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Volume 12, Issue 1 **January - June 2026**

EDITORIAL

First Cousin Marriages in Pakistan — Are We Suicidal?!

Professor Serajuddaula Syed

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First-cousin (consanguineous) marriages are very common in Pakistan. As per published data, the rate of such marriages is about 50 percent[1]. In reality, it is over 70 percent. An honest country-wide survey is required. Cousin marriages are highly prevalent in rural areas. During a short survey in rural Sindh, when respondents were told that children of both paternal and maternal uncles and aunts are your first cousins and should not be married to, the answer was amazing. Most of them said, "then who is left?". It is said that marrying outside the close family may lead to being labeled "Kari (honour killing)"[3].

It is a fact that genetic diseases are much more common in the children born in close communities and first-cousin marriages[1-2]. One in four children will have the disease if both parents are carriers of a particular disease[1,4]. The chances of becoming a carrier are higher in cousins[1]. Just to name a few genetic diseases -- thalassemia, muscular dystrophy, neuropathies, cystic fibrosis, osteogenesis imperfecta, and mental disabilities (special children) are common in Pakistan[1-2]. Clinical observations by ENT surgeons performing cochlear implantation suggest that most children presenting with congenital deafness are born in first-cousin marriages, reflecting the strong association between consanguinity and hereditary hearing loss[5].

Thalassemia used to be very common in Cyprus, where close community marriages are common. About seventy years ago, they made a law and implemented it. All brides and grooms must undergo hemoglobin electrophoresis. Both must be a carrier[6]. Only one or none could be carriers to eradicate thalassemia. This disease is now rare in Cyprus. Pakistan also has the law of premarital hemoglobin electrophoresis[7]. It is implemented hardly anywhere.

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In England, first cousin marriages are still common in Pakistani-origin families living in Bradford, Manchester, and Birmingham. The increased incidence of genetic diseases in these areas led to an enquiry (BBC documentary). The British parliament discussed this issue of unnecessary burden on the NHS and warned against first-cousin marriages[4]. Now the concerned families hide the consanguinity!

First cousin marriages also lead to sub-fertility and an increased rate of miscarriages, nature's act to avoid "special children"[1]. Parsi people hardly marry outside the community. Their number is ever decreasing. In Pakistan, out of 100 marriages, 70 are consanguineous[8]. Suppose each marriage produces four children. Thirty non-consanguineous marriages will produce 120 reasonably normal children. Seventy consanguineous marriages will produce 280 children. Out of these 280, seventy will have some kind of genetic disease. This is almost one in seven children born in Pakistan being genetically abnormal. This is preventable. And we have not given it a serious thought. Are we not suicidal?!

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ORIGINAL ARTICLE

Comparative Study to Assess the Practices Regarding Fad Diets Among Medical and Non-Medical Students of Lahore

Saba Nadeem Dar[1], Aqsa Nadeem[1], Urooj Arshad[1], Sayyeda Aatika Ejaz[1], Hooria Akhter[1], Aqsa Waheed[1], and Arooba Naeem[1]

ABSTRACT

Objectives: To compare the practices and assess the experiences regarding fad diets among medical and non-medical students

Methodology: A cross-sectional study was conducted on 378 students (189 from medical and 189 from non-medical institutions) from different universities of Lahore, through an online survey.

Results: Majority of the medical (47%) and non-medical (56%) students were satisfied with their weights. The majority of respondents did not follow fad diets. Among those who did, medical students were 17.5% and non-medical students were 21.69% but p-value (>0.05) showed insignificant difference between them. Both medical (24.87%) and non-medical (19.58%) students reported no changes in their weights resulting from the fad diets. However, the majority of medical (31.75%) and non-medical (21.6%) students also did not experience any side effects. A total of 25.93% medical and 28.04% non-medical students recommended these diets to others.

Conclusion: This study states that majority of the students of both medical and non-medical domains did not follow any fad diet, while those who tried fad diets, experienced few health consequences and therefore their majority will recommend it to others. This study demonstrates that there is no significant difference between medical and non-medical students in terms of practices and experiences regarding fad diets.

Keywords: fad diet; medical students; non-medical students

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INTRODUCTION

Obesity is a rapidly growing global health issue and Pakistan is experiencing a similarly increasing trend[1]. Obesity increases focus on dietary practices because social interactions influence body image and weight concerns in both genders. According to a study, female models feel social pressure of staying thin and are concerned about their body image[2]. Another study found a relation between internet use and body perceptions. Young people pay considerable attention to their body shape, as social media often portrays slim and fit individuals as the ideal. Those who perceive themselves as overweight may experience feelings of insecurity and find it difficult to feel accepted by others. As a result, many turn to various weight-loss methods, including fad diets, in an attempt to achieve their desired body image. To overcome this situation, many individuals adopt fad diets in the hope of achieving rapid weight loss without engaging in regular physical exercise[3].

In the last two decades, interest in dieting has increased worldwide. According to a study, the number of internet searches on weight loss increased significantly between 2004 and 2018[4]. According to a study, 62% people were influenced by interpersonal interaction that led to adoption of fad diets, while 92% were influenced by the social media. In addition, 15% respondents felt that social norms influence unhealthy weight control behaviors[5,6].

Social media promotes many diets that claim to lose weight without any exercise; these diets are called Fad Diets. Fad diet is not a scientific term but a popular dietary pattern that is perceived as a magic bullet to lose weight. Characteristics of Fad Diets can be easily differentiated from a healthy diet. Its nutritional

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adequacy is questionable and it lacks any scientific evidence to support its claims. Some food groups are even eliminated in these diets and these diets are for short term satisfaction rather than long term results[7].

Fad diets claim to deliver unrealistic goals like weight loss in a short period of time[8]. It is a collective term which includes different diets like Ketogenic diet, intermittent fasting, Atkins (low carbohydrate diet), Liquid diet, Egg diet and Banana diet etc. These diets claim to help in weight loss and improve your body shape in the short term. Some diets can improve your health to some extent but are nutritionally inadequate due to elimination of one or more food groups or dominant consumption of a single food. There are also some concerns of co-morbidities. Some side effects that are associated with these dieting patterns are decreased calcium balance and urine pH, feeling faint, and heart beat alterations etc[7].

One study estimated that 30% of the obese population did not follow any diet programme[9]. The rest of the respondents followed different fad diets including vegan and keto diets to achieve a certain body image.

Among those who followed fad diets, a majority were females, as women are more conscious about their body weight and body shape compared to males. Having a restricted diet and not eating balanced meals definitely causes nutrients deficiencies essential for various body systems. These diets are a short-term solution to obesity and when you stop the diet, the weight is easily restored.

Previous studies did not evaluate differences in practices between medical and non-medical students. However, medical students are typically expected to possess a higher level of awareness regarding the body's nutritional and health needs, in view of their medical training and their future responsibilities as healthcare professionals. To cover this research gap, a survey was performed on 378 participants out of whom half were medical students. This study compares the practices and experiences of medical and non-medical students regarding fad diets.

METHODOLOGY

ERC/IRB Approval: This study was conducted after approval of Research Ethics and Support Committee (RESC) and office of ORIC from University of Management and Technology, Lahore, Pakistan, 2023 with Ref No. RE 084-2023.

A comparative cross sectional study was conducted by the Department of Nutrition and Dietetics at the School of Health and Sciences, University of Management and Technology, Lahore to analyze the practices and experiences regarding fad diets among medical and non-medical students. The study was conducted from April 6, 2023 to June 20, 2023.

Convenience sampling technique was used for this study which included both male and female medical and non-medical students between the ages of 18 and 26 years belonging to universities in Lahore. Those having co morbidities (diabetes, hypertension, heart diseases, respiratory diseases, joint diseases, and mental health issues) were excluded.

Sample size was 378, calculated by Raosoft (online calculator). Data was collected through online survey, which comprised both close- and open-ended questions. Data analysis was done statistically by using chi-square test on SPSS version 23.

Participant's data were used with their permission in this study, however, their identity will remain confidential.

RESULTS

Table 4.1: Demographics

Demographics	Frequency (%)
Gender	
Male	125 (33.1%)
Female	253 (66.9%)
Marital status	
Single	342 (90.5%)
Married	35 (9.3%)
Divorced	1 (0.3%)
Age	
18-20	64 (16.9%)
21-23	166 (43.9%)
24-26	148 (39.2%)

As Table 4.1 shows, 66.9% participants were female. As many as 90.5% were single, 9.3% married and 0.3% divorced participants. Mean age range for the study was 21-23.

Total sample was divided into two categories. Among them 50% were medical students and 50% were non-medical students.

Table 4.2: Have you ever tried a fad diet?

Degree Program	Medical Degree	Non-medical Degree
No (you are finished with the survey) n (%)	156 (82.5%)	148 (78.3%)
Yes (continue with the survey) n (%)	33 (17.5%)	41 (21.6%)

p-value = .300

Chi-Square Tests

The majority of the medical (82.5%) and non-medical (78.31%) students had not tried any fad diets but among those who had tried, no significant difference was found between medical and non-medical students as P-value was 0.300 .

Table 4.3: What was the result of the longest fad diet you have tried?

Degree Program	Medical Degree	Non-medical Degree
n (%)	102 (54%)	106 (56%)
Lost weight and kept it off n (%)	22 (11.6%)	23 (12.2%)
Lost weight, but gained it back n (%)	18 (9.5%)	23 (12.2%)
No change n (%)	47 (24.9%)	37 (19.6%)

p-value = .594

The p-value of 0.594 as shown in Table 4.3 indicates insignificant difference between both groups. While among those who followed fad diets, the majority from both the groups reported no change in their weights.

Table 4.4: Did you experience any side effects? If so, please check which ones.

Degree Program	Medical Degree	Non-medical Degree
n (%)	101 (53.4%)	101 (53.4%)
Diarrhea n (%)	3 (1.6%)	4 (2.1%)
Heart Problems n (%)	2 (1.1%)	6 (3.2%)
Irritability n (%)	6 (3.2%)	4 (2.1%)
Joints Problems n (%)	3 (1.6%)	10 (5.3%)
None n (%)	60 (31.7%)	41 (21.7%)
Other n (%)	10 (5.3%)	9 (4.8%)
Vomiting n (%)	4 (2.1%)	14 (2.1%)

p-value = .030

P-value is significant (0.030) in table 4.4. Majority of both domains experienced no side effects. As compared to non-medical students (21.7%), more medical students (31.7%) reported no side effects.

Table 4.5: Would you recommend a fad diet or weight loss programme to somebody trying to lose weight?

Degree Program	Medical Degree	Non-medical Degree
n (%)	98 (51.9%)	98 (51.9%)
No n (%)	42 (22.2%)	38 (20.1%)
Yes n (%)	49 (25.9%)	53 (28%)

p-value = .837

P-value is insignificant (0.837) in table 4.5. The majority of students who followed fad diet will recommend fad diets or weight loss programmes to others and no significant difference between medical and non-medical students was found.

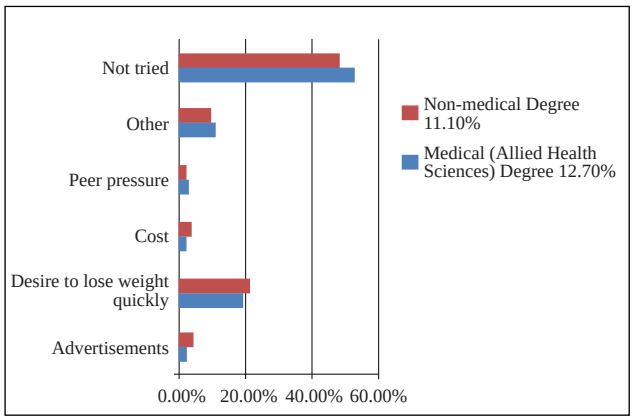

Figure 4.1: If you tried a weight loss programme. what made you decide to try it?

The figure 4.1 shows the motivation behind starting fad diets. The options provided were cost, advertisement, desire to lose weight quickly, peer pressure, not tried and other. Among medical students, 1.587% participants were persuaded by advertisements, 2.646% tried because of cost, 19.05% stated their desire to lose weight quickly, 2.646% succumbed to peer pressure, 52.38% participants did not try and 10.58% participants tried for other reasons. Among non- medical students, 4.233% participants tried because of advertisement, 3.175% were tempted by cost, 21.16% wanted to lose weight quickly, 1.587% gave in to peer pressure, 48.15% participant did not try and 8.995% tried for other reasons.

DISCUSSION

The current study compares medical and non-medical students for their practices, experiences, and results of their longest fad diet. In contrast to a previous study, Table no 4.2 shows that the majority of the students did not try any fad diets and this may be because the majority was satisfied with their body weights. Whereas a previous study had shown that the majority of

respondents had tried different fad diets including Atkins, vegan, keto, liquid, and other types of fad diets[9,10]. In a previous study, very few people were in favor of fad diets contrasting with the current study in which the majority will recommend fad diets to those who want to lose weight as shown in table 4.5[3]. Regarding the results of the longest fad diet tried, current research found that the majority experienced no change in their body weights as shown in Table no.4.3 while an earlier study had stated that the majority experienced temporary weight loss, but did not achieve desired results. A similarity in both the researches is that the participants did not achieve the desired results, while a contrast is that the current research participants reported no change but the respondents in the previous research had experienced temporary weight loss[11].

The present study demonstrates findings similar to those reported in previous researches. In a previous study, most participants did not report any adverse health effects associated with fad diets, although some noted behavioral changes. Likewise, in the current study, the majority of participants who followed fad diets experienced no adverse health effects; however, a few reported mild signs and symptoms, as shown in Table 4.4[3]. With regard to social media, previous studies reported that most participants became aware of different fad diets through social media platforms, advertisements, peers, and friends, which motivated them to adopt these diets[12]. Figure 4.1 shows that those who tried different weight loss programmes were influenced by several factors including advertisements as the most effective. When compared with previous research, the present study demonstrates similar findings regarding the influence of social media and advertisements on the adoption of various diets and weight-loss programmes. Several earlier studies have reported that exposure to social media and advertisements encourages individuals to engage in weight-loss practices to attain their desired body image and physical appearance.

The previous study found that majority of the participants used social media as a major source of information about different types of diets, while role models, influencers, friends, and family also played roles in informing the respondents about different diets[3]. Another study states that media is a major motivating factor which influences people to follow different diets[5]. Previous research had also demonstrated that exposure to body images on social media, including those of actors, role models with slim body types, and individuals sharing idealized personal images, can shape individuals' perceptions of their own body image and weight[13,14].

More studies have reported that those who viewed different advertisements regarding fad diets were more likely to adopt these which agrees with our current study[9,15,16]. Therefore, it is safe to say that most of the findings of this current study were similar to the previous studies except that majority of the participants did not try fad diet, experienced no change in their weights and majority will recommend it to others, as they experienced no serious health issues.

Since the survey was conducted online, there is a possibility of bias in the self-reported data. Due to the online format, there is no certainty regarding the accuracy of participants' reported weights. Additionally, the short time period allocated for the study may have further limited the reliability of the findings.

CONCLUSION

A fad diet is a weight-loss approach that many people try, often influenced by advertisements, peer pressure, and personal body image concerns. It has remained a popular trend over time. This study aimed to explore how medical and non-medical students use fad diets and what their experiences are like. The findings showed that most students in both groups had never tried a fad diet, mainly because they were satisfied with their body weight. Among those who had tried one, most did not notice any change in their weight, which may be because they followed the diet for a very short period, usually less than a week. Interestingly, many students from both groups said they would still recommend fad diets to others, as they did not report major health issues from them. Over all, the study suggests that there is no meaningful difference between medical and non-medical students in their use of fad diets or their willingness to recommend them.

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Authors' Contributions: SND: supervised the study, provided overall guidance, and contributed to data analysis. **AN, H,** and **AW:** contributed to the interpretation of results. **UA** contributed to study objectives, data collection, and data analysis. SAE contributed to the study concept, design, and data collection. **ARN:** performed statistical analysis using SPSS. All authors reviewed and approved the final manuscript.

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ORIGINAL ARTICLE

Assessment of Thyroid Gland Enlargement Through Clinical Grading and its Correlation with Ultrasonographic Thyroid Volume among Pregnant and Non-Pregnant Women in Local Population in Karachi

Hira Ahmed[1], Shazia Fahmi[1], Faiza Nafis[2], Asma Aijaz[3], Talat Samreen[1], Zaheer Amjad[4], and Hina Jabeen[5]

ABSTRACT

Objectives: The aims of this study were to evaluate thyroid gland enlargement using clinical grading methods in both groups, measure thyroid volume through ultrasonography, and determine the correlation between clinical grading and ultrasonographic thyroid volume.

Methodology: This cross sectional study was conducted at the Gynea OPD, Ojha campus, DIMC, DUHS, Karachi from June 2018-May 2020 through consecutive sampling and separated into two groups, Pregnant and Non Pregnant women. The gross examination of thyroid gland was performed and this examination purpose to evaluate the size of thyroid lobes through standard methods of inspection and palpation. The Thyroid volume was measured by ultrasound and then calculated by WHO co-factor V (ml) =0.479*L*W*D by applying inclusion / exclusion standard.

Results: The examination were made on thyroid grading with correlation of total thyroid volume (TTV) among pregnant and non-pregnant women. Thyroid gland was examined by inspection and palpation. The grading (from 0-2) of enlargement of gland was done. 50 pregnant women were examined among which 34 (68%) were found to have Grade 1 while 16 (32%) had no thyroid enlargement (means neither visible nor palpable). On the other hand, in the non-pregnant group, not even a single enlargement was observed. The mean difference was considered significant in pregnant women with p-value (< 0.01). In non-pregnant women, mean TTV was 5.58±2.41, where as in pregnant women, it was 7.02±3.21, which was significantly increased in pregnant women with p-value 0.01*.

Conclusion: The study proposed that physical clinical examination of thyroid gland is a valuable method, and it also finds that even pregnant women who looks healthy in this population may still have some degree of iodine deficiency. The volume of the thyroid gland is enhanced during pregnancy, suggesting an iodine deficiency. A combined approach (clinical + imaging) is recommended for accurate diagnosis in antenatal care.

Keywords: Non pregnant women, pregnant women, thyroid gland, usg imaging

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INTRODUCTION

The thyroid gland is placed superficially in the center of the neck, around the larynx and trachea. It consists of two symmetrical lobes, right and left that are attached by isthmus and located anterior to the second and third tracheal rings. The gland is fixed in place due to its encapsulation within the pretracheal fascia and its attachment to adjacent structures, including the cricoid cartilage and the upper tracheal rings, via the suspensory ligament of Berry[1]. The thyroid gland is modulated by under control of hypothalamic–pituitary-thyroid axis. The hypothalamus secretes thyrotropin releasing hormone (TRH) which act on pituitary gland to activate thyroid stimulating hormone (TSH). The TSH release the thyroid hormone (T3) and (T4). These iodine containing hormones are vital for regular growth and metabolic function. The basic role of gland is to synthesize, store and secretes this two iodine based hormone. The sufficient dietary iodine uptake is required for this process, as iodine is a key component of both T3 and T4.

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Thyroid hormones play a pivotal role in both prenatal and postnatal life, supporting normal physical growth and neurological development during intrauterine life as well as after birth. Once secreted into the bloodstream, thyroid hormones function as chemical messengers that reach in all tissues and organs of the body.

They regulate the metabolism of fats, proteins and carbohydrates at the cellular level. By influencing metabolic rate, oxygen consumption, and energy production, thyroid hormones ensure the proper functioning of cells and support overall homeostasis throughout the body[2]. Iodine is a substance, necessary for the thyroid hormones production.

These hormones play critical role in body's biochemical processes. The thyroid gland becomes enlarged and apparent in pregnancy due to iodine insufficiency, known as pregnancy iodine deficiency goiter or Iodine deficiency Diseases (IDD). The IDD are popular in Pakistan and about 70% of population are at risk of it. A study disclosed that the prevalence of IDD in pregnant women is about 79.8% in Pakistan[3]. When IDD occurs in pregnancy, it is referring to maternal and fetal hypothyroidism, mental disability, increased neonatal and infant fatality rate[4].

The thyroid glands show several level of enlargement due to deficiency of thyroid hormones production and iodine, leading to hypothyroidism[5]. The large size of thyroid gland is mostly due to dietary iodine inadequacy and if it occurs during pregnancy, it may cause harmful effect in the growing fetus. Hence, it is essential that the consumption of iodine should be enhanced in pregnancy[6]. For this reason, adequate iodine intake is required according to WHO criteria and ICCIDD has recommended the dose of 200 mcg iodine/day (range between 200-300 mcg) in pregnant women for the fetal evolution[7]. USG is the imaging technique that provides accurate information and the most specific method for measuring the thyroid volume[8].The thyroid gland is an appropriate organ for the examination with USG due to its superficial location in the body.

The determination of thyroid volume during pregnancy is evidential in the diagnosing and controlling of thyroid condition and iodine deficiency disorders (IDD)[9]. It can be explored through with physical examination by assessing the grading of thyroid enlargement and by ultrasonography to measure the thyroid volume[10]. Hence, to evaluate the grading of gland and to measure the thyroid volume by USG among two groups, to avoid harmful consequences in the fetus.

Thyroid gland enlargement can be accessed via different methods, each method important for diagnosis. Physical examination for grading involves inspection and palpation, classifying the thyroid into grades based on visibility and feel. It is easy, ready and cost-effective, making it helpful for screening. When thyroid volume is assessed by clinical grading, it provide only an approximate estimate. Although easygoing to use and widely available, clinical grading is less accurate and is usually confirmed by ultrasound for a precise assessment.

Thyroid enlargement is common clinical finding and is usually evaluated through physical examination. In regions like Karachi, where iodine deficiency still persists, thyroid disorders may be more prevalent, especially in pregnant women due to increased physiological requirement. Pregnant women are more assailable to thyroid changes due to high hormonal and metabolic demands. These changes can modify thyroid size and function, making accurate assessment important for both maternal and fetal health. Although Ultrasonography is a reliable procedure for measuring thyroid volume, its availability may be limited in many healthcare settings. Therefore, It is important to see how closely the results of clinical examination match those of ultrasound. The study purpose is to find out how consistent the physical examination is and whether it can be used as a easy and useful screening procedure rather of ultrasound in the local population.

METHODOLOGY

ERC/IRB Approval: This study was conducted after approval of Institutional Review and Ethics Board (IREB) from DIMC, DUHS, 2018 with Ref No. IRB-1064/DUHS/Approval/2018/82.

This cross-sectional study was done in two years, from June 2018-May 2020, at the Gynea department during antenatal checkup and the Radiology Department, Ojha campus, Dow university of health sciences (DUHS), Karachi through consecutive sampling. The total sample size of 100 women was calculated by using OpenEpi software and divided into two groups (A and B). Among the recruits, 50 were pregnant women (in the first and second trimesters) and 50 were non-pregnant controls of reproductive age (14–45 years)y. Non-pregnant women who visited the gynecology OPD for other reasons and those women whose thyroid gland was clearly visible on ultrasonography were included in the study. Participants with a history of thyroidectomy, Verbal Informed consent was taken from each participants. The basic clinical assessment implicated, gross examination of thyroid gland, which was performed in the Gynae OPD by trained healthcare professionals. This examination purpose to evaluate the size of thyroid lobes through standard methods of inspection and palpation. The palpation of thyroid gland was done in seated position with slightly extended

neck and movements of the gland were noted during deglutition. The enlargement of gland was classified by using the World Health Organization (WHO) grading system, which provide a standard approach for assessing goiter:

- **Grade 0**: No goiter is present; the thyroid gland is neither palpable nor visible upon clinical examination.
- **Grade 1**: The thyroid gland is enlarged and palpable but not visibly enlarged; it moves up and down during the act of deglutition (swallowing).
- **Grade 2**: The thyroid gland is both palpable and visibly enlarged, seen prominently in the middle of the neck region.

This clinical grading provided an essential baseline for correlating physical findings with ultra-sonographic measurements of total thyroid volume (TTV), conducted subsequently in the Radiology Department. The Ultrasonography of thyroid gland was carried out under the supervising of a highly experienced radiologist. The imaging method were concluded by using linear probe at a frequency of 7.5 MHz via GE Voluson S6 ultrasound machine, at Radiology department. The each lobe of thyroid gland was examined in two planes, transverse and longitudinal. The dimensions (length, width, and depth) of both the right and left thyroid lobes were carefully measured. The Total thyroid volume (TTV) was then calculated via the ellipsoid formula for each lobe and summed accordingly. The isthmus was not included in the volume calculation. To evaluate the volume of each lobe, the WHO-recommended formula was applied, V (ml) = 0.479 × L × W × D (L= length, W= width D= depth). This correction factor (0.479) provides a standardized technique for calculating thyroid volume. The volume of each lobe were sum up to calculate the Total Thyroid Volume (TTV) for each participants.

RESULTS

Thyroid gland was examined by inspection and palpation in both groups. Among non-pregnant women (n=50), all participants (100%) had Grade 0 thyroid enlargement, indicating no detectable goiter on clinical examination. In contrast, among pregnant women (n=50), only 16 (32%) had Grade 0, while the majority, 34 (68%), had Grade 1 enlargement, suggesting mild thyroid swelling detectable on palpation but not visible. None of the participants in either group showed Grade 2 enlargement. A p-value of < 0.05 was considered statistically significant in both groups. These findings show that thyroid enlargement was found in pregnant women, as shown in figure I.

The comparison of total thyroid volume (TTV) between the two groups showed that pregnant women had a higher mean TTV (7.02 ± 3.21) compared to non-pregnant controls (5.58 ± 2.41). This difference was statistically significant (p=0.01), indicating that thyroid volume is significantly increased in pregnant women compared to non-pregnant women as shown in figure II.

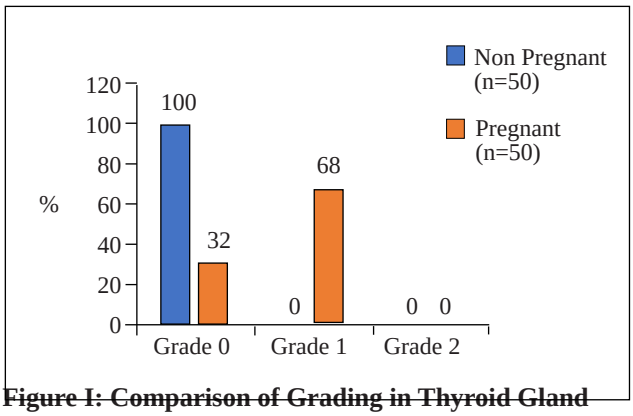

Figure I: Comparison of Grading in Thyroid Gland Enlargement Between Two Groups

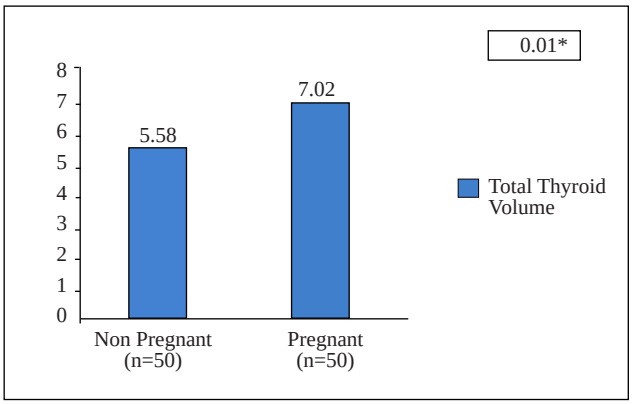

Figure II: Comparison of Total Thyroid Volume Between Pregnant and Non Pregnant Women

DISCUSSION

It has been generally known that the recognized stimulators of thyroid gland secretions in pregnancy are TSH, hCG and iodine. There are various physiological alteration that happen in thyroid gland to increase the thyroid size, volume and thyroid hormones in pregnancy as there is addition need for iodine and energy to meet the metabolic and hormonal modifications by the maternal organs[11]. The thyroid gland volume (TGV) is adjustable among several populations. The variations that occur in thyroid gland are because of iodine deficiency in pregnancy[12]. After 2023, the inappropriate calculation of thyroid size by inspection and palpation is primarily replaced by determination of TGV by USG[13].

USG is the first line imaging modality for the determination of thyroid size and calculation of thyroid volume. If there is decrease iodine for the synthesis of thyroid hormones, most likely it increases the risk of

maternal thyroid hypo function in the iodine-deficient women. However, even in iodine sufficiency, there are some reports on thyroid enlargement throughout pregnancy[14]. The iodine status is a universal health concern, mainly in underdeveloped countries and importance should be given to diagnosis at the community level due to the advanced effect on fetal neurological development and pregnancy outcomes. Thyroid gland need iodine and amino acid tyrosine to make thyroid hormones, which keep the level of metabolism in the tissues that is optimal for their regular function[15].

The thyroid hormones T3 and T4 has an essential role in regulating the physiological processes. During intrauterine life, insufficient levels of thyroid hormone due to maternal iodine deficiency can lead to serious consequences such as cretinism, low birth weight, and developmental delays.

After birth, thyroid hormones continue to influence metabolism, energy production, and overall homeostasis. These hormones function as chemical messengers, carried via the bloodstream to target cells throughout the body. They regulate the metabolic activity of nearly all cells, influencing basal metabolic rate, protein synthesis, lipid metabolism, and carbohydrate utilization. By enhancing mitochondrial activity and oxygen consumption, thyroid hormones ensure that the cells meet their energy needs efficiently. Furthermore, they contribute to cardiovascular health, gastrointestinal motility, reproductive function, and thermoregulation[16]. In our study, the grading of thyroid gland on gross examination showed no enlargement of gland in non-pregnant women (grade-0). While enlargement was observed in pregnant women which showed 68% of females having enlargement (grade-1) and 32% of which showed no enlargement (grade-0). We found increased size in pregnancy due to increase demand of thyroid hormones which utilizes iodine for its synthesis. This may be the reason of enlargement of thyroid gland. A study done on thyroid gland enlargement in 2019, observed that pregnant women had 70% visible and palpable enlargement of the thyroid gland[17]. In 2022, conducted a study on enlargement of thyroid gland (goiter) by inspection and palpation in pregnancy. They found 23% enlargement of thyroid gland in pregnant women and 19% in controls[18]. In 2024, a study done on thyroid gland enlargement by inspection and palpation. They observed 70% enlargement of thyroid gland in pregnant and 38% in controls[19]. In the present study, we evaluated the changes in thyroid volume in corresponding to normal pregnancy.

We also found a significant increase in TTV in pregnant group than controls. Corresponding to our findings, a study done by Ollero MD, Toni M, in 2019, found significant changes in the size of thyroid lobes[20,21]. The thyroid size is not sufficient to be noticed by physical examination or by palpation of the gland but should be assessed by USG.

Similarly, a study done in 2023, reported increase in thyroid gland volume in asymptomatic pregnant women that might develop iodine deficiency goiter. They also found a change in thyroid volume among pregnant and non-pregnant controls due to dietary iodine insufficiency during pregnancy[22].

The study was conducted on enlargement of thyroid gland by inspection and palpation in pregnancy. We found 23% enlargement of thyroid gland in pregnant women and 19% in controls[23]. So, Gynecologist must perform thyroid examination to evaluate thyroid grading during pregnancy to prevent maternal from adverse consequences. The research is also done on the thyroid volume to assess the iodine levels in pregnancy to prevent them from adverse mental and fetal consequences.

Numerous studies have established that iodine deficiency in pregnancy is related to harmful maternal and neonatal consequences. Inadequate iodine levels can contribute to complications such as spontaneous abortions, congenital anomalies, low birth weight, impaired cognitive development in the fetus, and placental abnormalities. These consequences highlight the critical importance of ensuring adequate iodine intake during pregnancy for both maternal health and fetal development[24,25].

To the best of our knowledge, there was no research conducted to examined the clinical grading of thyroid gland enlargement in correlation with total thyroid volume (TTV) as measured by ultrasonography (USG) in both groups, in our local population. By valuating and comparing total thyroid volume in both women groups, this research contributes valuable vision into the prevalence of iodine deficiency among pregnant women. The findings may help in distinguishing at-risk populations and investigate the routine thyroid evaluation and iodine supplementation for the antenatal care to prevent health complications related to thyroid diseases.

CONCLUSION

The authors advocate for increased awareness among healthcare providers, particularly gynecologists involved in antenatal care, to recognize the risks associated with iodine deficiency. As a preventive strategy, they recommend that iodine sufficiency be ensured not only during pregnancy but also in the preconception phase, to mitigate the risk of thyroid gland hypertrophy and associated maternal- fetal complications. The study concludes that thyroid gland enlargement is more common in pregnant women compared to non-pregnant women. Pregnant women also showed a significantly

higher total thyroid volume (TTV) than non-pregnant controls, indicating that clinical examination remains a useful screening tool. However, ultrasonography provides a more accurate and objective assessment and should be used for confirmation when available.

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Conflict of Interest: Author declares that there is no conflict of interest.

Authors' Contribution: **HA** conceived the study, prepared the manuscript, and handled correspondence. **SF** and **FN** provided guidance and reviewed the manuscript. **AA, TS, ZA**, and **HJ** performed the in vitro quality tests and contributed to data collection. All authors reviewed and approved the final manuscript.

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ORIGINAL ARTICLE

Weekly Assessment of Acute Oral Mucositis on Concurrent Chemo Radiotherapy in Oral Cavity Cancers

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ABSTRACT

Objective: This study aimed to assess the weekly progression of acute oral mucositis in patients with oral cavity cancer undergoing concurrent chemoradiotherapy.
Methodology: A descriptive study was conducted at the Department of Oncology, Dr Ziauddin University Hospital from September 2020 to December 2021. Patients with SCC of oral cavity with ages between 18 and 70 years, planned for radiation therapy as adjuvant (after surgery) with chemotherapy were recruited. The severity of mucositis was evaluated weekly during the treatment according to RTOG/EORTC criteria of adverse events. The severity of mucositis was compared between patients aged below and above 45 years and between genders.
Results: Total 126 patients were included in the study. At Week 07, Grade 3 radiation induced oral mucositis constituted the highest proportion in patients aged <45 years, i.e. 74.4%, followed by Grade 4 (23.3%), and Grade 2 (2.3%) oral mucositis, which seemed similar to the oral mucositis grades in patients who are >45 years of age. The p-value (0.783) indicated that the observed differences in grade distribution between the age groups were not statistically significant. At Week 07, the percentage of Grade 3 XRT induced oral mucositis was higher than Grade 4 and Grade 2 oral mucositis in both female and male patients. The p-value (0.979) indicated that the observed differences in grade distribution between genders were not statistically significant.
Conclusion: Acute oral mucositis is a common and severe toxicity in oral cavity cancer patients undergoing concurrent chemoradiotherapy, typically peaking at Grade 3 by Week 7, with no significant differences observed across age or gender, highlighting the need for uniform supportive care strategies.

Keywords: Oral cavity Squamous cell carcinoma (OCSCC), Chemo radiation CCRT, Oral mucositis (OM)

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, and its incidence is projected to increase 30% by 2030, reaching approximately 1.08 million new cases annually, according to GLOBOCAN[1-3]. The elevated incidence of HNSCC in regions like Southeast Asia is linked to the habitual use of products that contain particular carcinogenic substances. Oral mucositis (OM) is a frequent and debilitating complication experienced by patients with HNSCC receiving radiation therapy (RT), whether administered independently or alongside chemotherapy. It is marked by severe inflammation and ulceration of the mucosa of the mouth and throat. It includes intense inflammation and ulceration of the oral and pharyngeal mucosa, leading to significant pain and discomfort. These symptoms often impair essential functions such as speaking, swallowing, and eating, thereby greatly affecting the patient's over all quality of life[4].

OM is associated with higher rate of opioid use, weight loss, feeding tube placement, and hospitalization among patients with HNSCC. These symptoms put patients at risk for treatment delays that can compromise patient outcome[5]. Currently, there is no universally accepted method for assessing the severity of OM. Various

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clinical and research-based scoring systems have been developed and reported in the literature, each differing in their criteria, sensitivity, and clinical applicability[6].

Earlier research employed assessment tools to explore the relationship between the severity of OM and patient-reported quality of life (QOL). With advancements in modern radiotherapy (RT) planning for head and neck cancers (HNC), normal tissue can now be preserved better. Prior to and during the treatment, all patients must receive education as per institutional management[7]. According to Western Literature, 80-83% of patients receiving radiotherapy to head and neck region, develop OM, out of whom 19% develop mild, 35% moderate, and 28% of patients develop severe mucositis[8]. The objective of this study is to determine the frequency and graded severity of acute oral mucositis developing during treatment in patients with head and neck cancers undergoing concurrent chemoradiotherapy. This evaluation aims to better understand the clinical burden of mucosal toxicity and its potential impact on treatment tolerance and over all patient well-being.

METHODOLOGY

ERC/IRB Approval: A descriptive study was conducted at the Department of Oncology, Dr Ziauddin University Hospital from September 2020 to December 2021 with ERC Reference Code 0050217FARONCO and CRC Reference Code: 0030116RDTH

Patients with SCC of oral cavity of ages between 18 and 70 years, planned for radiation therapy as adjuvant (after surgery) with chemotherapy, were recruited to assess the incidence and severity of acute oral mucositis (occurring during treatment). This condition, characterized by inflammation of the oral mucosa due to radiotherapy, commonly presents with symptoms such as swelling, redness (erythema), ulceration, and bleeding.

In this study, only those patients were included who were treated with 3-Dimensional Radiotherapy techniques (conventional form of radiotherapy). Approximately one-third of the study patients received concurrent chemotherapy consisting of weekly cisplatin at a dose of 40 mg/m², administered alongside radiation therapy. Pertinent clinical pathologic data were collected from the medical records and stored in a secure Research Electronic Data Capture (REDCap) database[9].

Currently, multiple grading systems exist for evaluating oral mucositis (OM), each with distinct assessment criteria. The World Health Organization (WHO) scale considers both objective indicators such as the presence of erythema and ulceration and functional aspects related to the patient's ability to eat and sustain oral intake. Likewise, the National Cancer Institute (NCI) introduced the Common Terminology Criteria for Adverse Events (CTCAE), which grades the severity of mucositis based on its anatomical site and the type of treatment responsible, including chemotherapy, radiotherapy, or combined modalities[10].

Oral mucositis is a prevalent and potentially severe adverse effect observed in head and neck cancer (HNC) patients undergoing radiotherapy, with incidence ranging from 85% to 100%. Considering a frequency of 85%, a 95% confidence level, and a 7% (0.07) margin of error, the calculated sample size was determined to be 100.

Sample size calculated for the estimated frequency of oral mucositis was larger than the one for severity of oral mucositis. Non probability purposive sampling was done. Therefore, a total of 126 head and neck cancer patients who had developed oral mucositis during the course of radiotherapy were recruited to be comprehensively evaluated, fulfilling the study's objectives.

Existing literature reported the frequency of OM in patients receiving radiotherapy for HNC as 80%[11]. This assumption gives the maximum sample size; therefore, to estimate the frequency, with 95% confidence level and with 7% (0.07) bound on error of estimation, a sample of 126 patients was calculated and recruitment was done.

All patients with HNC referred to us for radiotherapy, were enrolled in this study on meeting the inclusion and exclusion criteria and after giving written informed consent. Assessments of patients for the severity of oral mucositis were done before starting radiotherapy, then on a weekly basis, and on the day of completion of radiation according to the toxicity pro forma based on RTOG criteria, displayed at the end. Each patient was examined and findings were collected for the severity of OM. Data form of each patient was filled every week according to the study protocol. Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 19. A descriptive analysis was carried out and results were presented as mean ± standard deviation for variables like age, gender, receiving concurrent chemoradiotherapy. For stratification of outcome, variables like age, gender receiving concurrent chemotherapy are presented in the form of cross tabulation.

The association of frequency and severity of oral mucositis to variables of treatment were done using cox-regression. A p-value of less than or equal to 0.05 was considered as statistically significant.

RESULTS

In this study of 126 patients, week 1 results showed that among patients younger than 45 years, 58.1% had Grade 0 and 41.9% had Grade 1 CCRT-induced oral mucositis. Among patients older than 45 years, a higher proportion had Grade 0 (61.3%) compared to Grade 1 (38.8%), indicating a slight skew toward milder presentation in the older age group. The p-value of 0.737 indicated that the difference between Grade 0 and Grade 1 CCRT induced oral mucositis is not statistically significant in week 1. However, at the end of week 1, Grade 0 CCRT induced oral mucositis was observed in 46.4% and Grade 01 in 53.6% of the female patients who underwent CCRT, as compared to the male patients who showed a notable decrease in the proportion of Grade 1 oral mucositis (35.8%). The p-value of 0.091 indicated a moderate level of significance, suggesting difference in proportions between Grade 0 and Grade 1 for males and females. The results of week 2 suggested Grade 0 (51.2%) and Grade 1 (48.8%) CCRT induced oral mucositis among patients younger than 45 years. However, the patients above 45 years of age showed an equal proportion in Grade 0 and Grade 1 CCRT induced oral mucositis (50%).

The high p-value (0.902) suggested that the variation among different age groups is statistically insignificant. At the end of week 2, an increase in proportion of Grade 01 oral mucositis was observed in female patients (60.7%), in comparison to the male (46.3%) patients. The p-value (0.181) at the end of week 2 suggested that the observed differences in gender distribution were not statistically significant.

At week 3, in patients of both age groups, Grade 01 oral mucositis (67.4% in less than 45 years and 80% in more than 45 years) showed the dominating pattern as compared to the Grade 02 oral mucositis.

The p-value (0.122) suggested that the observed differences in age distribution between <45 and >45 groups were not statistically significant.

However, a higher number of patients with Grade 01 CCRT induced OM were observed in both females (67.9%) and males (77.9%), in comparison to Grade 02 OM.

The p-value (0.277) at the end of week 3 also suggested that the observed differences in gender distribution were not statistically significant.

At week 4 of radiotherapy, a higher proportion of patients in both age groups exhibited Grade 1 OM compared to Grade 2, with (67.4%) in patients younger than 45 years and (90%) in those older than 45 years. Therefore, the low p-value (0.047) suggests that the observed differences in age distribution between <45 and >45 year groups are statistically significant. In contrast, at the end of Week 4, the p-value (0.247) suggested that the observed differences in gender distribution are not statistically significant. At week 5, in <45 years group, Grade 3 OM (53.50%) slightly exceeded Grade 2 OM (46.50%). While in >45 years group, Grade 2 (53.80%) slightly exceeded Grade 3 (46.30%).

The p-value (0.444) suggests that the observed differences in grade distribution between <45 and >45 groups are not statistically significant.

At week 5, the Grade 3 OM (60.70%) exceeded Grade 2 OM (39.30%) in female patients. However, Grade 2 OM (54.70%) exceeded Grade 3 (45.30%) in male patients.

The p-value (0.151) at week 5 also suggested that the observed differences in grade distribution between females and males were not statistically significant. By week 6, Grade 3 radiotherapy-induced OM had the highest proportion (70.0%) in patients older than 45 years, followed by Grade 2 (18.8%) and Grade 4 (11.3%). A similar distribution of OM grades was observed in patients younger than 45 years. A p-value of 0.187 indicated that there was no statistically significant difference in the distribution of Grades 2, 3, and 4 OM between <45 and >45 age groups. On the contrary, at week 6, the data indicated that within the female patients, Grade 3 OM had the highest proportion (64.30%), followed by Grade 2 and Grade 4, both at 17.90%, fairly comparable to OM in male patients. A p-value of 0.595 indicated no statistically significant difference in the distribution of Grades 2, 3, and 4 OM between females and males in this study.

At week 7, the patients aged <45 years showed Grade 3 CCRT induced OM in the highest proportion, i.e. 74.4%, followed by Grade 4 (23.3%) and Grade 2 (2.3%), which seemed similar to the OM grades in patients who are >45 years of age. The p-value (0.783) indicated that the observed differences in grade distribution between the age groups were not statistically significant. At week 7, the percentage of Grade 3 CCRT induced OM was higher than Grade 4 and Grade 2 OM in both female and male patients.

The p-value (0.979) suggested that the observed differences in grade distribution between genders were not statistically significant.

DISCUSSION

Radiation therapy serves as a cornerstone in the treatment of oral cavity cancers, utilized in both definitive and adjuvant settings to maximize local disease control, especially when administered alongside

Table 1: Stratification of Weekly (Week 1 and 2) assessment of oral mucositis according to according to age group to age and gender

	Age Group	Grade 0	Grade 1	p-Value	Gender	Grade 0	Grade 1	p-Value
Week 1	<45	25 (58.1%)	18 (41.9%)	0.737	Female	13 (46.4%)	15 (53.6%)	0.091
	>45	49 (61.3%)	31 (38.8%)		Male	61 (64.2%)	34 (35.8%)	
Week 2	<45	22 (51.2%)	21 (48.8%)	0.902	Female	11 (39.3%)	17 (60.7%)	0.181
	>45	40 (50%)	40 (50%)		Male	51 (7.5%)	44 (46.3%)	

Table 2: Stratification of Weekly (Week 3 and 4) assessment of oral mucositis according to according to age group to age and gender

	Age Group	Grade 1	Grade 2	p-Value	Gender	Grade 1	Grade 2	p-Value
Week 3	<45	29 (67.4%)	14 (32.6%)	0.122	Female	19 (67.9%)	9 (32.1%)	0.277
	>45	64 (80%)	16 (20%)		Male	74 (77.9%)	21 (22.1%)	
Week 4	<45	33 (76.7%)	10 (23.3%)	0.047	Female	22 (78.6%)	6 (21.4%)	0.247
	>45	72 (90%)	8 (10%)		Male	83 (87.4%)	12 (12.6%)	

Table 3: Stratification of Weekly (Week 5) assessment of oral mucositis according to according to age group to age and gender

	Age Group	Grade 2	Grade 3	p-Value	Gender	Grade 2	Grade 3	p-Value
Week 5	<45	20 (46.5%)	23 (53.5%)	0.444	Female	11 (39.3%)	17 (60.7%)	0.151
	>45	43 (53.8%)	37 (46.3%)		Male	52 (54.7%)	43 (45.3%)	

Table 4: Stratification of Weekly (Week 6 and 7) assessment of oral mucositis according to according to age group to age and gender

	Age Group	Grade 2	Grade 3	Grade 4	p-Value	Gender	Grade 2	Grade 3	Grade 4	p-Value
Week 6	<45	13 (30.2%)	23 (53.5%)	7 (16.3%)	0.187	Female	5 (17.9%)	18 (64.3%)	5 (17.9%)	0.595
	>45	15 (18.8%)	56 (70%)	9 (11.3%)		Male	23 (24.2%)	61 (64.2%)	11 (11.6%)	
Week 7	<45	1 (2.3%)	32 (74.4%)	10 (23.3%)	0.783	Female	1 (3.6%)	21 (75%)	6 (21.4%)	0.979
	>45	3 (3.8%)	62 (77.5%)	15 (18.8%)		Male	3 (3.2%)	73 (76.8%)	19 (20%)	

concurrent chemotherapy[12]. Precise prediction of toxicity severity is crucial for guiding effective management strategies and enhancing over all patient outcomes[13]. According to Globocan 2012 cancer fact sheet regarding Pakistan, the lip and oral cancer was the most common head and neck cancer in males and the second most common in females.

In our study, 76% of participants were male and 24% were female, yielding a male-to-female ratio of 3:1. Over all, men are two to four times more likely to develop head and neck squamous cell carcinoma (HNSCC) than women[14]. The median age at diagnosis for non–virus-associated HNSCC is approximately 66 years, while cancers related to HPV and Epstein-Barr virus (EBV) tend to occur at relatively younger ages[15]. The installation of mucositis in our study resulted in a few of the patients experiencing Grade II OM at the end of the first week of treatment whereas, it was observed two weeks after the start of radiotherapy in literature[16].

Patients 45 years of age and above were more vulnerable to mucositis in our study. Similar results were found in another study suggesting acute toxicity in older patients with more severe OM (Grades III and IV) than in their younger counterparts, suggesting a decrease in both tolerance and immunity. This was consistent with other studies with similar findings in about 50% patients having experienced Grade II or Grade III oral mucositis[17]. A Brazilian study found that oral mucositis most commonly occurred during the third and the sixth weeks of treatment, predominantly as Grade 1 and Grade 2 reactions[18].

Typically, acute OM begins to manifest within 5 to 14 days of the initiation of conventional fractionated radiotherapy. However, in this study, the dose delivered per fraction appeared to have played a key role in the earlier onset and higher frequency of Grade I OM. Notably, all patients developed OM during the course of radiation therapy, with the majority exhibiting Grade II and Grade III severity levels[19]. In our group, 20%

developed Grade IV OM towards the completion of treatment and at three months follow up. A recently published Indian study reported treatment gaps in up to 39% patients due to extreme grades of OM[20]. In the current study, the absence of treatment interruptions among patients was primarily attributed to the proactive insertion of feeding tubes prior to the initiation of radiotherapy, which effectively minimized nutritional deficiencies. It was also observed that Grade I and II OM occurred more frequently during the early phase of treatment (weeks 1-4), whereas the incidence of Grade III OM became more prominent in the later phase (weeks 5-7).

In another study done in M. D. Anderson Cancer Centre, the results were suggestive of the higher incidence of OM mainly between the third and the seventh week with high percentages of Grade III OM[21-22]. After 1-2 weeks of radiotherapy treatment (i.e. after receiving 20Gy dose), edema was observed. After the completion of three weeks treatment and receiving about 30 Gy radiotherapy dose, mainly vascular permeability was increased leading to a rise in edema formation[23].

Keeping the results of studies mentioned above and comparing them with our study, the conclusion is that the signs of OM become more visible from the third week onwards. A wide spectrum of predictive factors has been identified for the severity of OM and dysphagia, highlighting their multifactorial and complex pathophysiology. Among these, genetic predisposition plays a significant role, as genome-wide association studies (GWAS) have revealed specific genetic variants that are correlated with an increased susceptibility to developing acute OM[23,24].

We hope that these findings would provoke an understanding of the incidence and toxicity of this debilitating effect of head and neck radiation therapy in our patient population. This will enable our treating oncologists to render more attention towards prescription, planning, and verification of radiotherapy treatment, especially for the group of patients who have undergone surgery and are planned to have chemotherapy along with radiation, thus minimizing acute toxicity. Furthermore, our findings would provide baseline statistics for comparison with late toxicities for the presence of any relation.

Modern radiotherapy machines equipped with advanced techniques such as IMRT and VMAT have significantly reduced the incidence of OM. Therefore, we recommend the use of these technologies, where available, to minimize its occurrence[25].

As a tertiary care hospital with radiotherapy facilities, we receive most patients as referrals from hospitals outside Karachi. Consequently, post-treatment follow-up for up to 90 days is typically conducted at their local centers for toxicity evaluation. Therefore, our study was limited to outcomes assessed up to the final day of treatment.

This study was a single-center investigation conducted in a tertiary care setting; therefore, the findings may not be generalizable to the broader population. The onset of severe OM was closely associated with the need for feeding tube placement, increased hospitalization rates, opiate dependence for pain control, and significant weight loss. These complications not only exacerbate the clinical burden but also lead to a marked decline in patients' quality of life (QOL) and impose substantial financial strain. Therefore, continued advancements in the prevention, early detection, and management of OM are essential to mitigate these detrimental outcomes and enhance overall patient well-being[26].

CONCLUSION

Acute oral mucositis remains highly prevalent in patients with oral cavity cancer undergoing concurrent chemoradiotherapy, with the majority developing Grade 3 OM by Week 7 of treatment. The severity and progression of OM did not differ significantly across age groups or gender, suggesting a broadly uniform risk profile among patients. These findings highlight the need for proactive, standardized OM prevention and management strategies to mitigate treatment-related morbidity and improve patient tolerance to therapy.

Future studies should focus on larger, diverse populations with external validation and standardized definitions for predictors and outcomes to strengthen evidence on OM in head and neck squamous cell carcinoma.

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Authors' Contribution: FA developed the concept of the research under the guidance of **JAM. AHO** conducted literature search and drafted the manuscript. **AH, SJ, AS, and HS** contributed to data collection. **QB** carried out data analysis and assisted in manuscript writing. **AHK** contributed to data analysis, literature search, and manuscript writing. **SH** also contributed to manuscript drafting. **JAM** provided over all supervision and final approval of the manuscript.

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ORIGINAL ARTICLE

Frequency and Clinical Association of Anti-CCP Positivity in Patients with Psoriatic Arthritis (PsA) and its Significance in Skeletal Involvement

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ABSTRACT

Objectives: To determine the prevalence of anti-CCP antibodies in patients with PsA and evaluate their association with clinical features.

Methodology: A cross-sectional study was conducted in the Department of Rheumatology, Liaquat National Hospital, Karachi. Sixty-one (61) PsA patients fulfilling CASPAR criteria were enrolled. Clinical assessments included joint distribution, axial/peripheral involvement, and skin manifestations. Anti CCP measured using ELISA Kits (cut-off value: <17 U/mL), X-rays, MRI, or CT scans were utilized where available, as part of routine clinical evaluation to characterize the pattern of skeletal involvement and were considered in conjunction with clinical findings to support the overall diagnosis. Associations between anti-CCP positivity and clinical features were analyzed using chi-square tests and logistic regression.

Results: Anti-CCP antibodies were detected in 31.1% of PsA patients. Anti-CCP positivity was significantly associated with the absence of skin involvement (p 0.008) and older age (p=0.025). No significant associations were observed between anti-CCP status and patterns of joint involvement, axial or peripheral disease. Logistic regression demonstrated significantly lower odds of anti-CCP positivity in patients with skin involvement (OR = 0.06, 95% CI: 0.006–0.582, p = 0.015).

Conclusion: A substantial proportion of PsA patients in this cohort tested positive for anti-CCP antibodies. Anti-CCP positivity may represent a distinct clinical subset in PsA and was associated with older age and an inverse relationship with skin involvement. It may also be associated with a distinct clinical pattern of musculoskeletal involvement. These findings highlight the potential role of anti-CCP antibodies in the clinical assessment of PsA and support their use in guiding diagnostic and therapeutic considerations.

Keywords: Anti-CCP antibodies, diagnostic biomarkers, joint involvement, psoriatic arthritis, rheumatoid arthritis, Pakistan

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INTRODUCTION

Psoriatic arthritis (PsA) is a chronic inflammatory disease in which arthritis is associated in most cases with psoriasis. The biological and clinical spectrum of PsA may present common elements with rheumatoid arthritis (RA; e.g., symmetrical arthritis of the hands, elevated acute phase proteins) or with the general class of spondyloarthropathies (e.g., dactylitis, enthesitis, sacroiliitis). Unfortunately, there is no specific serologic test for PsA. Rheumatoid factor (RF) contributed to the designation of PsA as an independent nosological entity, in the sense that patients with arthritis and psoriasis were usually seronegative for RF, differentiating them from RA patients, who are usually seropositive for RF, but its low specificity for RA motivated the search for a more reliable serologic test. Anti-cyclic citrullinated peptide antibodies (anti-CCP) met the demands: they proved a similar sensibility for RA (55-80%) but a higher specificity (96-98%)[1,2]. Anti-CCP antibodies have been detected in the early phase of RA and have been associated with severe radiological damage. A clinical prediction model, discriminating between self-limiting persistent non-

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erosive and persistent erosive arthritis, also includes the evaluation of anti-CCP antibodies[3]. Interestingly, citrullinated fibrin has been identified as one of the major citrullinated proteins in RA synovium, and anti-filaggrin, antikeratin antibodies, or anti-CCP antibodies have been detected in the synovial fluid (SF) of RA patients. Despite reports of the high specificity of the anti-CCP test, these antibodies have recently been detected in the serum of patients with psoriatic arthritis (PsA), suggesting relevant considerations about the correct diagnosis of this disease[4]. Anti-CCP antibodies are mainly produced in the synovium by the local plasma cells and are designed to bind to citrulline-containing antigenic determinants of synovial proteins. The enzyme peptidyl-arginine-deiminase generates citrulline residues by acting on the normal arginine residues[5].

In clinical practice, the titer of antiCCP antibodies is determined by an enzyme-linked immunosorbent assay (ELISA), using synthetic citrullinated peptides. The detection of anti-CCP antibodies may precede by several years the clinical onset of RA, for which they have a high positive predictive value and a strong association with female gender, disease activity, functional impairment, and erosive disease[6]. The studies that evaluated anti-CCP antibodies in PsA patients, reported a prevalence of 5.6-20%[7,8]. While PsA has been clinically studied in Pakistan, data on anti-CCP antibodies in this context remain scarce. A study at a tertiary care center reported 46% joint involvement in 100 psoriatic patients using Moll and Wright criteria[9]. Another study found a 46.4% PsA prevalence among 140 patients, all seronegative for RF, without assessing anti-CCP status[10].

Treatment-focused research has emphasized GRAPPA guidelines and individualized therapy, yet has overlooked anti-CCP antibodies. This gap highlights the need for evaluating anti-CCP antibodies to enhance diagnostic precision and aid differentiation of PsA from seronegative or atypical RA presentations, where clinical overlap can complicate diagnosis. While anti-CCP antibodies are highly specific for RA, their presence in PsA patients suggests potential diagnostic value.

In Pakistan, despite a growing body of clinical research on PsA, the prevalence and significance of anti-CCP antibodies in these patients remain largely uninvestigated. This study aims to address this gap by evaluating the presence of anti-CCP antibodies in PsA patients, thereby contributing to improved diagnostic accuracy and better-informed clinical decision-making in local rheumatology practice.

METHODOLOGY

IRB/ERC Approval: This cross-sectional study was conducted in the Department of Rheumatology, Liaquat National Hospital, Karachi, from December 2024 to June 2025. The study was approved by the Ethical Review Committee of Liaquat National Hospital (App#1122-2024-LNH-ERC), dated 4th December 2024.

The procedure was explained to each participant and informed consent was taken. Detailed clinical examinations were conducted to assess joint involvement, enthesitis, and psoriasis severity. Blood samples (1 to 5 milliliters (mL) per sample) were collected to determine aCCP positivity . Anti CCP was measured using ELISA Kits (cut-off value: <17 U/mL), X-rays, MRI, or CT scans where available, as part of routine clinical evaluation, , to characterize the pattern of skeletal involvement and were considered in conjunction with clinical findings to support the overall diagnosis. Radiological findings were not considered primary outcome measures, however, used only to support the overall clinical diagnosis. Anti-CCP testing was not available for all patients due to clinical practice variability within the institute, and only patients with available results were included in the anti-CCP analysis. Demographic and clinical characteristics of the patients were obtained from medical records. All the findings and examinations were recorded on a pre-designed pro forma. The inclusion criteria were:

1. Age > 18 years;
2. Diagnosis of PsA fulfilling CASPAR criteria;
3. Duration of disease greater than 6 months;
4. Availability of anti-CCP antibody testing (Either current or within the past 12 months);
5. Consent to participate.

The Exclusion Criteria were:

1. Patients with undifferentiated arthritis or overlap syndromes (e.g., PsA with lupus or systemic sclerosis);
2. Incomplete clinical or serological data (If core data points such as CCP status, RF, joint distribution, and psoriasis history are missing);
3. Pregnant or lactating individuals.

The data was entered and analyzed using Statistical Package for Social Sciences (SPSS-27). Chi-Square test and logistic regression were used to assess the association between aCCP positivity and the pattern of skeletal involvement, and to determine the odds ratio p-value <0.05 was set for statistical significance. Data was entered using SPSS (ver 27). Frequencies and percentages were computed. Chi-square test was used to find the association between the demographic and clinical outcomes with the frequency of aCCP. Logistic regression was utilized to find the possible effect of demographic/clinical outcomes to aCCP.

RESULTS

OpenEpi was used to determine the sample size with a prevalence of 4.5% of aCCP positive among PsA patients, and 95% confidence interval, ±5% maximum error in estimate, and 80% power of the test. This was a cross-sectional study. Convenient sampling was utilized to collect the information from the patients. Sixty-one (61) patients participated in the study. Twenty-six (26) were male, and the majority of them were between 18 and 40 years (49.2%) (Figure 1). Skin involvement was detected in 55 participants (90.2%). Regarding joint involvement, 44 participants (72.1%) had small joint involvement in terms of distal interphalangeal (DIP) joints. Seventeen participants (27.9%) were affected, compared to 44 (72.1%) who were not. Intermediate joints were involved in 19 participants (31.1%), and large joint involvement was observed in 44 participants (72.1%). (Figure 2) Inflammatory back pain was reported by 26 participants (42.6%). Peripheral involvement was present in 51 participants (83.6%), and axial involvement was reported by 26 participants (42.6%).

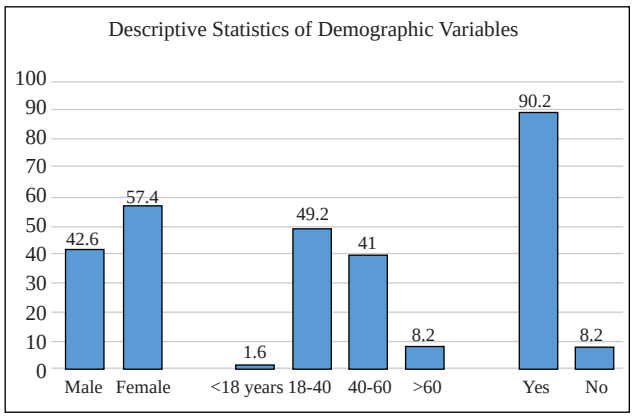

Figure 1: Descriptive Statistics for gender, age-groups, and skin involved

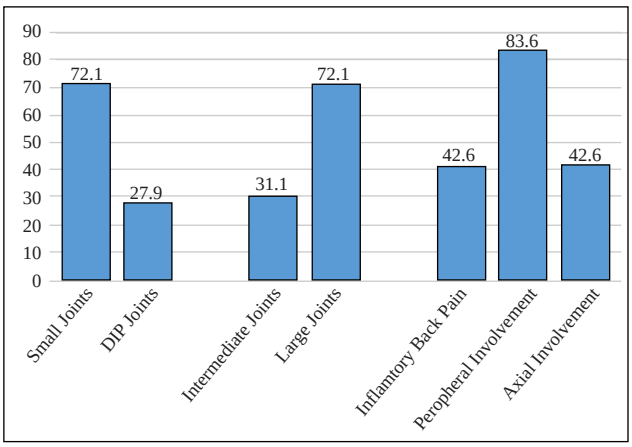

Figure 2: Descriptive Statistics of Joints Involved and Pain

Among the 45 participants tested for anti-CCP antibodies, 14 (31.1%) were positive for anti-CCP antibodies.

Table 1 shows the association of anti-CCP antibodies with various demographic and clinical factors. Anti-CCP antibody levels were assessed in a sample of 45 participants. Among males, 4 (25%) tested positive for anti-CCP, while 10 (34.5%) females tested positive. The difference was insignificant (p=0.738). The older patients (> 60 years) showed significantly higher positive anti-CCP (47.6%) than younger patients (16.7%) (p=0.025).

Table 1: Association of Anti-CCP with Clinical Features

Variable	Anti-CCP Positive n (%)	Anti-CCP Negative n (%)	Total (n)	p-value
Gender				
Male	4 (25.0)	12 (75.0)	16	0.738
Female	10 (34.5)	19 (65.5)	29	
Age Group				
18–40	4 (16.7)	20 (83.3)	24	0.025*
>60	10 (47.6)	11 (52.4)	21	
Skin Involvement				
Yes	9 (23.1)	30 (76.9)	39	0.008*
No	5 (83.3)	1 (16.7)	6	
Small Joint Involvement				
Yes	13 (38.2)	21 (61.8)	34	0.132
No	1 (9.1)	10 (90.9)	11	
DIP Joint Involvement				
Yes	6 (46.2)	7 (53.8)	13	0.286
No	8 (25.0)	24 (75.0)	32	
Intermediate Joint Involvement				
Yes	5 (45.5)	6 (54.5)	11	0.277
No	9 (26.5)	25 (73.5)	34	
Large Joint Involvement				
Yes	8 (28.6)	20 (71.4)	28	0.637
No	6 (35.3)	11 (64.7)	17	
Inflammatory Back Pain				
Yes	7 (33.3)	14 (66.7)	21	0.763
No	7 (29.2)	17 (70.8)	24	
Peripheral Involvement				
Yes	10 (28.6)	25 (71.4)	35	0.700
No	4 (40.0)	6 (60.0)	10	
Axial Involvement				
Yes	7 (33.3)	14 (66.7)	21	0.763
No	7 (29.2)	17 (70.8)	24	

Of those with skin involvement, 9 (64.3%) tested positive for anti-CCP, while 5 (83.3 %) without skin involvement tested positive. The association was statistically significant (p=0.008). None of the other factors (skin involved, small joints involved, DIP joints involved, intermediate involved, large joints involved, inflammatory back pain, peripheral involved, and axial involved) showed any statistically significant association with anti-CCP antibodies (p>0.05).

Binary logistic regression analysis was used to evaluate the odds of testing positive for anti-CCP in relation to various demographic and clinical factors (Table 2).

Table 2: Odds For Patients With Positive Anti Ccp (N=45)

	OR (95% CI)	p-value
Gender		
Male	0.633(0.162-2.483)	0.512
Female	Ref	
Age Group		
18-40 years	0.400(0.029-5.547)	0.495
40-60 years	2.000(0.153-26.187)	0.597
>60 years	Ref	
Skin		
Yes	0.060(0.006-0.582)	0.015*
No	Ref	
Small Joint involved		
Yes	6.190(0.708-54.157)	0.099
No	Ref	
DIP Joints Involved		
Yes	2.571(0.665-9.944)	0.171
No	Ref	
Intermediate Joints Involved		
Yes	2.135(0.565-9.484)	0.243
No	Ref	
Large Joint Involved		
Yes	0.733(0.202-2.662)	0.637
No	Ref	
Inflammatory Back Pain		
Yes	1.214(0.343-4.298)	0.763
No	Ref	
Peripheral Involvement		
yes	0.600(0.139-2.590)	0.494
No	Ref	
Axial Involvement		
Yes	1.214(0.343-4.298)	0.763
No	Ref	

None of the demographic or clinical factors showed any statistically significant odds ratio, except the skin involved. Skin involved (yes) showed a significantly low odd ratio of 0.06 (CI: 0.006 – 0.582) with p value of 0.015.

Since the univariate analysis showed no significant difference for any explanatory variable except the skin involved, a multivariate binary logistic regression was attempted.

Table 3 shows the bivariate correlation ; that only age, skin, and small joints involved had significant correlation with aCCP, keeping p-value <0.1, as the standard for entry of variable in the equation. The multivariate logistic analysis showed that the skin involved is the only variable with p<0.05.

Table 3: Model Developed by the Multivariate Logistic Regression

B		S.E.	Wald	df	p-value	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
Skin	3.005	1.274	5.562	1	.018	20.184	1.661	245.233
F			4.502	2	.105			
Age(1)	-.105	1.722	.004	1	.952	.901	.031	26.322
Age(2)	1.577	1.723	.837	1	.360	4.839	.165	141.766
Constant	-.425	1.560	.074	1	.785	.654		

DISCUSSION

Prevalence and Clinical Significance of Anti-CCP Antibodies in PsA

Based on our study findings, 31.1 % of patients with psoriatic arthritis (PsA) tested positive for anti-cyclic citrullinated peptide (anti-CCP) antibodies. This percentage of anti-CCP positivity is notable higher than the previously reported range of 7-17 %. In comparison to rheumatoid arthritis (RA), where 70 - 85 % of patients show positive results for anti-CCP antibodies, the rate in PsA is considerably lower. Anti-CCP antibodies are commonly known as an indicator for RA, yet their significance in PsA remains unexplored[11,12]. A research study involving 77 individuals with PsA, found that 20% tested positive for anti-CPP antibodies. This percentage is higher than that seen in the general population but is similar to that of psoriasis patients without joint complications[4]. These results indicate that although anti-CPP antibodies are less frequent in PsA than RA, they still play a significant role within the spectrum of psoriatic diseases.

Various reasons contribute to the differences in the presence of anti-CCP antibodies among individuals with PsA. Variances in the criteria used to select participants for studies have an impact on research outcomes. Moreover, factors such as standards and testing techniques influence the reported prevalence rates significantly. Genetic elements also play a role,

with the presence of the HLA-DRB1 shared epitope being linked to anti-CCP positivity and potentially influencing how these antibodies develop in PsA patients[13]. The differences may stem from variations in the detection kits and positivity thresholds used. This further adds to the challenge of maintaining consistency in prevalence data[14,15]. By establishing updated detection methods and criteria for diagnosis, we can potentially address these variations, thereby enhancing the reliability of anti-CCP testing.

Though not as common in PsA, anti-CCP antibodies hold significant clinical importance due to their association with more severe disease manifestations in patients. Literature also indicates that individuals testing positive for anti-CCP antibodies are more likely to experience a polyarticular form of arthritis and be at risk of developing joint erosions and substantial deformities[11,13,16]. Analysis has revealed that PsA patients with anti-CCP antibodies typically have a more extensive joint involvement and experience heightened disease activity compared to individuals without these antibodies[17]. Therefore, this connection indicates that there might be a more aggressive spectrum of PsA when the level of serum anti-CCP antibodies is elevated.

It is also important to mention that individuals with PsA who test positive for anti-CCP antibodies are typically older, 62.43 years of age on average, compared to those who test negative and are usually 47.59 years old on average. This age contrast could signify a more chronic disease progression in individuals with anti-CCP antibody positivity. Furthermore, PsA patients with anti-CCP antibodies tend to experience broader and more severe manifestations of joint disease as they tend to show involvement of metacarpophalangeal, elbow, and shoulder joints. In our study, anti-CCP positivity demonstrated a significant inverse association with skin involvement, with patients lacking cutaneous manifestations more likely to be anti-CCP positive, a finding further supported by regression analysis showing significantly lower odds in patients with skin involvement. These findings suggest that anti-CCP antibodies could be a marker for more extensive disease in PsA.

Comparison with Rheumatoid Arthritis

The function of anti-CCP antibodies in PsA shows a notable contrast to their function in RA. In the case of RA, specifically, anti-CCP antibodies serve as an indicator of the disease, predicting the advancement and severity of the condition[13,17]. These antibodies are associated with increased disease activity, erosive effects, and more pronounced functional impairment compared to individuals who do not possess these antibodies[18]. Contrary to this, anti-CCP antibodies, despite being associated with severe disease characteristics like polyarthritis and erosive alterations, show a less pronounced effect in PsA when compared to RA[12,19]. For instance, a study revealed that PsA patients with anti-CCP positivity often experience severe clinical symptoms but do not consistently display significant radiographic changes[12,20]. The results indicate that anti-CCP antibodies can be associated with disease severity in PsA, although not to the same intensity or in the same way as in RA. In PsA, the connection between anti–CCP antibodies and radiological results presents a complex scenario, unlike RA. In RA, anti-CCP antibodies strongly correlate with bone erosion and joint deterioration, serving as a distinct indicator of disease advancement[21,22].

In PsA, however, the link between anti-CCP antibodies and radiologic consequences appears definitive. Patients with PsA who test positive for anti-CCP antibodies tend to experience more severe clinical manifestations, such as increased joint swelling and higher chances of developing erosive arthritis, compared to those who test negative for the antibody. It is important to understand that these symptoms may not always result in changes when viewed through imaging[10,19,20]. This discrepancy suggests differences in the underlying mechanisms of PsA and RA despite some overlapping characteristics between the two conditions.

In RA, specifically, the presence of anti-CCP antibodies is linked to bone damage through processes involving the activation of osteoclasts and the production of inflammatory cytokines[21]. In PsA, a similar mechanism takes place, but more moderate results are observed. Some research indicates that certain PsA patients possess anti-CCP antibodies in their system. However, they may not be pivotal in the disease process compared to rheumatoid arthritis RA[20].

PsA is distinguished by the joint's asymmetrical inflammation and heightened cytokine levels like IL-17 and IL-23. In contrast, RA manifests with symmetrical joint involvement and increased levels of IL-2, IL-33, and TNF á-levels[6]. The lower occurrence and inconsistent connection of anti-CCP antibodies with the consequences of PsA raises the need to explore other elements, such as genetic predisposition and environmental influences that could impact the advancement of the disease[11,18].

Diagnostic and Management Challenges

The presence of anti-CCP antibodies often impacts the response to treatment in PsA patients. Various studies show that PsA patients with anti-CCP antibodies are

less likely to exhibit favorable responses to TNF inhibitors than PsA without these antibodies. The activation of inflammatory pathways by anti-CCP antibodies interferes with TNF inhibitors' effectiveness in independently targeting these pathways. This resistance against TNF inhibitors can be attributed to the immune response triggered by the presence of anti-CCP antibodies[2]. The pressing issue is the importance of gaining an insight into the immune pathways triggered by anti-CCP antibodies to explore other treatment options or additional approaches for PsA patients who test positive for anti-CCP antibodies. Tailoring treatment plans based on the detection of anti-CCP antibodies can show the potential to enhance disease outcomes.

The presence of anti-CCP antibodies in PsA introduces a new complexity to the diagnosis and treatment process. It can be difficult to distinguish enthesopathy in patients with psoriasis from cases of overt PsA since as many as half of all psoriasis patients may have subclinical enthesopathy that could develop into PsA[5]. Identifying and addressing these issues early is crucial since delayed diagnosis can result in worse consequences, such as erosion of peripheral joints and reduced physical capabilities[8,9]. Therefore, tackling these diagnostic hurdles and ensuring early interventions for improved patient outcomes is essential. Research is still being conducted on the genetic basis of PsA. Although certain genetic loci, like PSORS1 on chromosome 6, are known, the full genetic makeup of PsA remains elusive[23]. It is pivotal to identify and comprehend how certain genetic variations impact disease vulnerability and response to treatment to progress further in this study area. Genome-wide association studies (GWAS) and further research examining HLA alleles could help connect the dots in understanding the intricate pathogenesis of PsA, thereby enhancing our capacity to provide customized and efficient therapies. It is recommended that forthcoming studies delve deeper into investigating the significance of anti-CCP antibodies and other biological markers in the pathogenesis of PsA to achieve improved treatment responses and disease outcomes.

CONCLUSION

In our study, anti-CCP antibodies were found in 31.1% of the PsA patients studied. Although this is far higher than previously reported figures, it still falls short of the percentage reported for RA. Anti-CCP positivity may represent a distinct clinical subset in PsA and was associated with older age and inverse relationship with skin involvement. Anti-CCP positivity is a determinant of disease outcome in PsA as it is associated with a distinct clinical pattern of musculoskeletal involvement in PsA. Patients with anti-CCP positivity tend to be insensitive to TNF inhibitors and should receive alternative or supplementary treatment. These results emphasize the potential of anti-CCP antibodies as a marker for more severe PsA and emphasize the requirement for tailored treatments for better patient outcomes.

Limitation

This study is limited by its single -center design and relatively small sample size, which may affect the generalizability of the findings.

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ORIGINAL ARTICLE

Clinical Efficacy of Azithromycin Versus Doxycycline in Patients of Acne Vulgaris

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ABSTRACT

Objective: To determine the clinical efficacy of azithromycin versus doxycycline in patients of acne vulgaris.
Methodology: A quasi-experimental study Was conducted at the Department of Dermatology, Dow University of Health Sciences (DUHS), Karachi, from 09-07-2025 to 10-10-2025. The patients diagnosed with acne vulgaris were randomly distributed into Group A (Azithromycin Group) and Group B (Doxycycline Group). Group A patients were treated with oral azithromycin in a dose of 500 mg once a day and three times a week, whereas patients in group B received oral doxycycline in a dose of 100 mg once a day. All patients were additionally given 0.1% topical adapalene. Follow-up of each patient was performed after 6 weeks and 12 weeks, and improvement was measured after 12 weeks of therapy.
Results: Of the 122 patients with acne vulgaris, males were 36.1% (n=22) and 31.1% (n=19), and females were 63.9% (n=39) and 68.9% (n=42) in the Azithromycin and Doxycycline groups, respectively. There was no discernible difference between the two groups' levels of severity before treatment (p-value=0.917) and after six weeks of treatment (p-value=0.994), while a significant difference is seen after twelve weeks of treatment (p-value=0.018). Improvement in terms of clinical efficacy was significantly (p-value=0.006) higher in the Doxycycline group than in the Azithromycin group.
Conclusion: The clinical efficacy of azithromycin and doxycycline was similar after six-weeks of treatment, while doxycycline showed significantly higher clinical efficacy than azithromycin after twelve weeks of treatment in the management of acne vulgaris.

Keywords: Acne vulgaris, adolescents, azithromycin, doxycycline

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INTRODUCTION

Acne vulgaris is currently the commonest dermatological problem internationally and nationally[1]. It is a common, chronic inflammatory condition of the pilosebaceous unit (which includes the sebaceous gland and hair follicle). It is largely characterized by excessive sebum production, bacterial colonization, follicular hyperkeratinization, and inflammation. The disorder is distinguished by the chronic or recurrent formation of pustules, erythematous papules, and comedones[2]. It is a relatively common skin condition that mostly affects the face but can also affect the upper arms, back and trunk. It can manifest as inflammatory and non-inflammatory lesions[3].

With an approximate global frequency of 9.79% (across all age groups), it is among the most prevalent skin conditions[4]. Acne prevalence varies significantly among nations and age groups; estimates range from 35% to almost 100% of adolescents experiencing acne at some time[5]. One of the most prevalent skin disorders, acne mostly affects people in their teenage years and early teens. In 2019, acne vulgaris caused 4.96 million DALYs worldwide including 3.52 million DALYs that belonged to those aged 15 to 49. Acne was the 27th leading cause of increasing DALYs in the 10–24 age group in 1990, and it rose to the 19th position in 2019[6,7]. According to a recent report, 16.8% of university students in Pakistan have acne. Acne may pose a significant burden by significantly impacting the quality of life as well as moods of affected

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individuals, including an increased risk of anxiety, depression, and suicidal ideation[5-8].

Acne vulgaris is normally thought of as a benign, self-limiting disorder; however, it can result in serious psychological issues as well as disfiguring scars. There are numerous formulations and therapeutic agents available, each of which targets a distinct aspect of the pathophysiology of acne. The severity of the condition, preference of the patient, and tolerance are taken into consideration while choosing a treatment[9,10]. The guidelines for acne therapy states that some systemic and topical treatments are still considered standard. However, new therapy approaches are still required[11]. Currently, oral antibiotics are widely used in managing acne vulgaris. Azithromycin is a macrolide antibiotic that binds to the 50S ribosomal subunit to prevent bacterial protein synthesis. It also has immunomodulatory properties that minimize inflammatory acne lesions. However, it may cause gastrointestinal problems and raise worries about antibiotic resistance. On the other hand, doxycycline is a tetracycline antibiotic that binds to the 30S ribosomal subunit to prevent bacterial protein synthesis. It also has anti-inflammatory properties that minimise inflammatory acne lesions. It may also cause gastrointestinal problems and photosensitivity, especially after prolonged treatment[12,13]. Systemic antibiotics with topical agents have better safety profile and fewer side effects. Therefore, Azithromycin and Doxycycline are the systemic antibiotics of choice for managing acne in clinical practice across the world[14,15].

The prevalence and treatment of acne differ by gender, ethnicity, and age group in different nations. This is mostly because of environmental variables, social behaviours, and genetic reasons. As of right now, not much is known about the prevalence, distribution, and treatment of acne in Pakistan. Thus, there is a need to evaluate various treatment modalities and compare their efficacy in management of acne vulgaris. Therefore, this study has been designed at dermatology department of Dow University of Health Sciences (DUHS), Karachi in order to determine the clinical effectiveness of azithromycin as well as doxycycline in patients of acne vulgaris.

METHODOLOGY

IRB/ERC Approval: This study was conducted with approval from the Dow University of Health Sciences, Karachi, Institutional Review Board (IRB), through letter number 4050 dated 08-07-2025.

A quasi-experimental study on acne vulgaris patients was conducted at the dermatology department of Dow University of Health Sciences (DUHS), Karachi, from 09-07-2025 to 10-10-2025. Sample size was calculated using Open EPI software available online. This software uses the proportion of a previous study by Arjel et al., which found a 42.5% improvement with Azithromycin and a 67.5% improvement with Doxycycline after 12 weeks of treatment[15]. By taking a two-sided significance level of 95% and a power of 80%, the sample size stands to be n = 122 (61 in each group).

The study included (1) diagnosed cases of acne vulgaris who had been suffering from acne vulgaris for the past three months, (2) patients aged 14 to 45 years, and (3) both male and female patients. The study excluded (1) pregnant or lactating mothers, (2) patients already on medications like isotretinoin, oral contraceptives, hormonal therapy or retinoids, (3) patients who refused to participate in the research, (4) patients with eczema and photosensitive disorders, and (5) patients with known hypersensitivity to Azithromycin or Doxycycline.

Outpatients visiting the Outpatient Department of Dermatology and meeting the inclusion criteria were recruited. Each patient's specific demographic information, including age, marital status, educational status, residence and job status, were obtained. Each acne patient was evaluated for the duration of acne, the location of acne, and for acne severity by using a simple acne grading system. Patients were included in a 12-week therapy plan and were randomly distributed (lottery method) into Group A and B. Group A patients were treated with oral azithromycin in a dose of 500 mg once a day and three times a week, whereas group B patients with oral doxycycline in a dose of 100 mg once a day. All patients were additionally given 0.1% topical adapalene. Follow-up of each patient was performed after 6 weeks and 12 weeks, and improvement was measured after 12 weeks of therapy. The Simple Acne Grading System was utilized for confirming the diagnosis and grade of acne vulgaris (Table 1). Clinical efficacy or improvement with treatment was evaluated at the end of treatment and confirmed by the presence of Grade zero (non-existence of the lesion). Safety of the drugs in acne vulgaris patients was confirmed by the presence of various side effects such as nausea, abdominal pain, diarrhoea, headache, photosensitivity, etc.

Table 1: Simple Acne Grading System

Grade	Explanation
Grade 0	Non-existence of the lesion
Grade I	Comedones, occasional papules
Grade II	Papules, comedones, few pustules
Grade III	Predominant pustules, nodules, abscesses
Grade IV	Mainly cysts, abscesses, widespread scaring

Data analysis was performed with Statistical Package for Social Science (SPSS) software, Version 25. Mean, and standard deviation was calculated for age (years) and duration of disease (months). Frequency and percentages were computed for gender, age classes, marital status, and educational status, and job status, duration of disease in groups, acne location, acne severity, and improvement in both groups. A post-stratification chi-square and Independent sample t-test test was applied with a p value of < 0.05 as significant to compare data of both groups.

RESULTS

Of the 122 patients with acne vulgaris, 61 were treated with Azithromycin, while 61 were treated with Doxycycline. In the Azithromycin group, male acne vulgaris patients were 36.1% (n=22) and female acne vulgaris patients were 63.9% (n=39), while in the Doxycycline group, male acne vulgaris patients were 31.1% (n=19) and female acne vulgaris patients were 68.9% (n=42). The mean age in the Azithromycin group was 20.9 ± 6.6 years, while the mean age in the Doxycycline group was 20.4 ± 6.6 years. The comparison of demographic variables between the two groups shows no significant differences, such as gender (p-value=0.565), mean age (p-value=0.672), age group comparison (p-value=0.835), marital status (p-value=0.389), educational status (p-value=0.900), job status (p-value=0.827), and residence (p-value=0.555) [Table 2].

Similarly, a comparison of acne vulgaris disease between the two groups does not show any significant difference, such that the mean duration of acne vulgaris in the Azithromycin and Doxycycline groups was 8.5 ± 2.6 and 7.9 ± 2.6 months (p-value=0.214), respectively. The most common acne vulgaris locations were the forehead, right cheek and left cheek in 44.3% (n=27) and 39.3% (n=24) of patients, followed by the forehead, right cheek, left cheek, nose, chin, chest and upper back in 23% (n=14) and 27.9% (n=17) of patients, the forehead, right cheek, left cheek and nose in 19.7% (n=12) and 23% (n=14) of patients, and the forehead, right cheek, nose and chin in 13.1% (n=8) and 9.8% (n=6) of patients (p-value=0.824) in the Azithromycin and Doxycycline group, respectively [Table 3].

Similarly, a comparison of acne vulgaris severity before treatment and after six weeks of treatment between the two groups does not show any significant difference. Before treatment, group II had the most common acne severity reported in 54.1% (n=33) and 52.5% (n=32) of patients, followed by group III in 31.1% (n=19) and 34.4% (n=21) of patients, and group IV in 14.8% (n=9) and 13.1% (n=8) of patients (p-value=0.917) in the Azithromycin and Doxycycline groups, respectively. After six weeks of treatment, grade I had the most common acne severity reported in 44.3% (n=27) and 44.3% (n=27) of patients, followed by grade II in 25 (41%) (n=25) and 24 (39.3%) (n=24) of patients, grade III in 8 (13.1%) (n=8) and 9 (14.8%) (n=9) of patients, and grade IV in 1.6% (n=1) and 1.6% (n=1) of patients (p-value=0.994) in the Azithromycin and Doxycycline groups, respectively [Table 4].

Table 2: Demographics in Acne Vulgaris Patients in the Azithromycin and Doxycycline Groups (n=122)

Demographic Variables		Azithromycin Group	Doxycycline Group	P-Value
Gender	**Male**	22 (36.1%)	19 (31.1%)	0.565
	Female	39 (63.9%)	42 (68.9%)	
	Mean ± SD	20.9 ± 6.6 (14-40)	20.4 ± 6.6 (14-40)	0.672
Age (Years)	**14-20**	33 (54.1%)	35 (57.4%)	0.835
	21-30	20 (32.8%)	17 (27.9%)	
	31-40	8 (13.1%)	9 (14.8%)	
Marital Status	**Single**	45 (73.8%)	49 (80.3%)	0.389
	Married	16 (26.2%)	12 (19.7%)	
	Primary	6 (9.8%)	7 (11.5%)	
Educational Status	**Matriculation**	26 (42.6%)	23 (37.7%)	0.900
	Intermediate	14 (23.0%)	17 (27.9%)	
	Graduate	15 (24.6%)	14 (23.0%)	
	Student	37 (60.7%)	39 (63.9%)	
Job Status	**Housewife**	10 (16.4%)	7 (11.5%)	0.827
	Indoor Job	8 (13.1%)	10 (16.4%)	
	Outdoor Job	6 (9.8%)	5 (8.2%)	
Residence	**Rural**	17 (27.9%)	20 (32.8%)	0.555
	Urban	44 (72.1%)	41 (67.2%)	

Table 3: Disease Details in Acne Vulgaris Patients in the Azithromycin and Doxycycline Groups (n=122)

Variables		Azithromycin Group	Doxycycline Group	P-Value
Duration of Disease (months)	**Mean ± SD**	8.5 ± 2.6 (3.5-12)	7.9 ± 2.6 (3.5-12)	0.214
Acne Location	**Forehead + Right Cheek + Left Cheek**	27 (44.3%)	24 (39.3%)	0.824
	Forehead + Right Cheek + Left Cheek + Nose	12 (19.7%)	14 (23.0%)	
	Forehead + Right Cheek + Nose + Chin	8 (13.1%)	6 (9.8%)	
	Forehead + Right Cheek + Left Cheek + Nose + Chin + Chest & Upper Back	14 (23.0%)	17 (27.9%)	

Table 4: Pre and Post Treatment Acne Severity in Acne Vulgaris Patients in the Azithromycin and Doxycycline Groups (n=122)

Acne Severity		Azithromycin Group	Doxycycline Group	P-Value
Pre-Treatment	**Grade II**	33 (54.1%)	32 (52.5%)	0.917
	Grade III	19 (31.1%)	21 (34.4%)	
	Grade IV	9 (14.8%)	8 (13.1%)	
Six Weeks After Treatment	**Grade I**	27 (44.3%)	27 (44.3%)	0.994
	Grade II	25 (41.0%)	24 (39.3%)	
	Grade III	8 (13.1%)	9 (14.8%)	
	Grade IV	1 (1.6%)	1 (1.6%)	
12 Weeks After Treatment	**Grade 0**	28 (45.9%)	43 (70.5%)	0.018*
	Grade I	27 (44.3%)	16 (26.2%)	
	Grade II	6 (9.8%)	2 (3.3%)	

* Statistically Significant P-Value

Table 5: Side Effects in Acne Vulgaris Patients in the Azithromycin and Doxycycline Groups (n=122)

Acne Severity		Azithromycin Group	Doxycycline Group	P-Value
Side Effects	**Yes**	7 (11.5%)	10 (16.4%)	0.433
	No	54 (88.5%)	51 (83.6%)	
Type of Side Effects	**Nausea**	3 (42.9%)	4 (40.0%)	0.645
	Abdominal Pain	2 (28.6%)	2 (20.0%)	
	Diarrhea	0 (0.0%)	2 (20.0%)	
	Headache	2 (28.6%)	2 (20.0%)	

After twelve weeks of treatment, a comparison of acne vulgaris severity between the two groups showed a significant difference (p-value=0.018). After twelve weeks of treatment, grade 0 was the most common acne severity reported in 45.9% (n=28) and 70.5% (n=43) of patients, followed by grade I in 44.3% (n=27) and 26.2% (n=16) of patients, and grade II in 9.8% (n=6) and 3.3% (n=2) of patients in the groups of azithromycin and doxycycline, respectively [Table 4]. Side effects were compared between the two groups which also showed no significant differences (p-value=0.433) [Table 5].

Improvement in terms of clinical efficacy was significantly (p-value=0.006) higher in the Doxycycline group than in the Azithromycin group. Improvement in the Azithromycin group was reported in 45.9% (n=28) of patients, while improvement in the Doxycycline group was reported in 70.5% (n=43) of patients [Figure 1].

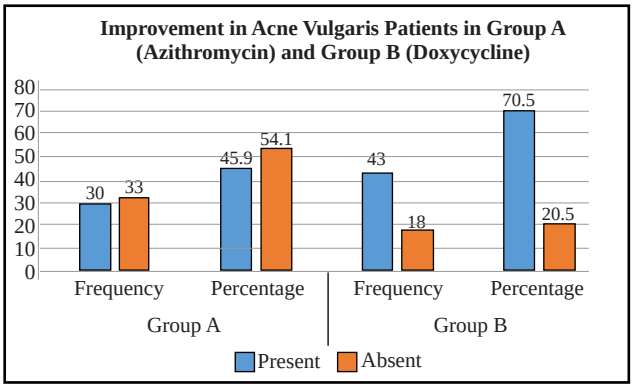

Figure I : Outcome in Acne Vulgaris Patients

DISCUSSION

Acne vulgaris is a common, chronic cutaneous inflammatory disorder of the pilosebaceous units, most commonly affecting the adolescents and young adults throughout the world[16,17]. Despite the fact that acne vulgaris is not fatal, it can significantly affect the health of patients and cause various health problems, including irritation, pain, or scars after completion of treatment, and psychological problems, including anxiety, depression, or low self-esteem, and negatively affect the quality of life[18,19].

Early diagnosis with appropriate management is always essential for controlling acne vulgaris. The efficacy, tolerance, and side effects of present treatments (such as hormone therapy, retinoid, and antibiotics) vary, and they might not be equally successful for all patients. It is normal practice to prescribe antibiotics for at least 6 to 8 weeks to assess their efficacy in treating acne vulgaris, but in cases of failure to achieve complete resolution of acne vulgaris, antibiotics may be prescribed for 12 to 18 weeks or longer to achieve complete resolution[12,13,20].

Therefore, this study compares the clinical efficacy of azithromycin and doxycycline for 12 weeks in patients with acne vulgaris. Of the 122 patients with acne vulgaris, 61 were treated with Azithromycin, while 61 were treated with Doxycycline. Female patients were predominantly suffering from acne vulgaris compared to male patients. 63.9% (n=39) and 68.9% (n=42) of female patients and 36.1% (n=22) and 31.1% (n=19) of male patients were presented with acne vulgaris in the Azithromycin and Doxycycline groups, respectively. The mean age in the Azithromycin group was 20.9 ± 6.6 years, while the mean age in the Doxycycline group was 20.4 ± 6.6 years. Most patients with acne vulgaris fall into the age group of 14-20 years. A similar female predominance and age group were reported by other researchers. A study by Arjel et al. reported 62.5% of female and 37.5% of male patients with acne vulgaris. The mean age was 20.1 ± 4.74 years in the Azithromycin group and 19.35 ± 4.89 years in the Doxycycline group[15]. A study by Raees et al. reported acne vulgaris in 55.3% of female and 44.7% of male patients and a mean age of 21.30±4.93 years[21]. A study by Iqbal et al. reported acne vulgaris in 55.0% of female and 45.0% of male patients and a mean age of 21.24±3.84 years[22]. Female predominance with acne vulgaris may be due to higher hormonal changes in females, particularly due to the menstrual cycle or polycystic ovary syndrome. This age group (< 20 years) indicates that most patients with acne vulgaris were in puberty and early adolescence, during which most hormonal changes occur, particularly increased androgen levels that trigger the sebaceous glands, resulting in the development of acne[15,23,24].

A comparison of acne vulgaris severity between the two groups showed no significant difference before treatment (p-value=0.917) and after six weeks of treatment (p-value=0.994), while a significant difference was seen after twelve weeks of treatment (p-value=0.018). Before treatment, group II was the most common acne severity reported in 54.1% (n=33) and 52.5% (n=32) of patients in the Azithromycin and Doxycycline groups, respectively. After six weeks of treatment, most patients improved to grade I [44.3% (n=27) and 44.3% (n=27)], and after twelve weeks of treatment, most patients improved to grade 0 [45.9% (n=28) and 70.5% (n=43)], in the Azithromycin and Doxycycline groups, respectively. Over all, improvement in terms of clinical efficacy was significantly (p-value=0.006) higher in the Doxycycline group (70.5%) as compared to the Azithromycin group (45.9%). A similar findings and efficacy of azithromycin and doxycycline in patients of acne vulgaris were reported by other researchers. A study by Arjel et al. reported the similar efficacy of azithromycin and doxycycline after six weeks of treatment (p-value=0.771), while a significant difference is seen after twelve weeks of treatment (p-value=0.035)[15]. A study by Raees et al. reported the similar efficacy of azithromycin (83.3%) and doxycycline (86.7%) in the management of acne vulgaris after twelve weeks of treatment[21]. A study by Iqbal et al. also reported the similar efficacy of azithromycin and doxycycline in the management of acne vulgaris after twelve weeks

of treatment[22]. The findings of both drugs indicate the equally effective management of acne vulgaris for a short period of time (six weeks), while doxycycline shows greater effectiveness in the management of acne vulgaris for a long period of time (twelve weeks). This indicates that doxycycline may be a superior option for long-term management of acne vulgaris, particularly in patients that require persistent improvement[15,23,24].

The comparison of side effects between the two groups also showed no significant differences (p-value=0.433). Side effects were non-significantly higher in the Doxycycline group (16.4%) as compared to the Azithromycin group (11.5%). The most common side effect was nausea reported in 42.9% (n=3) and 40% (n=4) of patients, followed by abdominal pain in 28.6% (n=2) and 20% (n=2) of patients, headache in 28.6% (n=2) and 20% (n=2) of patients, and diarrhoea in 0% (n=0) and 20% (n=2) of patients in the Azithromycin and Doxycycline groups, respectively. A study by Arjel et al. reported a similarly high rate of side effects in the management of acne vulgaris with doxycycline (22.5%) compared to azithromycin (15%)[15]. A study by Amatya et al. also reported a similarly high rate of side effects in the management of acne vulgaris with doxycycline (22.6%) compared to azithromycin (20%)[24]. This indicates that doxycycline may be a superior option for twelve weeks of treatment of acne vulgaris but is also associated with more side effects than azithromycin.

There are some limitations associated with this quasi-experimental study. First, the small sample size and single-centre design of this study may limit generalisability of the study. Second, the lack of long-term monitoring to evaluate long-term treatment efficacy and potential side effects of Azithromycin and Doxycycline in the management of acne vulgaris are shortcomings of our study.

CONCLUSION

The clinical efficacy of azithromycin and doxycycline was similar after six weeks of treatment, while doxycycline showed significantly higher clinical efficacy than azithromycin after twelve weeks of treatment in the management of acne vulgaris. This indicates that doxycycline may be a superior option for long-term management of acne vulgaris, particularly in patients that require persistent improvement.

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Authors' Contribution: **NK** developed the concept of this research; **NK** and **FAK** searched literature and drafted the manuscript; **TI** provided supervision, financial support, and critical guidance; **SAA** contributed in the validation of data and final review of the manuscript.

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REVIEW ARTICLE

Epigenetic Regulation of Antibiotic Resistance in Bacteria

Syeda Fatima Zohair

ABSTRACT

Objective: The objective is to understand how epigenetic mechanisms, such as DNA methylation and nucleoid-associated protein modifications, which regulate gene expression without altering the underlying DNA sequence, are associated with persistence and antibiotic resistance phenotypes in bacteria, which enable them to evade host immune responses and resist antimicrobial agents. The study aims to understand how epigenetic processes that control bacterial gene activity without changing DNA, help bacteria survive antibiotics and avoid immune system attacks.

Methodology: Peer-reviewed research articles, systematic reviews, and meta-analyses published between 1996 and 2024 were prioritized for inclusion, along with earlier foundational studies where necessary. Global health reports from organizations such as WHO and CDC were also consulted for epidemiological context. The keywords, *"bacterial epigenetics," "DNA methylation," "histone-like protein modification," "nucleoid-associated proteins," "RNA regulation," "epigenetic inheritance," "antibiotic resistance,"* and *"antimicrobial tolerance"* were used to search literature.

Results: This review finds that while the well-researched genetic factors are major influencers of bacterial resistance, genetic factors alone do not fully determine virulence due to the growing number of resistant strains. Epigenetic mechanisms also contribute by regulating gene expression without introducing permanent mutations. Rapid bacterial adaptations to antibiotic environments, and the transmission of resistance-associated phenotypes to daughter cells, have been shown to persist in some bacterial species across multiple generations under sustained selective pressure. These findings suggest that incorporating epigenetic targets into existing antimicrobial treatment strategies may improve therapeutic outcomes against resistant bacterial infections. They highlight that this dual-targeting approach may reduce the likelihood of pathogen adaptation, as it simultaneously disrupts multiple resistance-associated mechanisms available for the cell to defend itself. The practical implications of these systems could potentially lead to a decrease in the global recurrence of resistance cases.

Conclusion: Targeting bacterial epigenetic mechanisms, either through inhibitors of methyltransferases and other regulatory enzyme, or through epigenome editing tools in combination with existing antibiotics, represents a promising way to enhance antibiotic efficacy and reduce the emergence of resistance.

Keywords: Antibiotic resistance, antimicrobial strategies, bacterial pathogens, epigenetics, gene regulation

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INTRODUCTION

Antibiotic resistance has emerged as a global modern medicinal challenge primarily due to bacterial evolution.[1] Antibiotic resistance refers to the ability of bacteria to survive or proliferate in the presence of antibiotic concentrations that would otherwise inhibit their growth or cause cell death. Bacterial cells adapt to the presence of these drugs, reducing their effectiveness and contributing to the increasing prevalence of difficult to treat bacterial infections in the population, which threatens the health of millions of people and livestock. The 2022 Global Antimicrobial Resistance and Use Surveillance System (GLASS) report, drawing on data from 76 countries, highlighted the widespread nature of this resistance[2]. It reported 42% third-generation cephalosporin-resistant *Escherichia coli (E. coli)* and 35% methicillin-resistant *Staphylococcus aureus*, alongside an increase in resistant *Klebsiella pneumoniae (K. pneumoniae)* strains, and decreased antibiotic susceptibility in *E. coli* isolates associated with urinary tract infections[3]. The ability of bacterial cells to adapt to antibiotics has widely been linked to genetic factors, primarily horizontal gene transfer (HGT) and genetic mutations[4]. In HGT, cells undergo theoretically similar processes by the names of conjugation, transformation, and transduction which refer, respectively, to cells acquiring resistance genes

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from other bacterial cells via a 'sex pilus', cells uptaking foreign DNA from their environment, and cells transferring DNA in a process mediated by viruses such as bacteriophages[5]. Besides that, resistance may be built by genetic mutations as bacteria have rapid reproduction rates which cause mutations to accumulate in their DNA[6].

Figure 1 illustrates the major genetic mechanisms underlying antimicrobial resistance in bacterial cells, including horizontal gene transfer, spontaneous mutations, IS element insertions, two-component systems and post-transcriptional attenuation, all of which collectively drive the acquisition and dissemination of resistance.

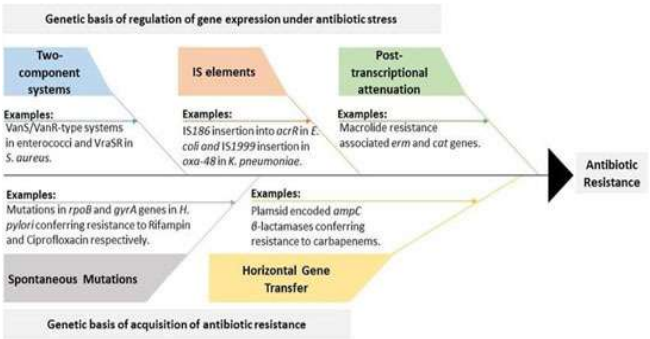

Figure 1: Shows genetic basis of antimicrobial resistance in bacterial cells. Besides mutations and HGT, transcriptional attenuation, a regulatory mechanism in which the elongation or termination of an RNA transcript is modulated in response to cellular signals before transcription is completed. (Adapted from Ghosh et al,[7]).

Purpose of the Review

The purpose of this review is to examine the expanding role of epigenetic mechanisms in shaping bacterial responses to antibiotic exposure. While genetic mutations and horizontal gene transfer are well-recognized contributors to antimicrobial resistance, emerging evidence shows that bacteria also employ non-mutational regulatory pathways to modulate gene expression, enhance survival, and transmit adaptive traits across generations. This review aims to synthesize current knowledge on how epigenetic mechanisms such as DNA methylation, nucleoid-associated protein (NAP) modifications, nucleoid remodeling, and RNA-mediated regulation contribute to virulence, persistence, and the development of resistance phenotypes. By integrating insights from molecular microbiology, genomics, and clinical research, the paper seeks to highlight underexplored pathways that may serve as novel diagnostic markers or therapeutic targets. Ultimately, the review intends to encourage the incorporation of epigenetic perspectives into conventional antimicrobial approaches to better address the global rise in resistant pathogens.

METHODOLOGY

This review employs a narrative review methodology with a defined search and inclusion protocol, drawing on secondary sources across major scientific databases, to consolidate evidence on epigenetic regulation in bacterial antibiotic resistance. A comprehensive literature search was conducted across major scientific databases, including PubMed, Scopus, Web of Science, Google Scholar, and PakMediNet with the search last updated in March 2024.

Boolean search strategies were applied using combination of keywords such as "bacterial epigenetics," OR "DNA methylation," OR "histone-like protein modification," OR "nucleoid-associated proteins," OR "RNA regulation," OR "epigenetic inheritance," AND "antibiotic resistance," OR "antimicrobial tolerance". An example search query used in PubMed was "bacterial epigenetics," OR "DNA methylation," OR nucleoid-associated proteins," AND "antibiotic resistance," OR "antimicrobial tolerance". The search strategy was adapted for each database to account for indexing differences. Filters were applied to include English-language, peer-reviewed articles including research articles, systematic reviews, and meta-analyses.

Studies published between 1996 and 2024 were prioritized for inclusion, along with earlier foundational studies where necessary. The starting point of 1996 was selected to capture early foundational work in bacterial gene regulations and the emergence of epigenetic concepts in microbiology, which gained increasing scientific attention during the late 1990's. Global health reports from organizations such as WHO and CDC were also consulted for epidemiological context.

Inclusion criteria encompassed studies that

1. investigated epigenetic mechanisms in bacteria;
2. examined their relationship to antibiotic resistance, persistence, or virulence; and
3. provided molecular, genetic, or biochemical evidence relevant to epigenetic regulation.

Studies were excluded if they

1. focused exclusively on eukaryotic epigenetics;
2. lacked mechanistic insights or empirical data; or
3. were non-English publications.

Firstly, abstracts of collected articles were reviewed for appropriateness. Studies with fewer than 10 subjects, unpublished abstracts lacking complete data, editorials, and review articles were excluded. All selected sources

were critically evaluated for scientific rigor, relevance and clarity. Where heterogeneity was encountered in study design, bacterial species examined or outcome definitions were interpreted cautiously and differences noted in the narrative synthesis. Extracted findings were thematically categorized into major areas like DNA methylation systems, RNA-based regulation, chromatin-associated proteins, epigenetic memory and inheritance, and therapeutic implications to create a cohesive synthesis of current knowledge. Meta-analysis was not possible because of the heavy heterogeneity of the selected studies.

Epigenetics and Mechanisms

In recent years, interest has grown in investigating mechanisms that regulate gene expression without altering the underlying DNA sequence, contributing to antibiotic resistance phenotypes in bacterial pathogens. This is due to the increasing prevalence of bacterial strains which are unaffected by the action of antibiotics and due to the fact that genetic changes alone are proving to be incapable of fully explaining the rapidity of the cell's response to developments in its environment[8].

When bacteria are exposed to sub-inhibitory concentrations of an antibiotic, they may gradually develop adaptive responses. These responses allow the cells to tolerate increasing drug concentrations without the acquisition of permanent genetic mutations, but as soon as the antibiotic is removed, they revert back to their susceptible phenotype, once again becoming a target for the drug. This phenomenon is termed adaptive resistance, defined as an auto-regulated process characterized by induction of resistance upon drug exposure and reversion to a susceptible phenotype upon its removal.[9]

These observations suggests that the bacterial cell is capable of large-scale adaptation without any significant genetic changes and the swift transition displayed by the cell also highlights the level of flexibility it shows, which contrasts with traditional views of genetic mutations leading to permanent changes, hence suggesting that there may be different factors such as epigenetics also influencing the development of resistance.

As illustrated in Figure 2, adaptive resistance is a reversible, epigenetically mediated process in which bacteria exposed to sub-inhibitory antibiotic concentrations gradually develop tolerance, only to revert to their susceptible phenotype upon removal of the antibiotic, underscoring the role of non-mutational mechanisms in resistance development.

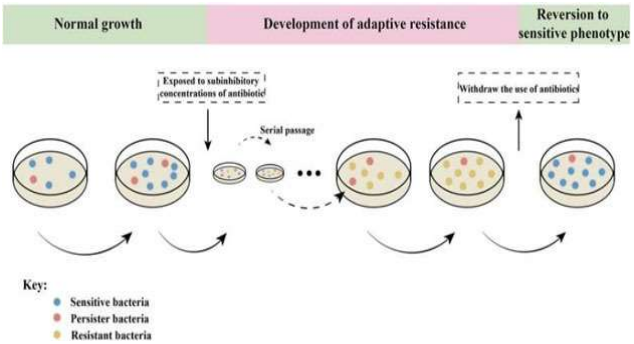

Figure 2: Adaptive Resistance is a term used to define the rapid nature of bacterial cell adaptations to its environment. *(Adapted from Wang et al,[10]).*

Epigenetics refers to the study of reversible changes in gene expression occurring without the permanent alteration of the underlying DNA sequence. It is a relatively newer method of understanding antibiotic resistance and could potentially offer therapies for existing antibiotic drugs to increase their lifespan. In bacterial cells, epigenetic changes occur through various ways such as efflux pump expression, DNA modifications, histone (histone-like proteins) modifications, and non-coding RNAs.

Efflux Pumps

Efflux, defined as the flowing out of a substance or particle, is an action carried out by efflux pump proteins in eukaryotic and bacterial cells, to remove unwanted substances from inside the cells. The expression of efflux pump proteins is controlled by operons, a group of cells transcribed together and controlled by a single promoter, and regulated by repressors and activators which are tasked with limiting and activating gene transcription[11]. Repressors and activators auto-regulate their transcription by binding to the promoter region[8]. When antibiotics are not present, the operon is expressed at low levels because repressor molecules have a binding affinity which is at least four times higher than that of activator molecules[12,13]. This limits the pump's expression.

When antibiotics enter the cell through membrane porins, they may act as inducer molecules, binding to a transcriptional repressor and preventing it from associating with the promoter region. This derepression permits RNA polymerase to initiate transcription of resistance-associated genes, including those encoding efflux pump activators or modifying enzymes. Owing to the activators' ability to express itself, its concentration multiplies along with the production rate of the efflux pumps, the activators also reduce the expression of membrane porins, which disallow antibiotics from entering the cell[8].

To date, six major families of efflux pumps have been discovered in bacteria. Namely, major facilitator superfamily (MFS), resistance nodulation division

(NDS), small multidrug resistance (SMR), ATP binding cassette transporter (ABC), multidrug and toxin extrusion (MATE), and proteobacterial antimicrobial compound efflux (PACE)[14,15]. Some of these are specific to their substrate while others can carry various, structurally dissimilar molecules.

Acinetobacter baumannii (A. baumannii) is an opportunistic bacterial pathogen frequently associated with healthcare-associated infections in immunocompromised individuals[16]. It yields a 29% to 73% mortality rate and is an important health concern for hospitals worldwide, with the Infectious Diseases Society of America classifying it as one of the six most resistant microorganisms on the planet[17,18]. This bacterium causes an infection which has an approximate incidence rate of 100,000 cases annually, globally and is associated with multiple classes of efflux pumps: MFS, RND, MATE, PACE, ABC, and SMR, all of which contribute to its growing resistance to antibiotics[19,20].

Figure 3 depicts the six major efflux pump families - MFS, RND, MATE, PACE, ABC, and SMR - and demonstrates the movement of their substrates across the inner and outer membranes of both gram-positive and gram-negative bacteria, highlighting the structural diversity that enables multi-drug efflux across bacterial species.

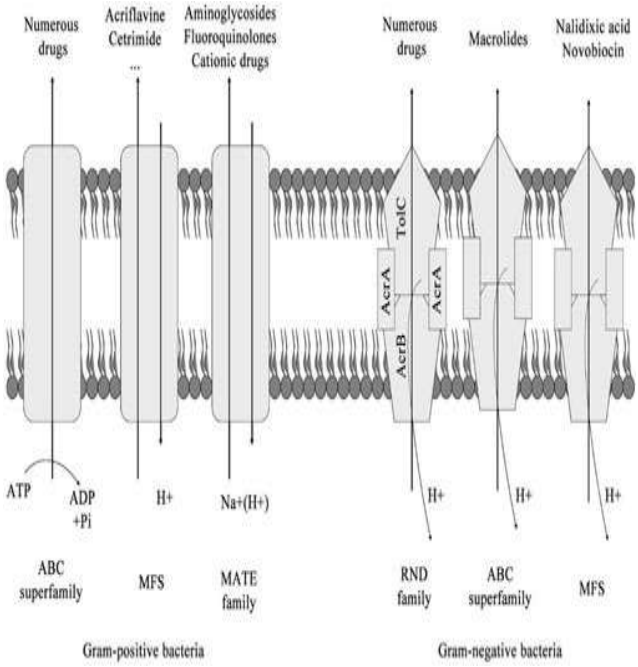

Figure 3: Shows six major families for both gram-negative and gram-positive bacteria and movement of their substrates across the inner and outer membrane (Adapted from Soto[16])

Out of these, the RND family pumps (AdeABC, AdeIJK, AdeFGH) are found to be the most clinically relevant as they are present in multiple resistant A. baumannii strains and are related to resistance to antibiotics such as aminoglycosides, chloramphenicol, tetracycline, erythromycin, and tigecycline lactams[21].

DNA Modifications

DNA Methylation: DNA methylation in prokaryotes involves the covalent addition of a methyl group to specific bases at the N6 position of adenine (yielding N6-methyladenine) or the C5 position of cytosine (yielding 5-methylcytosine), catalyzed by DNA methyltransferases (MTases)[22].

In bacterial cells, methylation can influence various factors such as virulence, cell cycle and growth, genome defense, etc. The restriction-modification (R-M) system is a well-studied model for investigating the role of DNA methylation in bacterial defense and its potential contribution to antibiotic resistance.

R-M systems are the bacterial cell's defense mechanisms that work by differentiating between self and foreign DNA through DNA methylation[23]. They consist of two components: restriction enzymes (REases) which cut foreign (unmethylated) DNA, and MTases which methylate specific sequences in the DNA, to prevent the cleavage of self-DNA (Stoddard Lab)[24]. Methylation in this system plays an important role in influencing HGT, phase variation and virulence, and persistence, all of which contribute to the cell's resistance. The methylation of R-M system, controls the uptake of foreign DNA, including plasmids which carry antibiotic resistance genes. When an incoming plasmid carries methylation patterns recognized by the host methyltransferase, it evades restriction endonuclease (REase) cleavage and is stably maintained, allowing the resistance genes it encodes to be expressed within the bacterial cell[25]. Furthermore, this process also influences the bacterial cell's responses to stress factors like antibiotics by regulating the action of persistence cells (discussed further in section 'Phenotypic Heterogeneity'), and forming biofilm, which is a cluster of bacteria encased in a slime-like coating, which increases the cell's resistance by decreasing permeability[26].

Non-Coding RNA: Non-coding RNAs (ncRNAs) are a structurally and functionally diverse class of RNA molecules that do not encode proteins, but play important roles in regulating gene expression at the transcriptional and post-transcriptional levels in both eukaryotic and prokaryotic cells. Certain ncRNAs bind to target mRNA transcripts, influencing their stability and translational efficiency by targeting transcripts that have a complementary base pairing[27]. In bacterial cells, small RNA (sRNA) carries out this function, affecting cellular responses to environmental stimuli. An example of such regulation in bacteria can be observed in the activities of the sRNA, MicF, a stress-induced gene present in E. coli and similar cells. It post-transcriptionally regulates OmpF expression by base-pairing with the ompF mRNA, thereby inhibiting its

translation and promoting its degradation. OmpF encodes an outer-membrane porin involved in antibiotic uptake[28]. This inhibition leads to reduced ompF levels which, in turn, decreases the permeability of the cell membrane, limiting the entry of certain external compounds into the cell such as antibiotics.

Table 1 summarizes the major categories of bacterial epigenetic modifications, including DNA and RNA-based mechanisms, the enzymatic systems responsible for each, their biological functions and representative examples across bacterial species, providing a consolidated reference for the mechanistic diversity discussed in this review.

Table 1: Summary of bacterial epigenetic modifications through employing various RNA and DNA modification methods (Adapted from Wang et al,[28])

Modifications	Type	Enzymatic Systems	Functions	Examples
DNA	Methylation	R-M Systems	Defense mechanism, regulate gene expression, virulence, biofilm formation	M.EcoGII. ModS, ModM, ModA, M.HpyIII, M2.HpyAII
	Phosphorothioation	Orphan Methyltransferases	Maintain EcoRII Plasmid stability, DNA repair, chromosome replication, Adenine and Cytocine Methyltransferases cause regulation of cell cycle	Dam, CcrM, Dcm, VchM, YhdJ.
		DNA Degradation	Defense mechanism, Oxidative stress, balance intracellular redox homeostasis, influence the transcriptional efficiency	dndABCDEFGH
RNA	Methylation	N^6– Methyladenosine Modification, N^1-Methyladenosine modification, 2 – Methylthiocytidine, modification, 5 – Methylcytosine modification	Regulate RNA stability, localization, transport, splicing, antibiotic resistance and translation	RlmF, RlmJ, RlmCD
	Non-Coding RNAs	Suppress or activate translation	Prevent RNA degradation	Fino/ProQ family, CsrA/RsmA family, Omp/ACF, MicACF

Phase Variability: Dam methylase (DNA adenine methyltransferase) is a well-characterized methyltransferase that adds methyl groups to adenine residues at GATC sequences throughout the bacterial genome. Methylation of promoter-proximal GATC sites can modulate transcriptional activity in a context-dependent manner either facilitating or impeding transcription factor binding and RNA polymerase recruitment, thereby enabling or restricting gene expression.

The modulation of genes in the aforementioned context is referred to as phase variability, a reversible and heritable process some bacteria undergo to adapt to the changes in their environment. *Neisseria meningitidis (N. meningitidis)* has phase variable MTases (mods) which are key epigenetic factors influencing the cell's gene expression, virulence, and immune evasion. The methylation of the promoter region impacts gene expression, altering bacterial phenotype and the interaction of the cell with its host[29]. The modA11 phasevarion regulates the expression of those genes involved in virulence and immune evasion, allowing the cell to adapt to the changes in its environment by evading immune defenses[30].

Type 1 fimbriae are hair-like protrusions present on Escherichia coli (E. coli) which act as links to host epithelial cells and are a crucial factor for the bacterium's virulence. Their expression is controlled by an inverted DNA element called fimS which contains promoters for the genes encoding the fimbrial subunits and is hence credited with being responsible for meditating the phenomenon of phase-variation in Type 1 fimbriae. As shown in Figure 4, fimS functions as a molecular switch controlling Type 1 fimbrial expression in E. coli; when the invertible promoter element is oriented in the 'ON' position, transcription of the fimbrial subunit genes is active, while reversal to the 'OFF' orientation silences their expression, exemplifying epigenetically regulated phase variation without any alteration to the underlying DNA sequence.

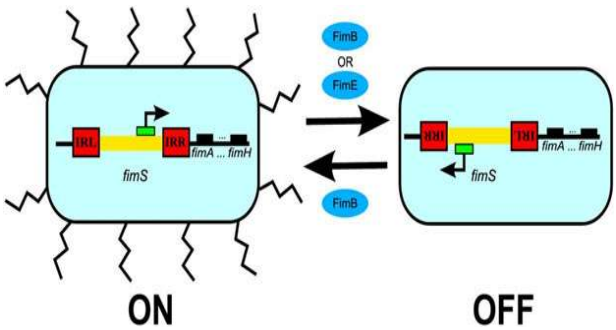

Figure 4: Shows position of fimS in bacterial cells (E. coli). FimS contains a promoter used to encode subunits like fimA and fimH (shown) and is in between two repeat invert sequences IRL, IRR. When the promoter is positioned correctly (upright) (as shown on LHS) transcription of structural fim genes is possible, enabling the expression of fimbriae (extensions on the surface of the cell). When the promoter is downwards, the system is 'off' (shown on RHS) and no fimbriae are visible. *(Adapted from Kuwahara[33]).*

When fimS is on, the promoter is active and the fimbrial genes are expressed. When it is off, the fimbrial genes remain unexpressed[31]. The applicability of the phenomenon

in this particular context is interesting to note as the expression of fimbrial genes allows E. coli to adapt to different locations in the host body without undergoing significant genetic alterations or permanent mutations.

Salmonella enterica (S. enterica), a gram-negative bacterium, has an outer membrane from which lengths of lipopolysaccharides (LPS) extend. These are large molecules made up of lipids and sugars which protect the cell from chemical attacks and stimulate the innate immune system[32]. The o-antigen present on the furthermost part of the LPS is highly changeable, with different lengths and compositions that influence the bacteria's response to multiple external factors such as the host immune system or the presence of antibiotics[33]. For the o-antigen to have changeable characteristics, it undergoes phase variability during which the expression of genes involved in o-antigen synthesis such as opvAB operon is regulated[34]. The o-antigen, although primarily associated with the evasion of the host immune system has been hypothesized to contribute to antibiotic resistance, as increased o-antigen chain length may reduce outer membrane permeability and promote biofilm formation, potentially limiting antibiotic penetration into the cell; however, this relationship requires further experimental validation.

Figure 5 shows the lipopolysaccharide (LPS) structure of gram-negative bacteria, with the o-antigen chain extending from the outer lipid layer; as described above, phase variability in o-antigen expression in S. enterica is epigenetically regulated through the opvAB operon, contributing to immune evasion and potentially to antibiotic resistance by altering outer membrane permeability.

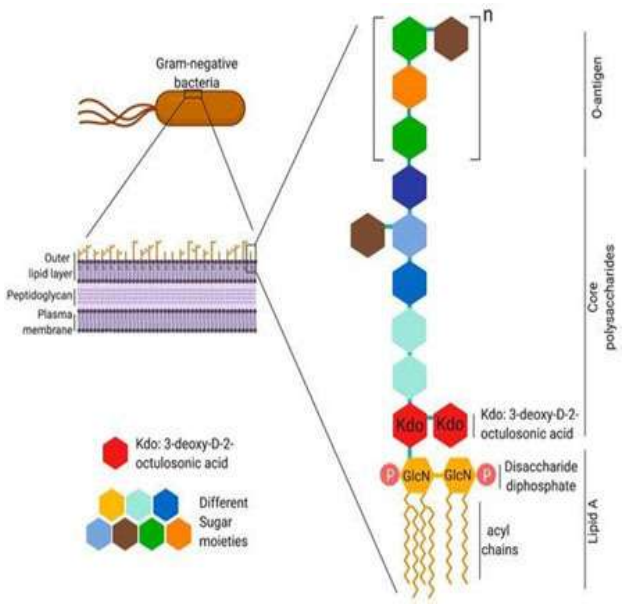

Figure 5: Shows gram-negative bacteria, magnifying the structure liposaccharide chain which is emanating from the outer lipid layer of the bacteria. The outermost part of the chain can be identified as the o-antigen. *(Adapted from Mazgaeen et al*[36])

Phenotypic Heterogeneity

Phenotypic heterogeneity refers to the variation in phenotype observed among genetically identical cells exposed to identical environmental conditions[35,36]. In the context of the bacterial population, this difference in expressed characteristics ensures the survival of the overall population as some individuals may be able to fare better under strenuous conditions such as antibiotics, allowing the species to thrive in the host.

Persister cells are a well-researched example of phenotypic heterogeneity in bacterial cells. These cells represent a small subpopulation of bacteria that enter a transient state of dormancy, enabling them to survive lethal concentrations of antibiotics that would otherwise eliminate genetically identical cells without acquiring permanent genetic changes associated with resistance[37]. Persister cells can emerge from a range of mechanisms, including stochastic processes, where certain conditions favor the formation of these variants[38].

Notably, studies have highlighted that one of the leading causes of persister cell formation is the induced antibiotic pressure on the bacteria, which facilitates individual survival and increases chances for the accumulation of genetic mutations in daughter cells[39]. Persister cells may contribute to resistance evolution by providing a surviving population under antibiotic pressure; however, the relationship between persistence rates and mutation rates is complex and not simply proportional. This association should be interpreted cautiously in the absence of direct evidence.

Furthermore, recent research shows that diversity exists within the population of persister cells as well. This suggests variation in cell responses when the population is exposed to different environmental and stress factors. The complexity of these phenotypic characteristics emphasizes the importance of personalized approaches in antibiotic therapy, as chances of treatment failure could increase if the diversity is left unaccounted for.

Histone Modifications

Histones are proteins, present in eukaryotic cells which help condense DNA into chromosomes. Though histones are absent in bacterial cells, they are replaced by nucleoid-associated proteins (NAPs) which, coupled with DNA methylation systems, can regulate gene expression by altering chromatin structure, thereby impacting the transcription of resistance-related genes[40]. Changes in histone marks could significantly impact the expression of antibiotic resistance genes, demonstrating that specific histone modifications are directly related to the development of resistance mechanisms in bacteria[37].

Bacteria package their DNA into a nucleoid, the bacterial equivalent of chromatin, through the help of NAPs such as HU in E. coli. The acetylation and methylation of NAPs, influence the expression of genes such as resistance genes, in the nucleoid, by either activating them (loosening the chromatin structure) or repressing them (tightening the chromatin structure)[41].

This could regulate the expression of resistance-related genes, influencing the cell's susceptibility to antibiotics.

Clinical Implications

The increasing prevalence of antibiotic-resistant bacterial strains represents a critical challenge in clinical medicine. When a pathogen shows resistance in vivo, current treatment guidelines suggest exploring alternative antibiotic regimens, combination therapy, or agents with broader or differing mechanisms of action. It is well established in the literature that infection with a resistant bacterial strain is linked with higher patient mortality rates. Conventional antibiotic therapy should be complemented by strategies targeting the epigenetic mechanisms that modulate bacterial susceptibility with the goal of sustaining and restoring antimicrobial efficacy[42,43,31].

Inhibition Drugs

Research into drugs designed to inhibit epigenetic modifications in bacterial cells to increase their susceptibility to antibiotics is progressing. It is driven by the findings that antimicrobial resistance is not solely genetic.

Epigenetic modifications which are characteristically reversible, present an important therapeutic opportunity. These drugs primarily operate in three ways[44].

i) by targeting enzymes like MTases or histone deacetylases which could potentially reverse resistance

ii) by altering the chromatin landscape and its associated proteins iii) by enhancing the effects of therapeutic agents to reduce drug resistance and boost the host immune response

By inhibiting enzymes responsible for DNA methylation such as methyltransferases and by disrupting NAP-mediated gene silencing, the expression of genes associated with antibiotic sensitivity may be restored, rendering the bacterial cell susceptible to antimicrobial agents. Combining epigenetic-targeting strategies with host-directed immunomodulatory therapies, may help augment immune-mediated clearance of resistant pathogens, allowing the pathogenic cell to become a target and the host system to fight the infection[45,46].

A promising strategy to administer these drugs to patients is, combining the inhibitors with existing antibiotics to revive older antibiotics already present in the system which are rendered ineffective due to their resistance[47]. This strategy could prolong the effectiveness of ongoing treatments and prove particularly useful when dealing with bacterial strains that are known to be resistant to multiple antibiotics such as K. pneumoniae and A. baumannii[48]. Studies dealing with the impact of natural compounds like epigallocatechin-3-gallate (EGCG) on Staphylococcus aureus show, that these compounds can disrupt cellular processes in the bacteria, which parallels the idea of using inhibitor drugs to alter and restore gene expression for increasing sensitivity to drugs[47].

However, despite this, the challenge remains in fully understanding the complex interplay between these epigenetic mechanisms and their influences on both the microbe and host responses, so off-target effects on host cells could be avoided and the effective delivery of the drug to bacterial cells could be ensured.

Epigenome Editing

Epigenomic editing involves the targeted modification of specific bacterial epigenetic markers such as, DNA methylation patterns at defined genomic loci or NAP-binding sites, to alter the expression of resistance-associated genes. Tools, such as, dCas9-fused effectors, have been investigated for their potential to selectively activate or repress such genes, though robust experimental evidence in bacterial systems remains limited. This method provides a more targeted form of therapy than inhibition drugs as it specifically focuses on a few genes rather than the entire cell.

CRISPR dCas

The CRISPR dCas system is a modified version of the CRISPR Cas system. In it, the Cas9 enzyme is catalytically inactivated (dCas9) by mutations in its nuclease domains, abolishing its ability to cleave DNA, while retaining its guide RNA-directed DNA-binding capability[44]. This system, when coupled with epigenetic regulators, can be programmed to target specific resistance-related genes in the bacterial cells and influence their expression[46].

While this approach offers greater target specificity, a significant challenge lies in developing effective delivery mechanisms. CRISPR-dCas systems are large macromolecular complexes, that face difficulties penetrating the bacterial cell envelope. Furthermore, bacteria harbor endogenous defense mechanisms including restriction-modification systems and CRISPR-based immunity that can recognize and degrade foreign nucleic acids, limiting the intracellular persistence of delivered constructs. The extent of these barriers varies considerably across bacterial species and warrants species-specific evaluation[47]. One delivery mechanism, genetically engineered bacteriophages, shows some promise and is currently being researched[48].

Bacteriophages can serve as delivery vectors for CRISPR-dCas constructs, exploiting their natural ability to infect bacterial cells and introduce foreign nucleic acids. Their ability to solely infect bacterial cells makes them an ideal carrier in cases of precision therapy because they do not harm human cells or beneficial microbiota.

However, even if the construct successfully traverses bacterial defense systems including restriction-modification barriers and endogenous CRISPR immunity, which may recognize and eliminate it as a foreign entity, it would still face challenges in reaching the target and evading additional bacterial defense responses[49].

Although innovations in biotechnology have enabled researchers to successfully modify previously existing medical techniques and use them to regulate the epigenetic mechanisms in bacteria, in-depth research still needs to be conducted in this field to perfect these techniques and make them suitable for clinical practice.

CONCLUSION

The emerging and rapidly growing crisis of antibiotic resistance calls for a deeper understanding of bacterial adaptation mechanisms, with epigenetic regulation emerging as a crucial factor. This paper has explored how DNA modifications, efflux pumps, phenotypic heterogeneity, and histone modifications, contribute to bacterial survival under antibiotic stress. Targeting these epigenetic mechanisms through inhibition drugs or epigenome editing to suppress enzyme function, alter DNA and gene expression, alongside administering preexisting antibiotics, presents a promising strategy to enhance antibiotic efficiency and reduce resistance. However, the development of precise therapeutic interventions remains a challenge, as bacterial adaptability and the risk of off-target effects continue to limit clinical translation. Detailed research and medical trials are needed to provide researchers with a well-rounded understanding of the interplay between genetic and epigenetic resistance mechanisms. Such insights may aid the development of medical techniques capable of controlling epigenetic changes and inform the rational design of next-generation antimicrobial strategies targeting both genetic and epigenetic determinants of resistance.

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Authors' Contribution: SFZ conceptualized the review, conducted literature search and study selection, performed data synthesis and interpretation, and wrote and revised the manuscript.

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CASE REPORT

Complete Surgical Excision of A Challenging Giant Intrathoracic Mass - Solitary Fibrous Tumor

Shifa Naz[1], Tanveer Ahmed[2], and Misauq Mazcuri[1]

ABSTRACT

Background: Solitary fibrous tumors are rare mesenchymal tumors that remain asymptomatic and are diagnosed incidentally. They grow at a slow rate and expand to giant size by the time of their presentation.
Introduction: This is a case of a female who presented with exertional dyspnea, orthopnea and dull chest pain for 4-5 years. Clinical examination showed reduced movement over the left chest along with dull percussion note and absent breath sounds. Computed tomography showed a large 20*11*12cm heterogeneous opacity occupying the left hemi thorax. Biopsy was inconclusive. A complete excision of the mass was achieved. Intraoperatively, mass was consistent with radiological findings, however, was adherent to surrounding structure with atelectasis. Patient had an uneventful recovery period with histopathological evidence of solitary fibrous tumor.
Conclusion: Complete surgical excision remains the mainstay of treatment for solitary fibrous tumors and can achieve favorable outcomes.

Keywords: Dyspnea, intrathoracic mass, solitary fibrous tumor

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INTRODUCTION

Solitary fibrous tumors (SFT) are rare mesenchymal tumors accounting for less than 5% of all pleural tumors[1]. It can develop in any body cavity with intrathoracic being the most common site[2,3]. Majority of the thoracic SFT are usually benign but they exhibit aggressive behaviour in terms of local invasion and recurrence as reported in 12 % of all cases[4,5]. SFT are often asymptomatic or have nonspecific symptoms like dyspnea, cough, and chest pain because of which they remain undiagnosed and are incidentally found on chest imaging[6]. Here, we present a rare case of large intrathoracic mass presenting as pleural effusion.

Case Presentation:

An elderly female, known hypertensive, presented with exertional dyspnea, orthopnea, and dull chest pain for the last 4 to 5 years. Her symptoms worsened over a year. She was initially managed by a medical team with needle thoracocentesis followed by a pigtail catheter placement as a case of loculated pleural effusion. On examination, reduced movement over the left chest was observed along with dull percussion note and absent breath sounds. A chest radiograph revealed a homogenous opacity in the middle and lower zone along with blunting of both angles and obscuration of cardiac silhouette (figure 1).

A computed tomography was done which was suggestive of a 20*11*12cm size heterogeneous opacity occupying the left hemi thorax. Additionally, the mass was abutting trachea, left main bronchus and a collapsed left lower lobe was seen along with a mediastinal shift towards the right side. Multiple radio-guided biopsies were inconclusive, likely due to necrosis or inadequate tissue sampling. It is worth mentioning, that surgery was already suggested by another institute which was refused by the patient. Eventually, because of the large tumor size and inadequate diagnosis, patient was referred to our institution for surgical intervention. Preoperative embolization was not performed as the tumor had multiple tortuous feeding vessels arising from both the intercostal arteries and pulmonary circulation, making selective embolization technically

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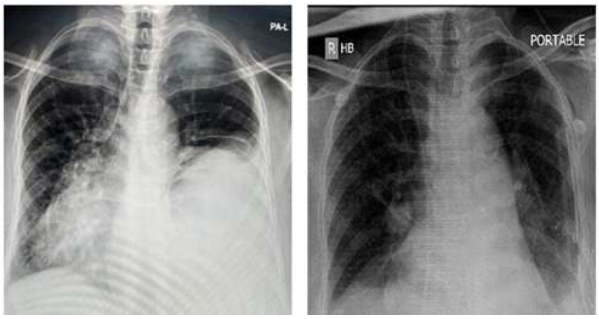

Figure 1: Preoperative Chest Radiograph Showing a Large Homogenous Opacity, Postoperative Chest Radiograph Demonstrating Complete Expansion of Lung

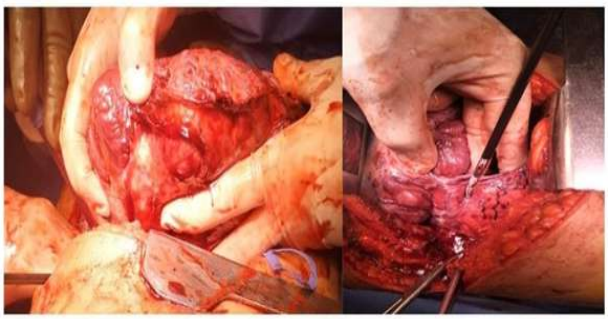

Figure 2: Intraoperative Image Showing a Giant Mass with Dilated Torturous Veins

difficult with uncertain benefit. In addition, the patient was hemodynamically stable, and complete surgical excision was considered achievable without prior embolization.

After multidisciplinary team meeting, general consensus was made among oncology, radiology, and thoracic surgery regarding complete excision of the tumor. Preoperative preparation was done including medical optimization of hypertension, breathing exercises, and obtaining high risk consent.

A standard left posterolateral thoracotomy was done with the excision of the 7th rib after advancing 32 FR left sided double lumen tube. Most importantly, graded posterolateral position was made to avoid sudden mediastinal shift to prevent hypotension. Intraoperatively, a tanned white encapsulated, lobulated, and highly vascular tumor was identified receiving dilated torturous supply from the upper lobe, chest wall, and intercostal arteries (aorta). Mass was adherent to diaphragm with neovascularization and a consolidated lower lobe due to prolonged compression was seen (figure 2).

A complete excision of the mass was achieved, resulting in expansion of lower lobe. Operative time was approximately 2.5 hours, with an estimated blood loss of 1200-1500ml requiring transfusion of 2 units of red blood cells intraoperatively.

Complete expansion of lung was noticed postoperatively. Patient had an uneventful recovery and was discharged later on 7th postoperative day. Histopathology confirmed the diagnosis of solitary fibrous tumor with immunohistochemistry positive for Cd34, Stat 6, BCL2, and CD99. During 6th months follow up, patient remained asymptomatic with no evidence of recurrence on clinical and radiological examination.

DISCUSSION

Solitary fibrous tumors are rare tumors that originate from mesenchymal cells within sub mesothelial tissue of pleura, distinguishing them from mesothelioma, which derives from mesothelial cells[4]. SFTs typically occurs in 4th to 6th decade of life and affects men and women equally[1]. Diagnosis and management of SFT are challenging as they are rare and are often discovered incidentally as small asymptomatic tumors but can grow to massive sizes[3]. The definitive diagnosis will, however, be made histopathologically[7].

Benign SFTs generally develop from the visceral pleura, are pedunculated, and extend into the pleural space, while malignant tumors more often originate from the parietal or diaphragmatic pleura and can invade the lung. The presence of symptoms like pleural effusion, and lack of pedicle are key indicators of potential malignancy[5].

Computed tomography usually reveals well defined lesions, but these findings are not definitive of diagnosis. CD34 and NAB2-STAT6 is molecular hallmark found in most of SFT, hence immunohistochemistry can be used for diagnostic purpose[2,8]. Rarely, SFTs is linked to paraneoplastic syndromes such as digital clubbing, and hypertrophic pulmonary osteoarthropathy (HPO), possibly due to tumor secreted cytokines, hyaluronic acid, or chronic hypoxia. Doege-Potter syndrome, characterized by refractory hypoglycemia, occurs in less than 5% of SFT, is associated with insulin like growth factor 2 secretion[9].

Radical surgical resection with wide clear margins is currently the cornerstone of treatment[10]. Depending on the vascularity of the tumor, surgical removal can be complemented by the endovascular embolization to prevent blood loss[5]. In some cases, minimal invasive techniques may be considered according to dimension,

position, and infiltration of adjacent structures, although there is risk of tumor seeding at the incision site[9]. Long term outcomes are generally favourable, but there is always a risk of local or distant recurrence[5]. Role of neoadjuvant/adjuvant chemo radiotherapy is still controversial in irresectable cases[7].

CONCLUSION

Fibrous tumors are often asymptomatic and may grow to be giant in size by the time of diagnosis. The gold standard treatment of thoracic solitary fibrous tumor is complete surgical resection. Due to rarity of tumor, longer follow up is required.

Patient Consent: Informed and written consent was acquired from the patient before the initiation of write-up of case report.

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Conflict of Interest: The authors declare that there is no conflict of interest.

Authors' Contribution: SN conducted the literature search, prepared the original draft, and contributed to manuscript writing. **TA** conceptualized the study and provided financial guidance. **MM** supervised the study process and contributed to review and editing of the manuscript. **NS** contributed to the literature search and manuscript editing.

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CASE REPORT

Atypical Presentation of Dermatofibroma: A Rare Case

Faiza Ahmed Khan[1], Sadaf Ahmed Asim[2], Madiha Sajid[3], and Narmin Khan[1]

ABSTRACT

Background: Dermatofibroma, also termed benign fibrous histiocytoma, is a common benign fibrohistiocytic tumor that typically presents as a firm reddish-brown nodule on the extremities. Atypical variants particularly atrophic and giant plaque-like forms occurring on the face are exceedingly rare and may be mistaken for inflammatory or malignant dermatoses. We report a 47-year-old woman who presented with a four-month history of an 8 × 4 cm hyperpigmented, indurated, atrophic plaque on the right side of her face, without antecedent trauma. Clinical differentials included morphea, lupus panniculitis, lichen sclerosus et atrophicus, and subcutaneous sarcoidosis. Histopathology revealed dermal spindle cells in a storiform pattern with collagen entrapment, and immunohistochemistry was positive for CD68 and ASMA and negative for CD34 and S100, confirming dermatofibroma. The lesion was completely excised with cervicofacial flap reconstruction. Recognition of such atypical facial variants is essential to guide accurate histopathological diagnosis.

Keywords: Atrophic plaque, atypical presentation, benign fibrous histiocytoma, CD68, dermatofibroma, facial dermatofibroma, giant dermatofibroma, histopathology, immunohistochemistry

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INTRODUCTION

Benign fibrous histiocytoma, another name for dermatofibroma, is a common benign skin tumor[1]. It often manifests as one or more normochromic reddish-brown or dark brown solid nodules (less than 2 cm) that primarily appear on middle-aged people's extremities, with a minor female preponderance. The dermatofibroma causes the distinctive "dimple sign" when two fingers are pressed side to side because the covering epidermis is tethered to the underlying lesion[2,3]. Facial involvement and giant plaque-like dermatofibroma, defined by its size of more than 5 cm, are uncommon and typically represent more aggressive variants that are difficult to treat and often lead to misdiagnosis. Only a few published cases describe the atypical presentations of dermatofibroma[4–6].

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The rationale for reporting this case lies in the diagnostic challenge posed by an atrophic, plaque-like dermatofibroma occurring on the face, a site at which such variants are seldom encountered and at which the morphology closely mimics inflammatory and connective-tissue disorders, increasing the risk of misdiagnosis and inappropriate treatment. The objective of this case report is to describe the clinical, histopathological, and immunohistochemical features of this rare atypical facial dermatofibroma, to highlight key differential diagnoses, and to provide a concise review of the literature that may aid clinicians in the early recognition and accurate diagnosis of similar lesions.

CASE REPORT

A 47 years old woman with no known co- morbidities presented to us with the complaint of a hyper-pigmented depressed lesion on the right side of her face for four months. She had initially developed asymptomatic skin-colored nodular lesions, subsequent to which she noticed gradual stiffening and hardening of that area, along with a slight depression and hyper-pigmentation. Apart from the progressive enlargement of the lesion, no other associated symptoms were noted. There had not even been any history of trauma on that site. No significant past medical, surgical, drug or family history was noted. Upon examination, a single erythematous

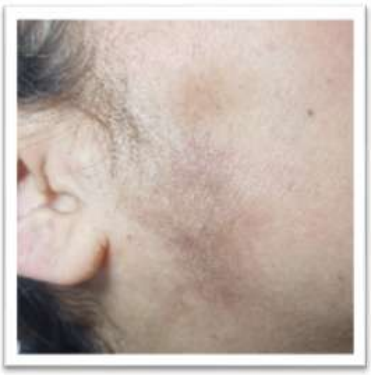

Figure 1: Lesion on Face

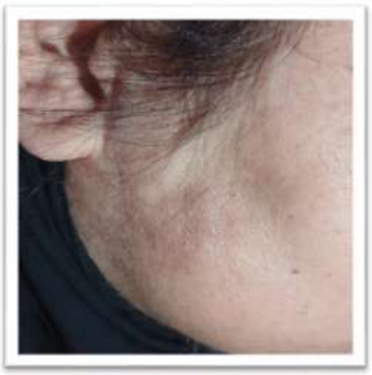

Figure 2: Lesion on Face

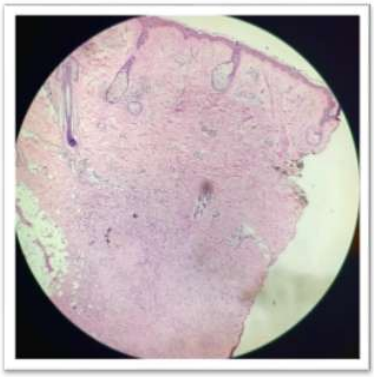

Figure 3

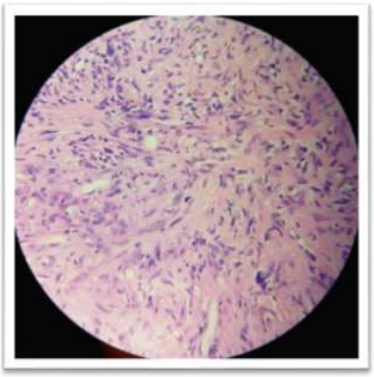

Figure 4

Figure 5: Immunohistochemistry with Positive Asma Stain Which is Made up of Fascicles of Plump, Spindle-shaped Cells with Oval to Irregular Nuclei

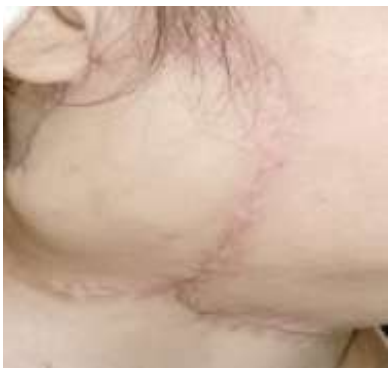

Figure 6: Three Weeks After Procedure

to hyper-pigmented indurated and atrophic plaque measuring 8x4 cm with multiple nodules on palpation, appeared laterally on right side of the face just above the lower jaw (Figure 1,2). The rest of the cutaneous and systemic examination was unremarkable.

The initial differentials include morphea, lupus panniculitis, extragenital lichen sclerosus et atrophicus, and subcutaneous sarcoidosis. An incisional skin biopsy was performed. Histopathology showed atrophic epidermis. The reticular dermis shows an ill-defined lesion, which is made up of fascicles of plump, spindle-shaped cells with oval to irregular nuclei and inconspicuous nucleoli.

Intersecting fascicles are arranged in a storiform pattern. Collagen trapping was also evident (Figure 3,4). Immunohistochemical stain CD68 and ASMA were positive, while CD4, CD34, and S100 were negative (Figure 5).

A final diagnosis of dermatofibroma was established based on clinical presentation and characteristic histopathological findings. After counselling the patient about her condition, she was referred to the Surgical Unit for complete excision of the lesion. Finally, a complete excision with cervicofacial flap was undertaken. (Figure 6).

DISCUSSION

Benign fibrous histiocytoma is recognized as one of the most prevalent benign skin tumors, with recurrence rates as low as 3–5%[4]. Dermatofibroma often presents asymptomatically and may appear following minor trauma or an insect bite. It predominantly involves the lower extremities[1].

Several clinical variants of dermatofibroma have been reported in the literature, including atrophic, atypical polypoid, giant, multinodular hemosiderotic, subcutaneous fibrous, keloidal, subungual, generalized eruptive, pleomorphic, ulcerated, erosive, multiple palmoplantar, and multiple clustered varieties.[7] The giant dermatofibroma was initially identified by Danckaert and Karassik. It presents as larger than 5 cm in diameter and appears as an exophytic skin lesion, most commonly affecting the lower limbs, followed by the back[3].

Histopathological analysis reveals that both the classical and giant types of dermatofibroma have several variants, including fibrous/fibrocellular, xanthomatized, aneurysmal, hemosiderotic, epithelioid, cellular, atrophic, lipidized, clear cell, palisading, and keloidal forms[8]. Review of the literature suggests that plaque-like dermatofibromas share a similar histopathological profile with the common fibrous/fibrocellular variant of typical dermatofibroma a poorly defined, non-encapsulated dermal lesion characterized by interwoven bundles of spindle-shaped fibroblasts and macrophages, often arranged in a storiform pattern within a loose collagenous stroma[2,3].

Immunohistochemical staining (IHC) with CD68 highlights the presence of histiocytic cells, while negativity for CD34 helps exclude dermatofibrosarcoma protuberans, which is CD34-positive in approximately 85% of cases and may be distinguished only by this feature. The cellular variant of dermatofibroma may, however, shows focal CD34 positivity at the tumor periphery. Factor XIIIa can also aid in differentiation, as it is typically negative in dermatofibrosarcoma protuberans.

Dermatofibromas of large plaque-like sizes might be a kind of giant dermatofibromas. To be exact, very few cases of such instances have been reported, which are defined by the presence of large, tough plaques that can develop either on their own or as a result of some trauma like an insect bite[2,3,5]. Dermatofibroma (DF) is rarely found on the face, although the literature has documented a few such cases[9]. Just at the beginning of the millennium, Mentzel et al. in 2001 defined more than thirty thousand cases of dermatofibroma and among them, only 34 cases were referred to as being in the facial area composed of the forehead, ear, cheek, eyebrow, and nose. Out of these, the majority (17 cases) showed very aggressive behavior which led to the invasion of soft tissue and muscle. Histologically, only nine cases could be classified under the distinctly typical storiform pattern, while most were positive for actin spindle-shaped myofibroblasts and made up of cellular fascicles. In the same year, Estela et al. documented 22 instances of dermatofibroma occurring in the facial region over a span of twenty years, observing no unusual features. Involvement of deep tissue was seen in only three cases[10]. Atrophic dermatofibroma is an uncommon type of dermatofibroma that was first identified in 1987. It represents about 2% of all types of dermatofibromas[11]. This variant of dermatofibroma is most often seen in middle-aged women (40–50 years) and is localized to the upper trunk and upper arms. When diagnosing inwardly puckered, depressed lesions, this variant of dermatofibroma should be considered. Only a handful of cases of atrophic dermatofibroma have been reported in the literature so far[11].

The diagnosis is mainly clinical; however, in the case of atypical presentations like this, the histopathological examination together with IHC becomes the mainstay for accurate diagnosis and ruling out other diseases. Even though it looks otherwise, it always has a benign course and, in most cases, simple excision is sufficient for curing the patient. So far, there have been no reports of recurrences after excision.

Comparison with previously reported cases is informative. AlQusayer et al. described a facial dermatofibroma presenting as a small nodular lesion on the cheek of an adult patient[4]. Whereas the present case is distinguished by a much larger 8 × 4 cm atrophic plaque-like morphology rather than a discrete nodule. Iqbal and Mudaliar reported a 7 cm giant dermatofibroma on the leg of a 29-year-old male presenting as a non-ulcerated scaly plaque[7]. Our case is similar in its plaque-like configuration but differs in two important respects—facial location and the predominantly atrophic, depressed surface rather than a raised plaque. Cavallo et al. recently described a 20 cm plaque-like dermatofibroma on the antecubital region with peripheral satellite lesions, again on an extremity[8].

Atrophic dermatofibroma, first described in 1987, has classically been reported on the upper trunk and arms in middle-aged women; Alzaidieen et al. reported one such case on the leg of a 44-year-old woman in 2025[9]. To our knowledge, the combination of giant plaque-like size, atrophic morphology, and facial location has not previously been reported. Compared with the historical series of Mentzel et al., in which 17 of 34 facial dermatofibromas demonstrated aggressive behaviour with soft-tissue and muscle invasion[4], our case behaved as a typical benign fibrous histiocytoma on histopathology and immunohistochemistry, with no evidence of deep invasion. The CD68 and ASMA positivity with CD34 and S100 negativity in our case. is concordant with the immunoprofile reported across the cited literature and supports exclusion of dermatofibrosarcoma protuberans.

LIMITATIONS

This report describes a single case and therefore cannot establish the prevalence, natural history, or recurrence rate of this atypical facial variant of dermatofibroma. Dermoscopic evaluation, which has been increasingly used as an adjunct in the assessment of fibrohistiocytic tumours, was not performed on our patient. Additional immunohistochemical markers such as factor XIIIa, which can further help differentiate dermatofibroma

from dermatofibrosarcoma protuberans, were not available in our setting. Long-term follow-up beyond the immediate post-operative period is also limited. Larger case series and prospective registries, focused on atypical and facial dermatofibromas, are needed to better characterize the spectrum and behaviour of this entity.

CONCLUSION

This case report describes an unusual presentation of dermatofibroma as a giant plaque-like atrophic lesion on the face, an uncommon site for this tumor. Although the clinical morphology was atypical and closely simulated several inflammatory and connective-tissue dermatoses, histopathological examination and immunohistochemistry confirmed the features of the typical fibrous/fibrocellular variant of dermatofibroma. The diagnosis of such atypical lesions is challenging and mandates careful clinicopathological correlation, with histopathology and immunohistochemistry serving as the cornerstone for precise confirmation. Awareness of this variant among dermatologists, primary-care physicians, and pathologists is important to include dermatofibroma in the differential diagnosis of atypical facial plaques, to avoid misdiagnosis as morphea, lupus panniculitis, or dermatofibrosarcoma protuberans, and to guide timely surgical management with reassurance regarding the benign course and low recurrence risk.

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Conflict of Interest: Authors declare that there is no conflict of interest.

Authors' Contribution: FAK worked on methodology and writing the original draft. **SAA** conceptualized, supervised, and guided. **MS** validated, reviewed, and edited the draft. **NK** worked on data curation.

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CASE SERIES

Modified Twin Occlusion Prosthesis—Case Series

Mehwish Khan[1], Mehmood Hussain[2], Syed Murtaza Raza Kazmi[3], Yasmeen Habib[4], and Muhammad Khalil[2]

ABSTRACT

The use of twin occlusion is commonly associated with maxillofacial prosthesis incorporating double row of teeth in posterior region where the inside or palatal row of teeth provide occlusal contact and hence helps in mastication and the outside or buccal row towards the cheeks provide support and improves the appearance. This clinical case series reports the treatment of patients with modified twin occlusion acrylic removable prosthesis to improve the patient's facial profile and achieve satisfactory aesthetics and function.

Keywords: Double occlusal plane, maxillofacial prosthesis, twin occlusion

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INTRODUCTION

Maxillofacial prosthesis with twin occlusion has been reported to be used generally for patients with mandibular defects following segmental mandibulectomy[1-4]. Deviation of mandible towards defect side observed in such patients, makes it difficult for the clinician to record a stable maxillo-mandibular relationship and achieve acceptable aesthetics and function[5]. In order to achieve satisfactory occlusion, a palatal ramp or a broader maxillary occlusal plane with double row of teeth (twin occlusion), is used along with the mandibular guide flange prosthesis[2]. Twin occlusion provides stable occlusal contacts with the opposing natural or artificial teeth facilitating mastication, whereas, the flanges of the prosthesis provide support and help in directing mandible towards a more stable position.[1,2,6,7] Several authors have reported the use of twin occlusion with the palatal ramp and mandibular guide flange prosthesis, and almost all of them have reported use of double row of teeth in posterior region[3,4,8-10]. The inside or palatal row of teeth provides occlusal contact and hence helps in mastication. Whereas, the outside or buccal row towards the cheeks, provides support and improves appearance[5,7,10].

In this case series, authors have presented three cases with a history of congenital and acquired defects, in which removable prosthesis is fabricated with twin occlusion, to improve the patient's facial profile and achieve satisfactory aesthetics and function.

CLINICAL REPORTS

Case 1

A 28-year-old male patient reported to the Prosthodontics department of a public sector university hospital in Karachi, Pakistan. He was wearing a removable denture and complained of small size of maxillary anterior tooth, in the prosthesis. He was also dissatisfied with the denture and wanted improvement in his facial appearance. Furthermore, he also complained of difficulty in speech with the existing prosthesis.

Patient had repaired bilateral cleft lip and palate and had received multiple surgeries in his childhood during the treatment. His medical history was not contributory. Dental history revealed that he had a filling of his maxillary molar teeth a few years back and had been using a palatal obturator since childhood which was replaced many times. Currently, he was wearing a palatal obturator which was made three years ago from a private hospital.

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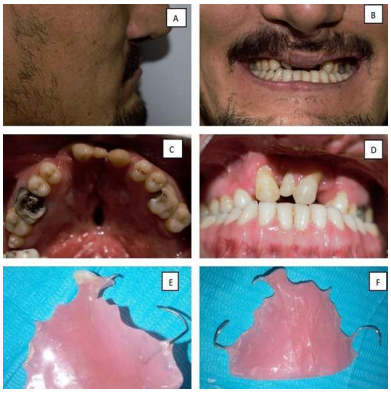

Figure 1: A, Extra-oral profile view. B, Extra-oral front view. C, Maxillary occlusal view. D, Anterior cross bite in occlusion. E, Existing prosthesis polished surface. F, Existing prosthesis intaglio surface.

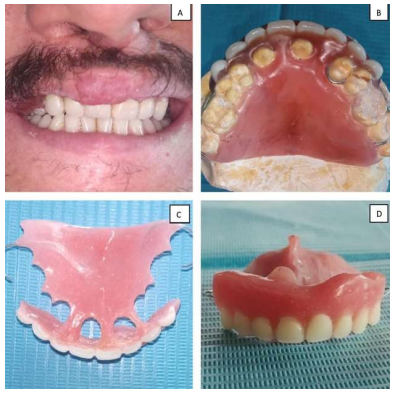

Figure 2: 2A, Clinical try-in stage. 2B, Finished wax-up. 2C-2D, finished prosthesis

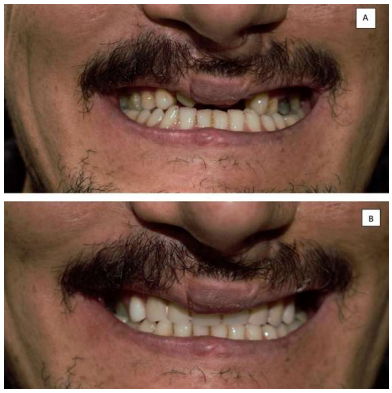

Figure 3: 3A, Clinical picture without the prosthesis. 3B, Clinical picture with the prosthesis

Extra-oral examination showed adequate mouth opening with no deviation. Nasal septum was observed to be slightly deviated towards the left side and there were bilateral scars from the previous surgeries of cleft lip on his upper lips. Straight facial profile, maxillary deficiency, inadequate upper lip support with average smile line was also observed (Fig. 1A-B). Intraoral examination revealed healthy well-keratinized mucosa with a small size palatal defect in the midline, in addition to a labial defect in the labial sulcus above the region of lateral incisors. He also had cross bite in intercuspal position with missing maxillary incisors, decayed mandibular left third molar, maxillary left first molar and amalgam filling in right first molar. A small peg shaped lateral incisor was present in the midline region with transposition of bilateral canines towards the midline. Remaining natural teeth in both arches were otherwise satisfactory (Fig. 1 C-D). Existing prosthesis exhibited adequate hygiene and was also replacing a central incisor in addition to closing the palatal defect (Fig. 1E-F). It was not sealing the palatal defect completely in the posterior region and there was no extension of the prosthesis in the labial defect, which might be a contributory factor to his nasal voice quality.

Due to financial limitations, the patient did not want any orthodontic or surgical intervention. Maxillary twin occlusion prosthesis with palatal obturator was planned, for which preliminary diagnostic impressions were made with irreversible hydrocolloid to obtain primary casts from Type II dental plaster. Diagnostic wax-up was done to show expected results to the patient. Suggested treatment plan was oral prophylaxis, extraction of decayed mandibular third molar, root canal treatment of maxillary first molars with full coverage crowns, followed by maxillary acrylic twin occlusion prosthesis with palatal obturator.

Patient refused endodontic treatment of molars as according to him he had no complaints with them and consented to proceed with the prosthetic treatment after oral prophylaxis and extraction of third molar.

CLINICAL TECHNIQUE

Impressions were made with irreversible hydrocolloid (Hygedent fast set) and master casts were obtained using type 3 dental stone. The articulation was done after bite registration. Six acrylic resin anterior teeth were trimmed in the form of labial veneer. Wax-up was completed by placing them labial to the existing anterior teeth. Additional wax was added in the labial region to provide lip support. At the clinical try-in visit, patient was not satisfied with lip support, so more wax was added in the labial and buccal region until the patient showed satisfaction. It was also observed during the try-in stage that he showed teeth up to first premolar during smile therefore, maxillary first premolars were also added labial to the existing ones in the form of acrylic shell for an improved smile (Fig. 2A-B). For retention purposes, an 18-gauge stainless steel wire was used for clasp fabrication on maxillary second molars. First molars were avoided in case the patient changes his mind about the endodontic treatment later. The prosthesis was processed in heat cure acrylic resin. After finishing and polishing, maxillary twin occlusion prosthesis (double rows of acrylic teeth in anterior region) was inserted after minor adjustments (Fig. 2C-D).

Anterior teeth in prosthesis were kept out of occlusion as the patient did not have any difficulty with mastication. Voice quality was improved but the patient still found it unclear. Palatal contact of tongue was observed after application of pressure indicating paste on the polished surface of the palate, areas of excessive pressure were relieved and prosthesis was polished again which resulted in more clarity in his speech sounds. Post insertion instructions were given regarding the maintenance of the prosthesis. Follow-up evaluation at 1, 3, and 6 months showed functional and psychological satisfaction of the patient (Fig. 3).

Case 2

A 30-year-old female came to the Prosthodontics department of a private dental hospital in Karachi, Pakistan for replacement of her missing right anterior tooth. She had a history of surgical resection of hemangioma in maxillary anterior region two years back. Her maxillary right central incisor was also removed during the surgical procedure. Medical history was not contributory. Previously, she had used an acrylic removable partial denture for some time but was not satisfied with the aesthetics of that denture

Extra-oral examination was unremarkable except the scars from the previous surgery on the philtrum region. Intraoral examination revealed healthy teeth with adequate oral hygiene and missing maxillary central incisor. She had an anterior crossbite in maximum intercuspation. Treatment options were discussed with the patient; anterior bone graft followed by implant supported fixed prosthesis, fixed orthodontics therapy, fixed partial denture, simple acrylic removable partial denture, acrylic twin occlusion prosthesis with labial flange to improve the patient's profile and address the anterior cross bite. Due to time constraint, the patient opted for acrylic twin occlusion prosthesis. Diagnostic wax-up was completed on diagnostic casts with six acrylic resin anterior teeth trimmed in the form of labial veneer and placed labial to the existing anterior teeth. Diagnostic wax trial was done and after patient satisfaction with the wax trial, final prosthesis was processed in heat cure acrylic resin. Patient showed satisfaction with the aesthetic improvement resulting with wearing of the twin occlusion prosthesis (Figure 4A-4B).

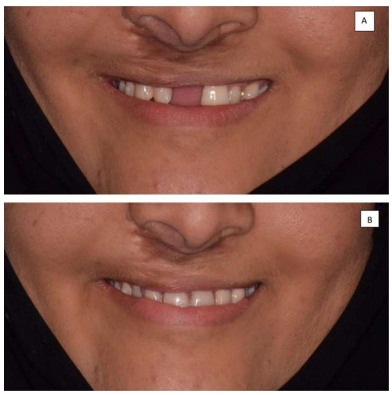

Figure 4: 4A, Clinical picture without the prosthesis. 4B, Clinical picture with the prosthesis.

Case 3

A 50-year-old male came to the Prosthodontics department of a private dental hospital in Karachi, Pakistan with complaints of difficulty in mastication with his current prosthesis. He had a history of myocardial infarction two months ago. He had been using his current acrylic removable partial denture in the maxillary arch replacing his missing teeth for one year. Extra-oral examination was unremarkable. Intra-oral examination revealed healthy teeth with adequate oral hygiene with completely edentulous mandible and multiple missing teeth in maxillary arch. Existing mandibular complete denture and maxillary partial denture exhibited adequate retention, stability, and support with satisfactory hygiene. On occlusal examination, buccal crossbite in posterior teeth with overjet in anterior region was observed with the prosthesis in place. There was no contact of maxilla-mandibular teeth in the posterior region with the existing prosthesis. Diagnostic jaw records revealed the same relationship of jaws as with the existing prosthesis. Correction of occlusal plane with extraction of mal-positioned teeth was advised before fabrication of new maxillary denture. Due to his medical condition, any surgical treatment was contraindicated. After discussion with the patient, a removable acrylic twin occlusion prosthesis was advised for the maxillary arch with double row of posterior teeth, to allow functional and stable contacts of existing mandibular complete denture with the new maxillary partial denture. The buccal row of teeth provided the needed cheek support whereas, the palatal row of teeth provided aid in mastication with wearing of the twin occlusion prosthesis. Patient exhibited satisfaction with the function and aesthetics of new modified maxillary partial denture (Figure 5A-5C).

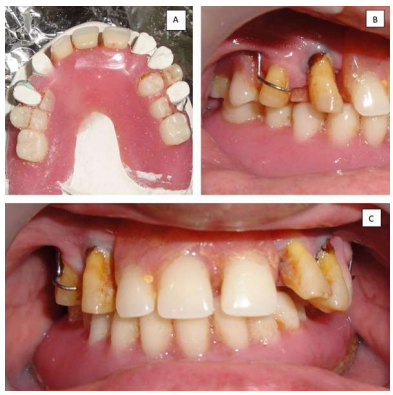

Figure 5: 5A, Maxillary twin occlusion prosthesis on cast. 5B-5C, Intra-oral view with the prosthesis.

DISCUSSION

Twin occlusion prosthesis has a long history of successful use in patients with hemi-mandibulectomy patients[7-10]. Authors of this study modified the conventional twin occlusion acrylic resin prosthesis for use in patients with maxillary defect and also in those with no history of surgery or trauma. Conventional prosthesis is designed for use in the posterior region to provide stable occlusal contacts and also guide the mandible into a more repeatable position[1-3,10].

Modification to provide a double row of teeth in the anterior region, provides improved lip support along with veneer effect to mask any morphological deformities in anterior teeth. Removable nature of prosthesis allows the patient to perform adequate oral hygiene and allows room for further modification in the prosthesis, where future surgical procedures are needed, as in patients with congenital anomalies or aggressive carcinomas. Furthermore, this modification can provide aesthetic improvement in those cases where complex procedures of bone grafting or surgical correction cannot be implemented due to financial, time or systemic health concerns.

The heat cure acrylic resin is used for the prosthesis as it has advantages of being readily available, easy to fabricate and repair, low cost, and aesthetic[10]. The presented prosthesis also has some limitations which include that it is not fixed which can be a major concern for some patients, also inadequate maintenance of the prosthesis may be associated with caries and periodontal compromise of natural teeth, mucosal lesions, fracture of prosthesis, and discoloration[6,8]. Authors recommend such modification in cast partial denture for improving strength and durability, whereas digital 3D printing techniques can be employed in future with novel materials for better precision and fit. Further studies with similar cases should be reported to strengthen and validate the findings of the present study in future.

CONCLUSION

Modified twin-occlusion prosthesis reported in this article provides an effective and functional prosthesis with significant psychological and aesthetic advantage to the patient. It is reversible, cost effective and simple in fabrication and will provide a conservative yet immediate treatment plan for patients with low socioeconomic background who cannot afford complex rehabilitative procedures.

Consent Statement: An informed written consent was taken from the patients for publication of images after hiding their identity.

Funding: Nil

Conflict of Interest: Authors declare that there is no conflict of interest.

Authors' Contribution: MK conceptualized, wrote original draft and collected resources. **MH** reviewed and edited the manuscript. **SMR** worked on validation and visualization. **YH** contributed to validation and resources. **MKH** supervised the study.

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LETTER TO THE EDITOR

Design, Translational, and Regulatory Barriers in Advancing Nanocrystal-Based Therapeutics

Yousra Shafiq[1], Huma Ali[2], Abubakar Jamshaid[3], Muhammad Nadeem[3], and Yumna Hydrie[3]

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Dear Editor,

Poor aqueous solubility is a critical issue that hinders the clinical integration of nearly 90% of the drug candidates in the discovery pipeline. Addressing that biopharmaceutical problem, nanocrystal-based drug formulations have emerged as a promising solution, particularly for BCS Class II and IV drugs, where oral absorption is compromised mainly due to inadequate solubility or permeability. Nanocrystals consist of crystalline particles of the pure drug, stabilized by minimal concentration of excipients and do not contain any carrier system[1,2]. By engineering these crystalline active ingredients into the nanometer range, dissolution velocity is significantly increased, resulting in better aqueous solubility, improved bioavailability and potential dose reduction[1-3]. However, several translational, design, and regulatory barriers continue to limit the seamless transition of nanocrystal therapeutics into clinical use[4].

The design of nanocrystal formulations poses multifaceted challenges because of their nanoscale physiochemical traits and sensitivity to both formulation and manufacturing parameters[3]. Firstly, the control of particle size in nanocrystal drugs is of considerable importance as minor variations can directly influence therapeutic efficacy of the product by altering dissolution rate, solubility, bioavailability, and cellular internalization. Another major challenge is aggregation which comes due to the greater surface area of nanocrystals, although it improves dissolution but simultaneously causes crystal growth, as high surface energy thermodynamically favors this phenomenon, compromising the desired advantages of nanocrystals[5]. Additionally, electrostatic stabilization and maintaining particle shape of nanocrystals is pivotal as the morphology and zeta potential affects biological activity. Stabilizers are used to control these parameters but currently their selection criteria is mainly empirical, rather than mechanistic insights which can lead to unpredictable toxicity or immunogenicity. Moreover, there is a definite lack of systematic QbD frameworks for nanocrystal-based product development. Opting Qbd and QRM driven design frameworks is necessary for a reproducible, high quality nanocrystal formulation[1,6].

The clinical success of nanocrystals is also restricted by many translational barriers, notably the incapability of current in vitro in vivo correlation (IVIVC) models to accurately predict the behavior of nanocrystals in complex physiological environment. Besides, the in vivo performance of nanocrystals differs greatly from the in vitro outcomes. Moreover, the phenomenon of Ostwald ripening during in vivo circulation of nanocrystals can highly increase the risk of embolism in fine capillaries, questioning their safety profile. In addition, the formation of a 'protein corona' as a result of interaction of nanocrystal drug particles with plasma proteins, may alter a nanoparticle's surface properties, mask the targeting ligands, and increase recognition and clearance by the immune system[2]. Furthermore, the field suffers from an over-reliance on animal models that lack clinical relevance, combined with a deficiency in long-term human safety data[6].

Lastly, regulatory barriers and insufficient pharmacovigilance studies complicate the global development of nanocrystals as drug delivery systems[1,7]. The lack of nanocrystal-specific guidelines brings about variability in classification and approval amongst defined regulatory bodies e.g. FDA and EMA[1]. Insufficiency of post marketing surveillance and reporting of nano specific adverse effects may result in compromised patient safety. Likewise, absence of

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validated analytical standards for characterisation compromises batch-to-batch consistency and large-scale production[6]. Focus on strengthening global harmonization, development of clear regulatory pathways, and establishing rigorous pharmacovigilance systems will ensure industry confidence[1,7].

All things considered, nanocrystals hold undeniable clinical potential to further enhance the prior mentioned gaps that must be addressed. Aligning the respective intent of academia, industry, and regulatory bodies, is an effective way to develop a future which is rational, safe, and effective in nano medicine[6,7].

FDA — Food and Drug Administration; EMA — European Medicines Agency; QbD — Quality by Design; QRM — Quality Risk Management; BCS — Biopharmaceutics Classification System

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