostic procedures in suspected cases. In the study by Y. Haviv *et al.* [13], the authors compared the effectiveness of nortriptyline and amitriptyline in the treatment of persistent muscle pain. The results show that both drugs are effective, but nortriptyline has a more favourable side effect profile. This study provides valuable information for the discussion of pharmacological strategies for the treatment of myofascial pain, helping physicians to make informed choices about treatment options. J.H. Huang-Lionnet *et al.* [14] provide an overview of pharmacological strategies for the management of myofascial pain. They discuss various treatments, including non-steroidal anti-inflammatory drugs, muscle relaxants, and antidepressants. The authors emphasise the need for individualised treatment plans that take into account the unique characteristics of each patient's pain, advocating a multidisciplinary approach to improve pain relief and functional outcomes.

G.M. Heir [15] investigates the effectiveness of pharmacological treatments for maxillofacial dysfunction. The results show that although medications can relieve symptoms, they should be part of a comprehensive treatment plan that includes physical therapy and behavioural interventions. This study emphasises that a multifaceted approach is required to treat temporomandibular disorder to ensure optimal management of the disorder. G. Affaitati *et al.* [16] conducted a retrospective analysis comparing the effects of topical agents and injectable therapies for the treatment of myofascial trigger points in patients with migraine. Their results suggest that both modalities are useful, but that injectable treatment may provide more significant and immediate relief. This study highlights the importance of individualised treatment strategies and suggests that injection therapy should be preferred in severe pain. L. Manchikanti *et al.* [17] evaluate the effectiveness of fluoroscopic epidural injections with and without steroids for the treatment of postoperative neck pain syndrome. Their study shows that steroid injections significantly improve outcomes compared to non-steroidal options. This study provides valuable evidence to support the use of fluoroscopic guidance in epidural injections, contributing to better clinical practices in the management of postoperative pain.

The study by L. Manchikanti *et al.* [18] discusses the need to change the Medicare healthcare payment policy in 2019. The authors emphasise that changes in the healthcare payment system can have a significant impact on the quality of care for patients with chronic pain. They propose a number of policy measures that could facilitate better integration of patients into the healthcare system and provide more effective treatment. The study by S.M. Moeen & A.M. Moeen [19] examines the use of intravenous lidocaine in combination with an improved recovery regimen in patients undergoing open radical cystectomy. The results show that this approach helps to reduce postoperative pain and shorten the length of hospitalisation, indicating its potential to improve clinical outcomes. Algorithms for the diagnosis and treatment of patients with pain syndromes in the lumbar and sacral regions were developed by S.Z. George *et al.* [20]. The authors propose a systematic approach to treatment, including both medical and non-medical methods, with an emphasis on the importance of individualised therapy for each patient.

Network meta-analysis is an important tool for evaluating the effectiveness of treatment in pain management. J. Watt & C. Del Giovane [21] emphasise its role in synthesising data from multiple studies to provide a comprehensive understanding of treatment outcomes. The study by M.J. Guzmán Pavón *et al.* [22] compares different manual therapeutic interventions for patients with myofascial trigger points, showing that some methods are more effective in reducing pain and increasing the threshold of pain sensitivity. Ü. Yalçın [23] studied the effectiveness of extracorporeal shock wave therapy and kinesiological taping in combination with exercise, demonstrating the positive effect of such combinations on improving functional performance and reducing pain. E. Ahn & H. Kang [24] discuss the basic concepts of network meta-analysis, emphasising its advantages and the need to control data quality.

The papers highlight different approaches to the treatment of chronic pain, pointing out the importance of comprehensive rehabilitation, a multidisciplinary approach and new diagnostic strategies to improve patient outcomes. The authors emphasise the need for coordination between specialists and individualised treatment.

Materials and Methods

The study involved 20 patients, including 12 women and 8 men, who suffered from chronic myofascial pain syndrome affecting the temporomandibular, shoulder and knee joints. The duration of pain in all participants was at least 6 months. The main criteria for inclusion in the study were the presence of chronic pain in these joints and the absence of internal joint damage, signs of osteoarthritis or serious postural disorders, which allowed to focus on patients with uncomplicated myofascial pain syndrome. All participants underwent a course of treatment consisting of a combination of physiotherapy and massage, the duration of which depended on the individual characteristics of each patient and their response to treatment, but ranged at least 12 weeks. Physiotherapy was aimed at relieving muscle tension and improving the range of motion of the affected joints, while massage was used to relieve pain, relax muscles and eliminate trigger points. All patients received the same number of treatments – three physiotherapy sessions and three massage sessions per week, i.e., 12 to 18 sessions of each type of therapy, depending on the duration of the course.

The study was not randomised, and no control groups receiving other treatments or no physiotherapy were used. The pain level was assessed using a visual analogue scale, as well as clinical examinations and functional tests that measured the range of motion in the joints. The self-report questionnaires included questions about pain intensity and frequency, the impact of pain on daily activities, and joint functional capacity. Statistical methods were used to analyse changes in pain scores, range of motion and overall functional status, including paired t-test to compare changes in scores before and after treatment, and Wilcoxon signed-rank test to assess data that did not have a normal distribution. Table 1 shows the main eligibility criteria for patients to participate in the study, which helps to focus attention on those with similar symptoms and health conditions.

Table 1. Inclusion criteria for study participants

Criteria	Description
Age	From 18 to 65 years old
Gender	Men and women
Health status	Absence of serious concomitant diseases
Chronic pain	Migration for at least 6 months

Source: created by the authors

The choice of the age range from 18 to 65 years is important for the study, as patients undergo significant physiological changes during this period that may affect pain perception and response to treatment. This category of people has a higher chance of maturity and readiness to participate in therapy, as adult patients usually have more experience in managing their health and are more active in seeking solutions to their problems.

With regard to the health status of the participants, it was decided to exclude those with serious comorbidities, as these conditions can significantly affect the results of the study. These conditions include cardiovascular diseases, such as hypertension and heart failure, which can reduce the overall endurance of patients and complicate the treatment process. Participants with cancer were also excluded, as their condition may be unstable and affect

their response to therapy. Other serious disorders that led to exclusion were systemic diseases such as diabetes and systemic lupus erythematosus, which can disrupt the body's functioning and interfere with the recovery process. Chronic lung and kidney diseases were also excluded from the study, as they can significantly affect overall health and treatment outcomes. Excluding such diseases helps to ensure the purity of the experiment and the accuracy of the data obtained, which is critical to the scientific validity of the study results.

Patients visited the physiotherapy clinic "Medical Centre for Rehabilitation of Movement" in Kyiv, where they underwent regular therapeutic procedures for six months. The study was conducted from January to June 2023. As part of the study, patients underwent weekly treatments that included both myofascial release and accompanying therapeutic massage techniques and exercises to improve joint mobility. Throughout the study period, patients were monitored by a team of specialists, including physiotherapists, massage therapists and doctors. All therapy sessions were conducted in specially equipped rooms of the clinic using modern rehabilitation equipment. Each patient underwent an individually tailored course of treatment, which took into account the condition of their joints, pain level and general physical condition. Data were collected using self-report questionnaires, clinical assessments of mobility and functional capacity, and pain scores on a visual analogue scale. Patients also received individualised recommendations for continuing exercises at home to maintain the effects of therapy. At the end of each month, an interim analysis of the treatment results was carried out, which allowed to adjust the therapeutic plan if necessary. The study adhered to ethical standards, ensuring compliance with the principles of human participation [25].

Physical therapy included included a set of relaxation and stretching exercises (the set of therapeutic exercises lasted from 30 to 40 minutes) for the masticatory and temporal muscles, which were performed under the direct supervision of a qualified physiotherapist. Patients performed these exercises daily, paying attention to the correct technique and intensity level. Each session also included elements of breathing exercises that helped to reduce overall muscle tension, which further enhanced the effect of the main exercises. The main goal of this complex was not only to restore the normal range of motion of the temporomandibular joint, but also to reduce pain during jaw movements and reduce the overall intensity of pain during exertion. Massage procedures were aimed at eliminating trigger points in the muscles that suffered from myofascial pain syndrome. Various techniques were used, including myofascial release, which ensured gradual relaxation of the tissues, and acupressure, which targeted specific painful areas to alleviate discomfort. Each massage session lasted from 45 to 60 minutes and was held three times a week throughout the course of therapy. The massage techniques were selected depending on the level of muscle tension and the location of trigger points, which allowed for a gradual reduction in pain and improvement of the overall mobility of the face and neck muscles.

The effectiveness of myofascial pain syndrome therapy was assessed based on three main criteria: pain level, range of motion in the temporomandibular joint and the amount of mouth opening. The effectiveness was evaluated at three stages: at the beginning of treatment to determine the baseline, immediately after completion of the course of treatment procedures, which allowed assessing the immediate therapeutic effect, and after six months to determine the duration and stability of the results. To measure the intensity of pain, patients were asked to use a visual analogue scale, where they independently determined the level of pain both at rest and during physical activity, on a 10-point scale, where 0 means no pain and 10 indicates the maximum pain intensity that a patient can feel. This approach allowed not only to quantify the pain but also to compare its changes at different stages of treatment, which provided clear data on the dynamics of pain. The amplitude of movements in the temporomandibular joint was assessed using a special method of gingliometry. This is an instrumental measurement that allowed objectively determining the mobility of the joints and recording the changes that occurred during the course of treatment. After completion of the therapy, the amount of mouth opening was reassessed, which was an important indicator of functional recovery.

Results and Discussion

Massage therapy had a comprehensive positive impact on the physical condition of patients, improving blood circulation in the muscles and promoting faster tissue healing and recovery. Increased blood circulation reduced swelling and improved metabolism in muscle tissue, which had a positive effect on the overall health of patients. The data show that the intensity of pain at rest decreased from the initial value of 7.8 ± 0.9 to 2.3 ± 0.7 after treatment, while during exercise the pain score decreased from 8.1 ± 1.1 to 3.1 ± 0.8 ($p < 0.01$), which confirms a statistically significant reduction in pain.

The range of motion in the temporomandibular joint increased from 27 ± 3 mm to 39 ± 4 mm ($p < 0.01$), indicating an improvement in the functional state of the affected areas (Table 2). Positive results after six months were maintained in 85% of patients, indicating the long-term effectiveness of the therapy. The high intensity of pain at the beginning of the study can be explained by the prolonged chronic course of myofascial pain syndrome and a significant load on the affected joints. The key indicators presented in the table, such as pain intensity at rest and during activity, joint range of motion, and the percentage of patients with positive results after six months, emphasise the importance of massage therapy in improving the overall functional status of patients. The results obtained are not only statistically but also clinically significant, as they provide real pain relief and improve the quality of life of patients with myofascial pain syndrome.

Table 2. Dynamics of changes in pain scores, amplitude of movements in the temporomandibular joint and percentage of patients with positive results after treatment of myofascial pain syndrome

Indicator	Before treatment (M ± SD)	After treatment (M ± SD)	p-value
Pain intensity at rest	7.8 ± 0.9	2.3 ± 0.7	<0.01
Intensity of pain during activity	8.1 ± 1.1	3.1 ± 0.8	<0.01
Range of motion in the temporomandibular joint (mm)	27 ± 3	39 ± 4	<0.01
Percentage of patients with positive results after 6 months	-	85%	-

Notes: M ± SD – mean value and standard deviation
Source: created by the authors

The effectiveness of chronic myofascial pain syndrome therapy was evaluated at three main stages, which allowed for a comprehensive analysis of changes in the symptoms and functional status of patients. At the initial stage of the study, before the start of treatment procedures, the average resting pain value measured by a visual analogue scale was 7 points, which indicated a significant intensity of pain experienced by patients in everyday life. The reasons for this high pain intensity could include chronic muscle tension, inflammation, and psychosomatic factors that increase the sensation of pain. During physical activity, the pain increased to 8 points, indicating significant discomfort and limitation in movement, making it difficult to perform normal tasks such as chewing and talking. It was also important to limit the amplitude of movements of the temporomandibular joint, the maximum amplitude of mouth opening did not exceed 30 mm, which significantly interfered with jaw function.

After the course of treatment, which included exercise therapy and massage, the average resting pain score decreased to 3 ($p < 0.01$), indicating significant symptom relief and a significant reduction in pain intensity. Pain during physical activity decreased to 4 points, indicating an overall improvement in the physical condition of patients and a decrease in discomfort during physical activity. These results confirm the importance of pain relief in improving patients' functionality and overall well-being. The range of motion in the temporomandibular joint also improved significantly: the maximum amplitude of mouth opening increased to 40 mm, which indicates a significant restoration of jaw mobility and improvement in its functionality. These changes indicate that muscle tension has decreased, and the symptoms associated with myofascial pain syndrome have become less pronounced. Six months after completion of the treatment, 80% of patients had a sustained positive result: their pain at rest either disappeared completely or decreased to at least two points, which is a significant improvement compared to the baseline.

Temporomandibular joint dysfunction was significantly reduced in 85% of patients, who reported a restoration of normal jaw function and reduced discomfort. In addition to improved joint function, 75% of patients also reported an improvement in their overall posture, indicating an overall positive impact of the treatment on their physical condition. For better clarity, the results of the therapy effectiveness assessment are presented in Table 2, which shows key quantitative indicators such as pain intensity at rest and during activity (pre- and post-treatment), temporomandibular joint range of motion, and the percentage of patients with positive results after six months. Table 2 provides a clear picture of the improvement that occurred after treatment and emphasises the importance of these indicators for the overall functional status of patients. The rationale for the significance of the results, including statistical analysis and its clinical relevance, confirms the effectiveness of the chosen approach to the treatment of chronic myofascial syndrome. Thus, the combined treatment met expectations, demonstrated a good long-term result and significantly improved the quality of life of patients.

These results indicate a significant improvement in symptoms and discomfort. The range of motion of the temporomandibular joint improved to 40 mm, indicating restoration of jaw mobility. Six months after treatment, 80% of patients had a stable effect. The pain disappeared or decreased to a level of at least two points, and the dysfunction of the temporomandibular joint significantly decreased. This confirms the effectiveness of the chosen approach to the treatment of chronic myofascial pain syndrome, as the combined treatment met expectations, demonstrated good long-term results and improved the quality of life of patients.

In the process of treating chronic myofascial syndrome, it is important to evaluate not only the effectiveness of the methods used, but also to compare them with the results of other studies. This allows to identify general trends, confirm or refute the findings, and adapt treatment methods according to the available data. This paper demonstrates the positive impact of physiotherapy and massage on pain relief and improvement of the functional status of patients with chronic myofascial syndrome. However, for a comprehensive understanding of the effectiveness of these methods, it is important to consider similar studies to help understand how the results compare with other scientific data in this area. For example, the study by G. Affaitati *et al.* [16] demonstrated the significant effectiveness of physiotherapy and massage in reducing pain in patients with chronic myofascial syndrome. The results of their work indicate a decrease in pain intensity, which confirms the results of this study. They noted that the average score on the visual analogue scale decreased, although this value is lower than the results of the present study, which indicates the effectiveness of the selected treatments, but calls into question the comparability of the study conditions.

Another study conducted by P. Valiente-Castrillo *et al.* [6] also confirms the positive effect of physiotherapy and massage on the functional state of joints in patients with chronic myofascial syndrome. In their study, a significant reduction in pain and improvement in range of motion were observed. These results emphasise that a comprehensive treatment that includes exercise and massage can significantly improve joint function and overall well-being.

However, not all studies support the idea that physiotherapy is the only effective method. L. Manchikanti *et al.* [17] noted that physiotherapy in combination with pharmacotherapy is more effective in reducing pain. The results showed a reduction in pain, which indicates the additional benefit of including medication in the overall treatment plan. This fact may explain why some patients in this study did not achieve the desired outcome when treatment was based on physiotherapy alone. Important conclusions are also contained in the study by L. Manchikanti *et al.* [18], which focuses on the treatment of vertebrogenic pain. The study showed that combined treatment, which includes physiotherapy, manual therapy and massage, leads to a significant improvement in the condition of patients. This reinforces the need for a comprehensive approach to treatment that includes a variety of methods to achieve maximum effect.

This would maximise the effectiveness of treatment, improve outcomes and sustain the improvements achieved; it is important to continue research in this area to improve the treatment and quality of life of patients with chronic myofascial syndrome, which is an important aspect of modern medicine and rehabilitation.

Conclusions

A study of 20 patients with chronic myofascial pain syndrome showed that the myofascial release technique is highly effective in treating this condition. Myofascial release is a manual therapy aimed at directly affecting specific tissues, which was used to relax and smooth out stiff areas of fascia. During the treatment, the specialist could focus on the patient's tactile sensations and symptoms to more accurately identify the affected area. Myofascial release techniques differed in pressure, duration of sessions, movements and tension intensity, depending on the characteristics of each patient. This variability affected the outcome of the treatment, but in general, the treatment was successful.

On average, patients reported a significant reduction in pain intensity and an improvement in their overall physical condition after completing the treatment. Palpation and symptom assessment allowed the specialist to identify areas of inflammation and fibrosis that required therapy. The main goal of the myofascial release was to reduce muscle tension and pain, which was achieved by relaxing and smoothing out the stiff areas of the body's soft tissues. The results demonstrated significant symptom relief in all patients, confirming its effectiveness as a therapeutic method. An assessment of the duration of effectiveness after six months showed that 80% of patients maintained a good therapeutic result, and the intensity of pain decreased to a minimum level or disappeared altogether. This indicates long-term positive changes that confirm the effectiveness of myofascial release in the treatment of chronic myofascial pain syndromes. In general, the results of this study confirm that myofascial release is a reliable and effective treatment method, especially when applied correctly and in a timely manner. Prospects for further research may include studying the long-term results of myofascial release in combination with other therapeutic methods, as well as analysing the possibility of personalising treatment approaches for different groups of patients.

Acknowledgements

None.

Conflict of Interest

None.

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Ефективність лікування міофасціального больового синдрому за допомогою лікувальної фізкультури та масажу

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Анотація. Метою дослідження було визначення ефективності методів фізичної терапії та масажу для зниження больових симптомів у пацієнтів із хронічним міофасціальним больовим синдромом, тривалість якого становила не менше 12 тижнів. Основний акцент було зроблено на усунення тригерних точок болю та довготривале розслаблення м'язів навколо уражених ділянок. У дослідженні брали участь 20 пацієнтів, які скаржилися на хронічний біль, що супроводжувався обмеженням рухливості у суглобах, зокрема скронево-нижньощелепного, дискомфортом під час жування та епізодичними нападами гострого болю. Пацієнтам був призначений 12-тижневий курс лікування, який складався з щотижневих сеансів фізичної терапії та масажу, спрямованих на зменшення напруженості м'язів і покращення рухливості суглобів. Протягом курсу проводилося три сеанси на тиждень, кожен із яких тривав від 45 до 60 хвилин. Фізична терапія включала вправи, спрямовані на зміцнення м'язів та розтягнення міофасціальних тканин для полегшення рухів. Масажні техніки включали глибокий масаж тканин, міофасціальне розслаблення та мануальну терапію, орієнтовані на розслаблення напружених м'язів навколо скронево-нижньощелепного суглоба та зменшення больових тригерних точок. Результати дослідження продемонстрували значне зменшення інтенсивності болю після лікування: середній показник болю за візуально-аналоговою шкалою знизився з $7,8 \pm 0,9$ до $2,3 \pm 0,7$ у стані спокою та з $8,1 \pm 1,1$ до $3,1 \pm 0,8$ при фізичному навантаженні ($p < 0,01$). Крім того, амплітуда відкриття рота зросла з 27 ± 3 мм до 39 ± 4 мм ($p < 0,01$), що свідчить про покращення рухливості суглоба, а показник функціональної активності за шкалою Хелкімо покращився з 3,5 до 1,2 ($p < 0,01$), вказуючи на загальне зменшення симптоматики та поліпшення функціональної здатності суглобів. Через шість місяців після завершення курсу лікування у 85 % пацієнтів зберігався стабільний терапевтичний ефект: у 12 % біль повністю зник, а у 5 % він залишався на низькому рівні. Також було зафіксовано відновлення нормальної функції м'язів у 80 % пацієнтів, покращення гнучкості суглобів та корекцію постуральної позиції у 75 % випадків

Ключові слова: тригерні точки; скронево-нижньощелепний суглоб; м'язове розслаблення; фізичні навантаження; відновлення рухливості у суглобах

International Journal of Medicine and Medical Research

Journal homepage: https://ijmr.com.ua/
Volume 10, No. 2. 2024

UDC 577.2:616-085(048.6)
DOI: 10.61751/ijmmr/2.2024.81

Article's History:
Received: 29.06.2024; Revised: 26.09.2024; Accepted: 26.11.2024

Achievements in molecular biology: CRISPR/Cas9 in gene therapy (literature review)

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Abstract. CRISPR/Cas9 provides high precision and efficiency in altering genetic sequences, therefore significant in gene therapy. Mutations can be corrected, pathological genes can be removed, and the functional ability of proteins can be restored by CRISPR/Cas9. The review aimed to analyse the possibilities of using gene editing technology to treat cancer, genetic, and infectious diseases in the available studies published in 2013-2024. This publication analysed the use of CRISPR/Cas9 in experimental models for treating Duchenne muscular dystrophy, cystic fibrosis, sickle cell anaemia, acquired immunodeficiency syndrome and cancer. In Duchenne muscular dystrophy, genome editing helps increase the level of utrophin, which compensates for the dystrophin deficiency. In cystic fibrosis, CRISPR/Cas9 is used to correct defects in the CFTR gene, and in human immunodeficiency virus therapy, it is used to remove proviral deoxyribonucleic acid from infected cells. However, the technology also has certain limitations, such as the risk of off-target changes in the genome and the difficulty of delivering CRISPR/Cas9 into cells. Therefore, in 2024, CRISPR/Cas9 requires further improvement in clinical practice. CRISPR/Cas9 has great potential to change the approach to the treatment of incurable diseases in the future. The practical value of the study conducted by the authors is the presentation of a ready-made summary of the CRISPR/Cas9 system and a thorough analysis of the results of its use in the treatment of various diseases, which can be used to assess what prospects this technology has for future use

Keywords: genome editing technologies; adeno-associated viruses; Duchenne muscular dystrophy; cystic fibrosis; haemoglobinopathies; acquired immunodeficiency syndrome; cancer

Introduction

As of 2024, more than 9,000 diseases associated with changes in the human genome were identified, and effective treatments were found for less than 800 diseases [1]. Therefore, scientists are increasingly considering gene therapy as a priority treatment. This increased interest is supported by numerous studies and searches for technologies that could replace current, sometimes ineffective treatments. Over the past thirty years (1990-2020), many discoveries in the fields of molecular biology and genetics were made, for instance, "genetic scissors", polymerase chain reaction, and others. Well-known genome editing methods, such as mononucleases (MN) and zinc finger nucleases (ZFN), received considerable coverage. Despite the proven effectiveness of these methods, they often require careful and thorough planning when selecting genetic targets and may present various obstacles to accurate genome editing [2].

Suggest Citation:
Voroshchuk O, Yaremchuk O. Achievements in molecular biology: CRISPR/Cas9 in gene therapy (literature review). Int J Med Med Res. 2024;10(2):81–9. DOI: 10.61751/ijmmr/2.2024.81

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H. Yang *et al.* [3] highlighted the new immune system CRISPER/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats with Cas9-associated protein). The discovery of this technology is based on the study of the bacterial immune system, which uses CRISPR DNA sequences to fight viruses. Compared with conventional gene editing methods, this system, discovered in 2012, has greater potential due to its low cost, high accuracy, greater efficiency than MN/ZFN and multifunctionality in genome editing.

The mechanism of CRISPER/Cas9 functioning, also named "gene scissors", is based on the target gene being recognised by an artificially created RNA (sgRNA). This RNA then directs the Cas9 protein to break the DNA double strand, creating double-strand breaks (DSBs) in the corresponding region of the genome. Then, a natural process of repairing the damaged DNA occurs. In this process, targeted changes are made to the genome, including the removal of the mutated region or its replacement. H. Jiang *et al.* [4] reviewed how this unique technology demonstrated excellent and promising results in gene therapy and is used in the treatment of a wide variety of diseases, such as AIDS, cancer, Duchenne muscular dystrophy and many other genetic diseases. In addition, CRISPER/Cas9 is also used in agriculture to create genetically modified crops with increased resistance to parasites and weather conditions, in pharmaceuticals to develop therapeutic molecules based on the results of genome editing, and in other areas not related to medicine [5].

In 2020, the Nobel Prize in Chemistry was awarded to Emmanuelle Charpentier, who heads the Division of Pathogen Sciences at the Max Planck Society in Berlin, and Jennifer Doudna from the University of California, Berkeley, for the discovery of one of the most powerful tools of gene technology – CRISPR/Cas9, or the so-called "genetic scissors". The technology of "genetic scissors" has brought life sciences to a new stage of development and is of great benefit to humanity. Even though genome editing technologies have certain drawbacks and limitations, and methods of their delivery to cells need to be improved, this discovery will allow for greater efficiency in gene therapy [6].

Despite all of the aforementioned advantages of this unique gene therapy approach, there are also many challenges and problems with its application, one of which is the difficulty of delivering the system to target cells, as discussed by W. Yang *et al.* [7]. As of 2024, the use of adeno-associated viruses (viral vectors) has significant limitations and the likelihood of transferring limited genetic material of the system, and the risks of inducing an immune response in the patient have also been noted. At the same time, the liposomal delivery system is inefficient and can damage the cell.

Nevertheless, there is an insufficiency, and sometimes the absence, of a comprehensive and structured presentation of the CRISPR/Cas9 technology. Most studies present either the structure of the system, its advantages, or its role in the treatment of one group of diseases. Therefore, this study was necessary to summarise most of this material in a less detailed form and present it in a structured and grouped way, from the mechanism of functioning and the structural design of the system to preclinical results in the treatment of various diseases. This is also necessary to provide a comprehensive assessment of the CRISPR/Cas9 gene tool and evaluate its prospects. Therefore, the purpose of this paper was to analyse the structure, advantages, disadvantages and medical applications of the latest promising CRISPR/Cas 9 technology according to studies published in 2013-2024.

The methodology of the study was as follows: the literature review was conducted by analysing numerous digital publications found on such library websites as PubMed, Scopus and Web of Science Core Collection. The analysis of literature sources covered the period from 2013 to November 2024; the search was carried out using MeSH (Medical Subject Headings) terms, synonyms and keyword searches. During the primary analysis, 55 literature sources were selected and reviewed. After further systematisation of the information using the methods of analysis, synthesis and generalisation, only 35 sources remained. Exclusion criteria: publications that did not meet the purpose of this study and the absence of significant results in the reviewed papers.

CRISPR/Cas9 Bacterial Immune System

CRISPR/Cas9 is a system that is a bacterial defence mechanism against phage infection [8, 9]. Based on the organisation of the effector protein, the CRISPR/Cas system is classified into two different classes. Class 1 CRISPR/Cas systems use multi-protein effector complexes, while class 2 CRISPR/Cas systems use single-protein effectors. These two systems use short DNA sequences (spacers) to guide the Cas proteins. The conserved sequence to facilitate targeting is called a protospacer flanking sequence (PFS) for RNA-targeted Cas proteins or a protospacer adjacent motif (PAM) for DNA-targeted Cas proteins [10]. The CRISPR/Cas9 system of *Streptococcus pyogenes* consists of Cas9 (CRISPR-associated protein 9) (Fig. 1), an endonuclease that causes double-stranded DNA breaks, which can be used to modify of the genome with a single guide RNA (sgRNA), which provides specificity [11].

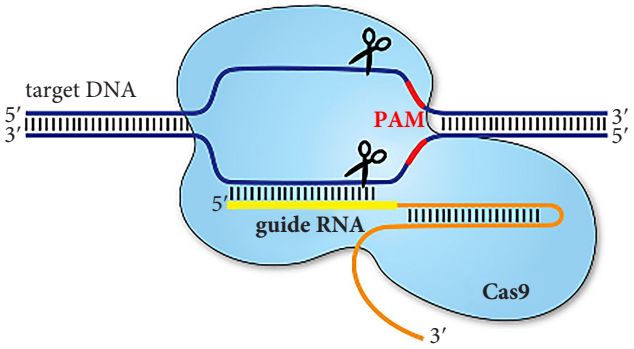

Figure 1. CRISPR/Cas9 technology
Source: M. Redman *et al.* [12]

WClustered Regularly Interspaced Palindromic Repeats (CRISPRs) are sequences in the bacterial genome

that, together with CRISPR-associated proteins (Cas), protect against viruses. One of these proteins is the Cas9 endonuclease, which cuts two DNA strands. Cas9 is guided to its target by a small-group RNA (sgRNA). To make a cut, a certain DNA sequence (from 2-5 nucleotides) must be located at the 3'-end of the sgRNA – known as RAM. DNA repair after the cut occurs in 2 ways: either by non-homologous end joining, which often leads to random DNA insertions/deletions, or by homologous repair. The latter uses a homologous DNA fragment as a template, allowing for precise editing. This pathway involves the delivery of a homologous DNA site with the desired modification (sequence change) using Cas9 protein and sgRNA, which ensures changes are made with an accuracy of one base pair [13].

CRISPRs protect against virus invasion in combination with a series of CRISPR-associated (Cas) proteins. Cas9, one of the associated proteins, is an endonuclease that cuts both strands of DNA. Cas9 is guided to its target by a stretch of RNA. This can be synthesised as a single strand called synthetic single-guided RNA (sgRNA); the stretch of RNA that binds to genomic DNA is 18-20 nucleotides. To cut, a specific DNA sequence of 2 to 5 nucleotides (the exact sequence depends on the bacterium that produces Cas9) must be located at the 3'-end of the guide RNA: this is called a protospacer adjacent motif (PAM). Repair after a DNA cut can occur in two ways: non-homologous end joining, which usually results in random DNA insertion/deletion, or homology-directed repair when a homologous DNA fragment is used as a repair matrix. It is the latter that allows for precise genome editing: a homologous DNA site with the required sequence change can be delivered using the Cas9 nuclease and sgRNA, theoretically allowing for changes as precise as a single base pair [13].

Bacteria develop this system by capturing DNA sequences and using it as a memory to identify themselves as an enemy and destroy it when they attack in the future. The great advantage of the CRISPR-Cas9 system is that it is not based on protein engineering (as in the case of ZFN) but on base pairing between sgRNA and DNA. CRISPR/Cas9 is easy to use as only a small new RNA fragment needs to be synthesised to target a new locus, which prevents the lengthy construction and cloning of complete protein domains, as in ZFN nuclease-based approaches. The synthesis of small fragments also allows for multi-complex applications, which allows for the targeting of multiple loci and the generation of chromosomal changes (deletions and translocations) [10, 11].

A bacterial immune mechanism for protection against bacteriophage infection and plasmid transfer in nature is the CRISPR/Cas9 system, which consists of the Cas9 endonuclease and a guide RNA (sgRNA) that provides specific recognition of the target DNA sequence. The Cas9 protein creates a double-stranded DNA break in the region complementary to the sgRNA in the presence of a protospacer adjacent motif (PAM). This technology allows not only for high-fidelity genome editing but also for modelling complex genomic changes, which makes it much easier to use than ZFN. The ease of use of CRISPR/Cas9, means that only sgRNA is needed to synthesise a precise target, therefore substantial for efficient genome editing and targeting multiple loci simultaneously.

Advantages of CRISPR/Cas9 in Genetic Engineering and Mechanisms of CRISPR/Cas9 Genome Editing

CRISPR/Cas9 is a revolution in the world of genetic research due to its versatility, accuracy and ease of use. Its success is based on the ability to precisely modify the genome by inducing double-strand breaks (DSBs) in DNA at predefined locations. This approach has opened new horizons for studying genetic functions, developing therapeutics, and creating organisms with specified characteristics [9, 14]. Unlike previous technologies, such as ZFN and TALEN, which required complex protein design for each new genetic locus, CRISPR/Cas9 uses a guide RNA (sgRNA). This RNA can be easily programmed to target any DNA sequence. As a result, the development of CRISPR tools requires less time and resources, which greatly simplifies the preparation for experimental studies [13, 14].

CRISPR/Cas9 provides a high level of editing accuracy. Efficiency is achieved using the Cas9 protein, which cuts DNA, and guides RNA, which provides specific targeting. Once a break is created, the cell triggers repair mechanisms such as nonhomologous end joining (NHEJ) or homologous directional repair (HDR). NHEJ usually causes small insertions or deletions that can shift the reading frame and cause gene knockout. HDR, in turn, can be used to insert specific sequences or make point mutations using donor DNA as a matrix [11, 13].

CRISPR/Cas9 can edit multiple genes simultaneously in a single experiment, which significantly accelerates the study of complex genetic networks. This creates opportunities for research into polygenic diseases caused by the interaction of many genes. In addition, CRISPR/Cas9 adaptations, such as CRISPRa (activation) and CRISPRi (inhibition), allow for changes in gene expression without editing their sequences. This is particularly useful for functional studies when it is important to assess the impact of changes in expression levels on cellular processes [15-16]. Compared to ZFN and TALEN, the development of CRISPR tools is much faster and cheaper. As of 2024, there are numerous ready-made sgRNAs for different genomes, which simplifies the preparation for experiments. Laboratories around the world can easily integrate CRISPR into their research, which contributes to its spread [9, 15].

CRISPR/Cas9 technology has enormous potential in medicine. It is being considered for the treatment of genetic diseases, cancer, infectious diseases, etc. The uniqueness of this technology is determined by its ability to create stable genetic modifications that do not disappear over time, contrary to the temporary changes typical of RNAi [10, 14]. CRISPR variants, such as base editing, can alter individual nucleotides without creating DNA breaks. Prime editing is another innovative approach that offers the ability to edit

DNA with high precision. These modernisations expand the range of capabilities of the technology [9].

CRISPR/Cas9 is not simply a genetic editing technique, but a system that is changing approaches to genome research and disease treatment. Its advantages in accuracy, versatility, speed and accessibility render this technology indispensable in modern and future biology and medicine [13]. The CRISPR/Cas9 genome editing mechanism is divided into three stages: the first is recognition, the second is cleavage, and the third is repair. The constructed sgRNA directs Cas9 and recognises the target sequence in the gene of interest using a complementary base pair of the 5'crRNA. The Cas9 protein is inactive in the absence of sgRNA. The Cas9 nuclease produces double-stranded breaks (DSBs) at 3 bases upstream of the PAM. The PAM sequence is a short (2-5 base pairs) conserved DNA sequence downstream of the cut site [16].

The Cas9 protein is a nuclease that is most used in genome editing technology, recognising the PAM sequence at 5'-NGG-3' (N can be any nucleotide base). When Cas9 finds a target site with a matching PAM, it triggers local DNA melting to form an RNA-DNA complex, but the mechanism of how the Cas9 enzyme melts the target DNA sequence is not yet clearly understood. The Cas9 protein is then activated to cleave the DNA. The HNH domain cleaves the complementary strand, while the RuvC domain cleaves the non-complementary strand of the target DNA to produce predominantly blunt-ended DSBs [17].

CRISPR/Cas9 is one of the most revolutionary technologies in the field of genetic engineering that combines versatility, precision and ease of use. Thanks to guide RNA (sgRNA) and the Cas9 endonuclease, this system can accurately target specific DNA sites, causing double strand breaks that stimulate the cell's natural repair mechanisms. This creates great prospects for creating gene knockouts, and point mutations, editing single nucleotides, and studying complex genetic networks. The advantages of CRISPR/Cas9, such as low cost, high efficiency and accessibility, render it an indispensable technology in molecular biology and medicine.

The Use of CRISPR/Cas-9 in the Treatment of Genetic Diseases

One of the areas of application of CRISPR/Cas9 is its potential use for the treatment of diseases caused by single gene mutations, certain infectious diseases, various types of cancer, etc. This approach was tested only in preclinical models, although expectations are that it can be applied in clinical practice. J. Wang *et al.* [18] investigated the impact of CRISPR/Cas9 on Burkitt's lymphoma. This is a cancer of the lymphatic system, in particular B lymphocytes, that develops in the germinal centre of the lymph node, caused by mutations in the cMyc gene. J. Wang and team used a CRISPR/Cas9 system against Epstein-Barr virus (EBV) and found a reduction in the effect of the virus on tumour proliferation. In addition, F.A. Khan *et al.* [19] determined that when editing the tumour suppressor gene *Trp53* in *Arf-/-Eμ -Myc* lymphomas, overexpression of the Myc gene is responsible for several types of lymphoma cancer. *Mll3* is another substantial tumour suppressor gene disruption by CRISPR *ex vivo* in acute myeloid leukaemia [20]. The use of the TALENs genome editing tool in a girl with leukaemia by C. Chen *et al.* [21] created grounds for more effective use of CRISPR in the clinic.

Another serious genetic disease is Duchenne muscular dystrophy (DMD). According to the research of Canadian scientists D. Wojtal *et al.* [22], CRISPR/Cas9 exhibits a therapeutic effect in Duchenne muscular dystrophy (DMD) caused by mutations in the dystrophin gene, thanks to its genome editing mechanism by increasing the expression of utrophin (UTRN). UTRN is a protein that is functionally similar to dystrophin and partially compensates for its loss. In these studies, CRISPR/Cas9 was used to activate the A and B promoters of the UTRN gene to increase the synthesis of utrophin. The increase in the level of urotrophin (1.7-6.9 times compared to baseline values) would allow compensation for the functions of dystrophin, in particular: restoring the amount of β-dystroglycan, a protein that is critical for the stability of muscle cell sarcolemma. This approach allows not to correct the dystrophin gene directly, but to compensate for its absence, reducing the progression of DMD [21, 22].

For the treatment of DMD, CRISPR/Cas9 can also be used to remove mutations in the dystrophin gene that cause a reading frame shift. Removing the defective exons can be used to restore the reading frame, resulting in the synthesis of a functional, albeit reduced, dystrophin protein. This approach resembles the phenotype of Becker muscular dystrophy, which is a less severe form of the disease. M. Tabebordbar *et al.* [23] used the delivery of CRISPR/Cas9 endonucleases using adeno-associated virus (AAV) to restore dystrophin expression in mice with experimental DMD by deleting the exon containing the original mutation. As a result, a shorter but functional protein is synthesised. Mice treated with CRISPR/Cas9 for DMD partially recovered functional muscle dysfunction. This study demonstrated that the dystrophin gene was edited in muscle stem cells that replenish mature muscle tissue. This is necessary to ensure that any therapeutic effects of CRISPR/Cas9 do not disappear over time.

Multiple studies by C.E. Nelson *et al.* [24] provide substantial evidence supporting the effectiveness of *in vivo* genome editing to correct destructive mutations in DMD in the corresponding dystrophic mouse model. Programmable CRISPR complexes can be delivered locally and systemically to terminally differentiated skeletal muscle fibres and cardiomyocytes, as well as to muscle satellite cells in newborn and adult mice, where they mediate targeted gene modification, restore dystrophin protein expression, and partially recover functional deficiencies in dystrophic muscles.

G. Schwank *et al.* [25] were the first to use CRISPR/Cas9 to study the treatment of cystic fibrosis (CF). Cystic fibrosis is caused by mutations in the CFTR gene, particularly the most common mutation F508 del, which leads to the formation of a non-functional CFTR protein. This

disrupts the transport of chlorine ions across cell membranes, causing the accumulation of thick mucus in the respiratory tract, digestive tract and other organs. In the study, adult intestinal stem cells were first taken from two patients with cystic fibrosis, and then intestinal organoids were grown from these cells to mimic the structure and function of real tissue. The CRISPR/Cas9 system was then used to correct F508 del. To fix the mutation, the researchers provided the cell with a donor DNA template that contained the correct sequence of the CFTR gene. The cell uses this template to repair the DNA break through the HDR mechanism, which replaces the F508 del mutation with a normal sequence. Adeno-associated viruses (AAVs) were used to deliver CRISPR/Cas9 components into cells. They effectively delivered Cas9 and sgRNA to organoid cells. As a result, they successfully corrected the most common mutation causing cystic fibrosis in intestinal organoids. The authors demonstrated that once the mutation was corrected, the function of the CF transmembrane conductor receptor (CFTR) was restored.

M.C. Canver *et al.* [26] conducted a study that demonstrated the possibility of using the CRISPR/Cas9 system to treat such serious diseases as sickle cell disease (SCD) and β-thalassaemia. Haemoglobinopathies are caused by mutations in the β-globin (HBB) gene, which causes disorders of haemoglobin (HbA) synthesis. Traditional treatment approaches include replacement therapy or bone marrow transplantation, but they are limited and ineffective. M.C. Canver *et al.* [26] also explored an alternative approach – reactivation of fetal haemoglobin (HbF) synthesis. This type of haemoglobin is expressed in the embryonic and perinatal periods and compensates for the functions of adult haemoglobin. With the transition from HbF to HbA in the postnatal period, HbF activity is inhibited by the protein BCL11A. The identification of the role of this protein as a key suppressor of fetal haemoglobin has become the basis for the development of therapeutic strategies [12]. M.C. Canver and the team used the CRISPR/Cas9 system to target the BCL11A enhancer, which is a regulatory element in the genome that controls the expression of this protein. In mice with a model of sickle cell anaemia, scientists used CRISPR/Cas9 to edit the BCL11A enhancer, substantially increasing HbF levels. The high level of fetal haemoglobin significantly reduced the symptoms of the disease, improved haematopoiesis and restored red blood cell function. In human cells, the researchers used primary erythroblasts, the cells involved in the formation of red blood cells (the so-called precursors). Editing the BCL11A enhancer using CRISPR/Cas9 led to the activation of HbF, which functionally replaced HbA. This proved that the technique can be effective for human cells as well. The results of their research are extremely promising. Reactivating HbF is a more realistic approach than introducing a full-length β-globin gene because of the difficulty of delivering such large genes into cells.

The use of the unique CRISPR/Cas9 system has yielded positive results and great potential in the future for the treatment of various genetic diseases and more. This technology can be used for precise changes in the genome, which creates new prospects for the treatment of diseases such as Duchenne muscular dystrophy, cystic fibrosis, sickle cell anaemia and various types of cancer. CRISPR/Cas9 can also be used to correct mutations, activate genes and change protein expression.

CRISPR/Cas9 in the Treatment of AIDS

Another potential clinical application of CRISPR/Cas9 is the treatment of complex infectious diseases such as HIV. Acquired immunodeficiency syndrome (AIDS) is one of the most serious infectious diseases in the world [27-28]. As of 2024, 41 years have passed since the discovery of human immunodeficiency virus type 1 (HIV), but there is still no vaccine against HIV infection. Combination antiretroviral therapy (cART) has been used for the past ten years to keep HIV replication under control. Although cART can inhibit the replication of the virus, the virus can persist in some cells. As a result, patients must take antiretroviral drugs for life [29].

Long-term use of antiretroviral drugs causes side effects. Thus, the search for alternative strategies to combat HIV is highly relevant. A prominent field is gene therapy, which is needed to deliver antiviral gene reagents that would prevent the virus from replicating in cells that may be infected with HIV. Technologies for modifying gene expression through RNA interference (RNAi) or genome editing based on clustered regularly interspaced short palindromic repeats (CRISPR) offer new opportunities for HIV inhibition.

Firstly, it is necessary to mention the fact that complex antiretroviral therapy does not remove the viral DNA integrated into the cell genome (although it actively suppresses HIV replication). This viral DNA creates latent reservoirs that remain a source of new infection after ART is stopped. E. Herrera-Carrillo *et al.* [27] examined the CRISPER/Cas9 technology in HIV therapy. The mechanism of action of CRISPR-Cas9 against HIV in their studies involves the use of a guide RNA (sgRNA) that provides precise targeting of the Cas9 protein to the proviral DNA integrated into the cell genome. Cas9 creates double-stranded breaks in the viral DNA, causing damage. Then, breaks in important regions of the viral genome, such as LTR (long terminal repeats) regulatory regions or genes encoding viral proteins (gag, pol, env), block the activation of the proviral DNA or cause its inactivation. This approach effectively inhibits virus replication and prevents its spread to new cells.

The results of *in vitro* experiments [29, 30] demonstrate a significant reduction in the amount of proviral DNA in infected cells, which indicates the effectiveness of CRISPR-Cas9 in fighting both active and latent forms of the virus. Even in the case of incomplete deletion of proviral DNA, editing causes mutations that make the virus replication defective. The use of multiple sgRNAs to simultaneously target multiple regions of the HIV genome reduces the risk of mutations that allow the virus to avoid CRISPR. Despite the encouraging results, several challenges need to

be overcome for the successful use of CRISPR in HIV treatment. These include the high mutability of the virus, which makes targeting difficult, the possibility of off-target effects that can damage the host cell genome, and the difficulty of delivering CRISPR-Cas9 to all infected cells in the body. Delivery is one of the key technical challenges, and methods based on viral vectors (e.g., adeno-associated viruses, AAV) or nanoparticles are being developed for this purpose.

E. Herrera-Carrillo *et al.* [27] argue that CRISPR/Cas9 has significant potential to become a revolutionary approach to HIV treatment, as it allows eliminating pro-viral DNA and blocking viral replication. At the same time, further research is needed to implement this technology in practice to optimise the safety and efficacy of the method, as well as to develop effective approaches to delivering CRISPR into the body.

CRISPR/Cas9 technology has proved to be effective in the treatment of AIDS and opens new prospects for treating this incurable disease. Thanks to its ability to precisely edit the genome, this system can not only block virus replication but also effectively destroy the pro-viral DNA that is integrated inside cells. Despite the encouraging results of *in vitro* studies, there are certain obstacles to the clinical application of CRISPR/Cas9 in the treatment of HIV and AIDS, such as the high mutability of the virus, problems with the delivery of the CRISPR/Cas9 system to the affected target cells, and others.

CRISPR/Cas9 as a Method of Fighting Cancer

Cancer is the primary cause of death due to genetic mutations in the world. Oncogenes, tumour suppressor genes and DNA repair genes are substantial in the mechanisms of carcinogenesis. By using CRISPER gene editing, research biologists found a way to treat cancer with immunotherapy. Gene editing is possible not only in cell cultures and mammals but also in humans. In addition, CRISPR/Cas9 has been used to create an oncolytic virus that is used to treat cancer due to its ability to specifically infect and lysate cancer cells, ideally preserving normal cells for cancer treatment [31-32].

According to research conducted by L. Wang *et al.* [31], CRISPR/Cas9 technologies achieved success in cancer immunotherapy by providing a precision genetic editing technology. This new method can be used to make precise changes to the genome and correct or remove mutations that cause cancer. The use of CRISPR/Cas9 can improve the ability of the immune system to detect and destroy cancer cells, which is progress in cancer treatment. The precision of CRISPR/Cas9 in genetic modification is beneficial for the treatment of T cells, which is an important aspect of cancer therapy. CRISPR/Cas9 improves the specificity and efficacy of chimeric antigen receptor (CAR) and T-cell receptor (TCR) agents that target tumour agents, thus enhancing the immune response against cancer. This approach inhibits the immune checkpoint inhibitors (PD-1/PD-L1) that cancers use to prevent them from being recognised by the immune system, thus allowing the immune system to effectively fight cancer [33].

In other studies, conducted by A. Saber *et al.* [32] in oncology, CRISPER/Cas9 demonstrated the ability to address tumour heterogeneity and drug resistance. Tumour heterogeneity, the diversity of tumour cells within cells in a single patient, hinders treatment by creating a pool of genetic variants that can lead to therapeutic resistance and ineffectiveness of treatment. Congenital and acquired drug resistance often leads to relapse and death [34]. CRISPR/Cas9 was used to explore therapeutic targets to create drugs that do not develop resistance. CRISPR/Cas9 is a method for improving existing cancer treatments and has all the prospects for successful application in the future in clinical oncology as well. T-cell therapy with chimeric antigen receptor (CAR), gene screening, and tumour modelling were improved, but the effects of genome editing remain poorly understood and may increase the risk of additional genetic mutations [35].

The CRISPR/Cas9 system created new opportunities in cancer therapy. By editing the genome, CRISPR/Cas9 can be used to modify immune cells to fight tumours more effectively, as well as to create oncolytic viruses that selectively destroy cancer cells. This technology can also overcome the problem of tumour heterogeneity and the development of drug resistance. Despite the significant results and great potential, CRISPR/Cas9 still requires further research to be studied in detail and approved for mainstream cancer treatment.

Conclusions

CRISPR/Cas9 technology has become a breakthrough in the field of genetic engineering, creating new opportunities for gene therapy by eliminating mutated genomic regions. The emergence of this innovative technology, based on the natural mechanism of bacterial defence against viruses, has become a vivid example of how basic research can change practical medicine. The CRISPR/Cas9 mechanism of action allows for genome editing with high precision, simplicity and efficiency, rendering this technology a unique treatment for genetics, cancer and many other diseases. According to the results of the analysed studies, in Duchenne muscular dystrophy, the use of CRISPR/Cas9 has increased the level of utrophin, which partially compensates for the loss of dystrophin, helping stabilise muscle tissue.

In cystic fibrosis, the F508 del mutation in the CFTR gene was successfully corrected in intestinal organoid cells of patients. The restored function of the CFTR protein improved ion transport in tissues. In haemoglobinopathies such as sickle cell anaemia and β-thalassaemia, CRISPR/Cas9 allowed reactivating foetal haemoglobin (HbF) by editing the BCL11A protein enhancer, which compensates for the β-globin deficiency. In HIV, CRISPR/Cas9 has effectively removed proviral DNA from the cell genome, reducing viral replication and inducing mutations that render HIV inactive.

At the same time, there are successful cases of this technology being used in cancer, where CRISPER/Cas9 is used to edit immune cells (T-lymphocytes) to increase their ability to detect and destroy tumour cells. In addition, CRISPR is capable of creating oncolytic viruses and modifying immune checkpoint inhibitors, which enhances

the effectiveness of immunotherapy. However, despite all the advantages, CRISPR/Cas9 requires further research, in particular, to study various possible side effects and reduce the risk of their impact on the body, improve the technology of CRISPR/Cas9 delivery into the cell, etc.

Acknowledgements

None.

Conflict of Interest

None.

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Досягнення молекулярної біології: CRISPR/Cas9 у генній терапії (огляд літератури)

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Анотація. CRISPR/Cas9 забезпечує високу точність і ефективність у зміні генетичних послідовностей, що робить її важливим інструментом у генній терапії. Завдяки CRISPR/Cas9 можна коригувати мутації, видаляти патологічні гени та відновлювати функціональну здатність протеїнів. Метою цього огляду було проаналізувати можливості використання технології редагування генів для лікування онкологічних, генетичних, інфекційних захворювань у наявних дослідженнях, які опубліковані в період 2013-2024 років. У даній публікації проаналізовано застосування CRISPR/Cas9 на експериментальних моделях у терапії м'язової дистрофії Дюшена, муковісцидозу, серповидно-клітинної анемії, синдрому набутого імунодифіциту та онкологічних захворювань. При м'язовій дистрофії Дюшена редагування геному сприяє підвищенню рівня утрофіну, що компенсує дефіцит дистрофіну. При муковісцидозі CRISPR/Cas9 використовується для корекції дефектів у гені CFTR, а при терапії вірусу імунодифіциту людини – для видалення провірусної дезоксирибонуклеїнової кислоти в інфікованих клітин. Однак технологія має і певні обмеження – ризик позацільових змін у геномі, складність доставки CRISPR/Cas9 у клітини. Отже, на 2024 рік залишається необхідність подальшого вдосконалення методів застосування CRISPR/Cas9 в клінічній практиці. CRISPR/Cas9 має великий потенціал в майбутньому змінити підхід до терапії невиліковних захворювань. Практичною цінністю проведеного авторами дослідження є подання готової короткої інформації про систему CRISPR/Cas9 та грунтовний аналіз результатів її застосування при терапії різних захворювань, що дозволяє оцінити, які перспективи має ця технологія у застосуванні в майбутньому

Ключові слова: технології редагування геному; аденоасоційовані віруси; м'язова дистрофія Дюшена; муковісцидоз; гемоглобінопатії; синдром набутого імунодифіциту людини; рак

INTERNATIONAL JOURNAL OF MEDICINE AND MEDICAL RESEARCH
Scientific-Practical Journal

Volume 10, No. 2
2024

Managing Editor:
T. Pyatkovskyy

Editing bibliographic lists:
T. Pyatkovskyy

Editing English-Language Texts:
K. Ubyikon

Desktop publishing:
O. Glinchenko

Signed to the print 26.11.2024
Format 60*84/8
Conventional Printed Sheet 10.5
Circulation 100 copies

Publisher: I. Horbachevsky Ternopil National Medical University
46001, 1 Maidan Voli, Ternopil, Ukraine
Tel.: +380 352 524492
E-mail: info@ijmr.com.ua
https://ijmr.com.ua/