ISSN 1024 – 6193

PAKISTAN JOURNAL OF PATHOLOGY

An Official Journal of Pakistan Association of Pathologists and all Societies of Pathology Specialties

Quarterly

Vol. 35, No. 2, Apr - Jun 2024

Indexed with Indexus Medicus for the Eastern Mediterranean Region (IMEMR), EBSCO Host, Register with International Standard Serial Number (ISSN-France), Indexed with PASTIC, Asian Digital Library, PakMediNet, , NLM Catalog (ID 9425966), Recognized by the Pakistan Medical and Dental Council (PM&DC) and Higher Education Commission (HEC) Islamabad (Category-Y)

Website: http://www.pakjpath.com

Pakistan Journal of Pathology

Vol. 35, No. 2, Apr - Jun 2024

An Official Journal of Pakistan Association of Pathologists

Recognized by Higher Education Commission in Category **'Y'**

CONTENTS

ORIGINAL ARTICLES

CASE REPORT

Original Article

Frequency and covariates of molecular subcategories of breast carcinoma - a referral tertiary care center study in Khyber Pakhtunkhwa, Pakistan

Hina Khan[1], Abdul Qadir[1], Sadia Khan[2], Shehla Akbar[1]

[1]Combined Military Hospital, Peshawar Pakistan

[2]School of Professional Psychology, University of Management and Technology, Lahore Pakistan

ABSTRACT

Objective: To assess the frequency of different molecular subcategories of breast cancer and establish correlations with clinical and pathological features at a tertiary care center in Khyber Pakhtunkhwa, Pakistan.

Material and Methods: This cross-sectional study was conducted at CMH, Peshawar, Pakistan (a tertiary care center of Khyber Pakhtunkhwa, serving as a referral center for Bannu, Mardan, Nowshera, Risalpur, Landikotal, and Kohat city) from January 2021 to December 2022. Non-probability consecutive sampling technique was used to collect breast cancer samples i.e., biopsies, lumpectomies, and mastectomies of 161 cases. Immunohistochemistry was applied to all cases using polyclonal antibodies for ER, PR, HER2, and Ki-67 stains by DAKO envision method. All the cases were classified into four molecular subtypes of breast carcinoma (Luminal A, Luminal B, Her2 enriched, and triple-negative) according to the 2011 St Gallen consensus report.

Results: In this study, 161 patients were enrolled, with a mean age of 51.20±13.20 yrs (range: 22 to 75 yrs). The distribution of molecular subtypes revealed Luminal A as the most prevalent (29.9%), followed by Luminal B (26.7%), Her2 enriched (25.5%), and Triple negative (18.0%). Luminal A subtype predominantly affected individuals aged 31-50 yrs and 51-70 yrs, while Luminal B was more common in the 51-70 yr age group. Her2 enriched subtype was prevalent among the elderly, whereas the Triple-negative subtype impacted younger individuals. Invasive ductal carcinoma was notably the most frequent subtype among Luminal A and Luminal B cases.

Conclusion: Our study found that the Luminal A subtype occurred in 48 cases (29.9%), followed by Luminal B with 43 cases (26.7%). We identified a notable association between increasing age and breast cancer incidence in this study.

Keywords: Breast cancer, Histological characteristics, Molecular classification, Treatment strategies

This article can be cited as: Khan H, Qadir A, Khan S, Akbar S. Frequency and covariates of molecular subcategories of breast carcinoma - a referral tertiary care center study in Khyber Pakhtunkhwa, Pakistan. Pak J Pathol. 2024; 35(2): 54-62.

DOI: https://doi.org/10.55629/pakjpathol.v35i2.814

INTRODUCTION

Breast cancer is a significant global health concern, accounting for a substantial number of new cases and deaths each year. In 2020, breast cancer surpassed lung cancer as the most common cancer worldwide, with 2.3 million newly diagnosed cases. Among female cancers, breast cancer accounts for 24.5% of all cases, highlighting its substantial impact.

Asia bears a significant burden of breast cancer, with an age-standardized incidence rate of 44.95% in this region [1]. Breast cancer poses a multifaceted public health challenge in Asia, demanding concerted efforts and effective interventions. The region's expansive population, diverse cultures, and abundant resources present both prospects and complexities in tackling this disease.

Pakistan, in particular, faces a considerable challenge, with a relatively high age-standardized incidence rate (38.4 per 100,000 population) and prevalence rate (87.6 per 100,000 population) for breast cancer [2]. The incidence and prevalence of breast cancer

Correspondence: **Dr. Hina Khan, Trainee Histopathology, Department of Pathology, Combined Military Hospital, Peshawar Pakistan**

Email: drhinaahtisham@gmail.com

Receiving Date: **07 Feb 2024**
Revision Date: **03 Apr 2024**
Acceptance Date: **07 May 2024**

in Pakistan are notably significant, with a growing population of women susceptible to the disease. However, barriers such as delayed diagnoses and limited healthcare accessibility contribute to elevated mortality rates. Mitigating these challenges necessitates a steadfast focus on early detection, heightened public awareness, improved healthcare provisions, and the development of tailored treatment approaches.

Understanding the molecular subtypes of breast cancer is essential for effective management and personalized treatment. These subtypes exhibit variations in behavior, clinical features, and response to therapy. However, the frequency and covariates of molecular subtypes may vary among populations and geographical regions, emphasizing the need for local studies to determine the prevalence and clinical characteristics of different subtypes [3].

In this study, we aim to identify the clinical and pathological features that are associated with different molecular subcategories of breast cancer and to determine the frequency of each subcategory from patients of a tertiary care center in Khyber Pakhtunkhwa (KPK), Pakistan. To the best of our knowledge, this is the only study conducted in this province of Pakistan with a greater sample size and includes study samples from six cities of KPK. This study's findings could contribute to a better understanding of the molecular epidemiology of breast cancer in the Pakistani population, and it could help tailor personalized treatment approaches that are specific to the molecular subtype. Moreover, it could provide useful insights for future studies and contribute to improving breast cancer management and outcomes in Pakistan.

MATERIAL AND METHODS

The Cross-sectional study was conducted from January 2021 to December 2022 at CMH, Peshawar, Pakistan (a tertiary care center of Khyber Pakhtunkhwa, serving as a referral center for Bannu, Mardan, Nowshera, Risalpur, Landikotal, and Kohat city) after taking ethical approval from Institutional Ethical Review Board (IERB). Sample size was calculated by using WHO sample size calculator by using the prevalence of female breast cancers (24.5%) [1], 95% confidence level and 5% margine of error. All excisional biopsies, lumpectomies, and mastectomies were included by using non probability consecutive sampling. Male patients, patients with incomplete medical records, and cases that resulted in Her2 2+ results were excluded from the study.

Data of patients including age, menopausal status, size of tumor, and laterality of breast involved was retrieved. Histological details including histological type, histological grade, presence of ductal carcinoma in situ/lobular carcinoma in situ including grade of in situ component, presence of lymph vascular invasion, and nodal metastasis were collected. For the specimens in which nodes were not submitted with the specimen, radiological reports were used to retrieve this information.

Immunohistochemistry was applied to all cases using polyclonal antibodies for ER, PR, HER2, and Ki-67 stains by DAKO envision method. The pressure cooker method was used for heat-induced epitope retrieval. Positive and negative controls were used for interpretation. ER and PR stains were interpreted according to the Allred scoring system (Figure-I, II). ER low was defined as a total score of 3-4 and ER high as a score of 5-8. The Her 2 staining was done according to the CAP protocol 2020 (Figure-III). Ki-67 was interpreted according to the joint guidelines of the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP). All the cases were classified into four molecular subtypes of breast carcinoma (Luminal A, Luminal B, Her2 enriched, and triple-negative) according to the 2011 St Gallen consensus report. The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 22. Frequencies and percentages were computed for qualitative variables, while means and standard deviations were calculated for quantitative variables. The association was evaluated through chi-square test, considering a p-value of less than or equal to 0.05 as significant.

RESULTS

A total of 161 patients were included in this study with mean age 51.20±13.20 years range from 22 to 75 years. Among the molecular subtypes, Luminal A was found to be the most common (29.9%), followed by Luminal B (26.7%), Her2 enriched (25.5%), and Triple negative (18.0%). For less than 30 years, the most common molecular subtype was Triple-negative breast cancer (57.9%), for 31 to 50 years Luminal A (35.4%), for 51 to 70 years Luminal B (35.2%) and for more than 70 years again Luminal A (50%) breast cancer. Most of the patients were in the age range of 51-70 years. A positive association was found between the increasing age of the patient and breast carcinoma, as p-value = 0.010. However, regarding the menopausal status and age of the patient, no significant association (p value > 0.05) between the molecular subtypes and these two parameters was found (Table-I). Most of the postmenopausal women had Luminal B breast cancer (32.2%), while premenopausal patients had Luminal A type cancer (32.4%). Concerning breast laterality, no significant differences were observed among the molecular subtypes (p value >0.05). The proportions of left breast tumors (90/161) were more than right breast tumors (71/161). Tumor size was categorized into three groups: <2 cm, >2 cm but ≤5 cm, and >5 cm. The majority of tumors (90/161) diagnosed had a size of >2 cm but ≤5 cm, (24/161) cases diagnosed, were having a size of <2 cm, and (47/161) had a size of more than 5 cm at the time of diagnosis.

When considering the molecular profiles, the Luminal A subtype was observed in 48 cases (29.9%), followed closely by Luminal B with 43 cases (26.7%), Her2 enriched with 41 cases (25.5%), and Triple negative with 29 cases (18.0%). Regarding the histological subtypes, the most common was invasive ductal carcinoma, accounting for 122 cases (75.8%). This was followed by invasive lobular carcinoma with 19 cases (11.8%) and mixed ductal and lobular carcinoma with 8 cases (5%). There were smaller proportions of cases represented by invasive cribriform carcinoma, metaplastic carcinoma, mucinous carcinoma, encapsulated papillary carcinoma, and tubular carcinoma. Among the cases of invasive ductal carcinoma, the most common molecular subtype was Luminal A (31.1%), followed by Luminal B (26.2%). Invasive lobular carcinoma cases predominantly belonged to the Luminal B subtype (42.1%). Among the cases of mixed ductal and lobular carcinoma, the Her2 enriched subtype was most frequent (37.5%), while the Luminal A subtype was observed in cases of invasive cribriform carcinoma, mucinous carcinoma, and tubular carcinoma. Metaplastic carcinoma exhibited a Triple negative molecular profile, whereas encapsulated papillary carcinoma cases showed both Her2 enriched and Triple negative profiles (Table-II).

The histological characteristics showed no significant correlation with various molecular subtypes (p>0.05). 91.9% of cases exhibited grade II tumors, followed by 8.1% of grade I tumors. Most of the Triple-negative tumors (16, 55.2%) were of grade III. Notably, 90 cases of Luminal A, Luminal B and Her2 enriched cases were classified as Grade II. Ductal carcinoma in situ (DCIS) was predominantly found in Her2 enriched (36.4%) and Triple negative cases (27.3%), while it was notably absent in most Luminal A (46.6%) and Luminal B cases (34.2%). The prevalent grade of DCIS among all molecular subtypes was low. In terms of lymph node metastasis, Luminal A cases exhibited lymph node involvement in 11 cases (14.9%), Luminal B in 25 cases (33.8%), Her2 enriched in 21 cases (28.4%), and Triple negative in 17 cases (23%). The lymphovascular invasion was present in 14.8% of Luminal A, 31.8% of Luminal B, 28.4% of Her2 enriched, and 25% of Triple-negative cases (Table-III).

Table-I: Correlation of clinicopathologic parameters of Luminal A, Luminal B, Her 2 enriched and Triple negative carcinoma (n=161)

Clinicopathological parameters	Luminal A	Luminal B	Her2 enriched	Triple-negative	Total	p-value
Age groups						
≤ 30 yrs	3 (15.8%)	2 (10.5%)	3 (15.8%)	11 (57.9%)	19	0.23
31-50 yrs	23 (35.4%)	14 (21.5%)	16 (24.6%)	12 (18.5%)	65	0.58
51-70 yrs	19 (26.8%)	25 (35.2%)	22 (31%)	5 (7%)	71	0.43
70 > yrs	3 (50%)	2 (33.3%)	0 (0.0%)	1 (16.7%)	6	0.29
Menopause						
Pre-menopause	24 (32.4%)	15 (20.3%)	19 (25.7%)	16 (21.6%)	74	0.56
Post- menopause	24 (27.6%)	28 (32.2%)	22 (25.3%)	13 (14.9%)	87	0.47
Breast laterality						
Left	23 (25.6%)	24(26.7%)	26 (28.9%)	17 (18.9%)	90	0.71
Right	25 (35.2%)	19(26.8%)	15 (21.1%)	12 (16.9%)	71	0.51
Tumor size						
<2 cm	13 (54.2%)	6(25%)	4 (16.7%)	1 (4.2%)	24	0.33
>2 cm but ≤ 5 cm	25 (27.8%)	28(31.1%)	20 (22.2%)	17 (18.9%)	90	0.61
> 5 cm	10 (21.3%)	9(19.1%)	17 (36.2%)	11 (23.4%)	47	0.74

Table II: Distribution of histological types of breast carcinoma among Luminal A, Luminal B, Her 2 enriched and Triple negative carcinoma (n=161).

Sr #	Histological type	Total	Luminal A	Luminal B	Her2 enriched	Triple-negative
1	Invasive ductal carcinoma of no special type	122	38(31.1%)	32(26.2%)	30 (24.6%)	22 (18.3%)
2	Invasive lobular carcinoma	19	4 (21.1%)	8 (42.1%)	5 (26.3%)	2 (10.5%)
3	Mixed ductal & lobular carcinoma	8	1 (12.5)	2 (25.0%)	3 (37.5)	2 (25.0%)
4	Invasive cribriform carcinoma	3	2 (66.6%)	0	1 (33.4%)	0
5	Metaplastic carcinoma	3	0	0	1 (33.4%)	2 (66.6%)
6	Mucinous carcinoma	3	2 (66.6%)	1 (33.4%)	0	0
7	Encapsulated papillary carcinoma	2	0	0	1 (50.0%)	1 (50.0%)
8	Tubular carcinoma	1	1 (100.0%)	0	0	0
	Total	**161**	**48 (29.8%)**	**43 (26.7%)**	**41 (25.5%)**	**29 (18.0%)**

Note. 161 cases divided into 4 groups across 8 histological types of breast carcinoma. Frequency (percentage)

Table-III: Correlation of histological features of Luminal A, Luminal B, Her2 enriched, and Triple negative carcinoma (n=161).

Histological features	Luminal A	Luminal B	Her2 enriched	Triple-negative	Total	p-value
Histological grade						
Grade I	36 (85.7%)	2 (4.8%)	4 (9.5%)	0	42	0.52
Grade II	12(13.3%)	35(38.9%)	30 (33.3%)	13 (14.4%)	90	
Grade III	0	6(20.7%)	7 (24.1%)	16 (55.2%)	29	
DCIS						
Present	14 (15.8%)	18 (20.5%)	32 (36.4%)	24 (27.3%)	88	0.43
Absent	34 (46.6%)	25 (34.2%)	9 (12.3%)	5 (6.8%)	73	
DCIS grade						
Not applicable	34 (46.6%)	25 (34.2%)	9 (12.3%)	5 (6.8%)	73	0.08
Low	12 (21.1%)	12 (21.1%)	23 (40.1%)	10 (17.5%)	57	
High	2 (6.5%)	6 (19.4%)	9 (29%)	14 (45.2%)	31	
LVI						
Present	13 (14.8%)	28 (31.8%)	25 (28.4%)	22 (25%)	88	0.47
Absent	35 (47.9%)	15 (20.5%)	16 (21.9%)	7 (9.6%)	73	
Lymph node metastases						
Absent	32 (46.4%)	14 (20.3%)	16 (23.2%)	7 (10.1%)	69	0.44
Present	11 (14.9%)	25 (33.8%)	21 (28.4%)	17 (23%)	74	
Unclear	5 (27.8%)	4 (22.2%)	4 (32.2%)	5 (27.8%)	18	
Total	**48**	**43**	**41**	**29**	**161**	**-**

Note: Chi-square run through significance test of Phi and Cramer V values. DCIS (Ductal carcinoma in situ), LVI (Lympho-vascular invasion).

Table-IV: Distribution of molecular subtypes of breast carcinoma by immunohistochemistry in local studies from different cities of Pakistan.

Author	Setting	Number of patients	Years	Luminal A	Luminal B	Her2 enriched	Triple-negative
Current study	Peshawar	161	Jan 2021-Dec 2022	48	43	41	29
Sharif N, *et al*.	Peshawar	60	2012-2013	20	11	14	14
AA Hashmi, *et al*.	Karachi	1951	2011-2016	37%	63%	---	---
Alam S, *et al*.	Lahore	110	Jul 2016-Jan 2017	41	69	---	---
Akbar A, *et al*.	Islamabad	50	Jan 2015-oct 2016	15	17	14	4
Khokhar S, *et al*.	Lahore	261	Oct 2013-Mar 2015	54	72	32	50
Akbar M, *et al*.	Abbottabad	60	Jan2010-Dec 2010	17	15	18	10
Hashmi A, *et al*.	Karachi	1104	Jan 2010-Dec 2012	45.8%	17.8%	17.8%	18.6%
Mushtaq M, *et al*.	Islamabad	278	2016	10%	51%	18%	20%
Gulzar R, *et al*.	Karachi	285	Dec 2012-Dec 2015	60	139	54	32
Henna N, *et al*.	Lahore	83	2019	20.5%	9.6%	15.7%	27.7%
Sikandar B, *et al*.	Karachi	1247	2008-2012	28%	20%	10%	36%
Tabassum S, *et al*.	Karachi	119	Jan 2013-Dec 2014	17	38	16	30

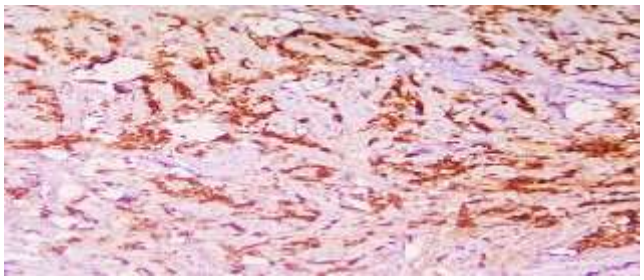

Figure-I: Immunostain for Estrogen receptor in Invasive Ductal Carcinoma (no special type), strong nuclear positivity, score 8/8

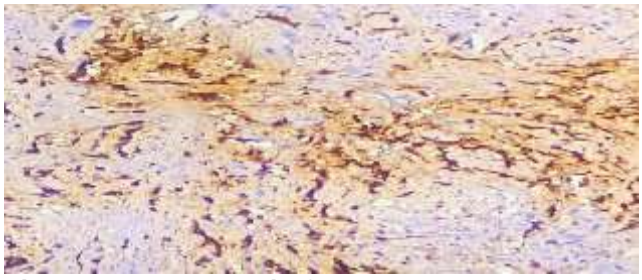

Figure-II: Immunostain for Progesterone receptor in Invasive Ductal Carcinoma (no special type), strong nuclear positivity, score 8/8

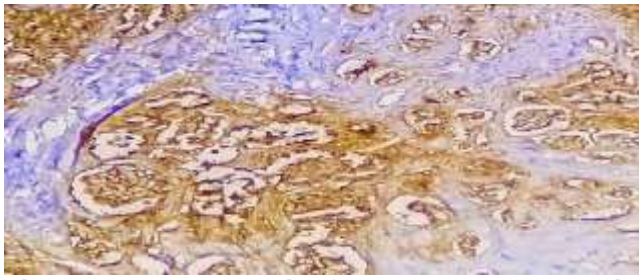

Figure-III: Immunostain for Her 2 receptors in Invasive ductal carcinoma (no special type), 3 + staining, strong, complete, membranous pattern.

DISCUSSION

Breast carcinoma is a heterogeneous disease with varying clinical and molecular characteristics. The identification and classification of molecular subtypes have revolutionized the field of breast cancer research and patient management. Additionally, histological features play a crucial role in understanding tumor behavior and guiding treatment decisions. In this study, we aimed to investigate the frequency of molecular subtypes in our population and the correlation between histological characteristics, molecular subtypes, and clinical parameters in breast carcinoma.

A total of 161 breast carcinoma cases were included in this study from CMH Peshawar Histopathology Laboratory. Our study showed that most of the cases of breast carcinoma were having an age range of 51-70 years with a mean age of 51.2 years ±13.2. Our findings are in accordance with several local and international studies [4-8]. However, they are in contrast to some recent local studies including a study by author Ullah Z, *et al* and Akbar F, *et al* [9,10].

Our study revealed that the Luminal A subtype of breast cancer was predominantly observed in the age group of 31 to 50 years (35.4%), with a secondary peak in the 50 to 70-

year age group (26.8%). These findings align with previous studies [11-13] conducted in this field. Notably, our study demonstrated an equal distribution of Luminal A cancer cases between premenopausal and postmenopausal patients, which contrasts with the findings of Dokcu Ş. *et al*. [14], who reported a higher prevalence of Luminal cancers among postmenopausal women and non-Luminal cancers among premenopausal patients. Histologically, Invasive ductal carcinoma was the most frequent subtype observed among Luminal A cases (38/48), followed by Invasive lobular carcinoma (4/48). Luminal A cancers are known to be hormone-responsive, typically exhibiting low-grade features and favorable prognoses [11, 15]. Consistent with this, our data indicated a significant proportion of Luminal A cases with absent lymphovascular invasion (13/48) and lymph node metastasis (32/48).

Our study showed most cases with a higher histological grade in comparison to Luminal A cancers, particularly with a predominant occurrence of invasive ductal carcinoma of no special type (32/43). Compared to the Luminal A subtype, the Luminal B subtype exhibits an intermediate prognosis and a higher likelihood of locoregional recurrence [16,17].

Her2-enriched carcinoma is characterized by genetic amplification and elevated expression of the HER2 protein. In our study, this subtype is prevalent in the older age group (50 -70 years) and comprising of most tumors with sizes> 2cm and > 5 cm. This molecular subtype is characterized by higher histological grade, increased proliferative index, and a higher propensity for metastasis, leading to shorter disease-free survival and poorer prognosis [18]. However, HER2-positive tumors have shown favorable responses to targeted therapies such as Trastuzumab (a humanized monoclonal antibody) and Lapatinib (a molecular receptor tyrosine kinase inhibitor) that specifically inhibit HER2 activity [19, 20]. Most cases in our data were invasive ductal carcinoma of no special type (30/41), followed by invasive lobular carcinoma (5/41) and mixed ductal and lobular carcinoma (3/41). This finding contrasts with a study by SM Fragomeni, stating that HER2 overexpression is exclusive to invasive lobular carcinoma [21]. Our study showed most cases with intermediate grade, lymph node metastasis as well as lymphovascular invasion.

Our study showed triple negative cases to be prevalent in the age range of less than 30 years and between 30 to 50 years. This finding is in accordance with other studies [22,23], where triple-negative breast cancer is more prevalent in younger patients. Triple-negative breast tumors were found more in the left breast, with most cases having sizes more than 2 cm and less than 5 cm, followed by cases of tumors having sizes more than 5 cm, indicating the aggressive behavior of this tumor. Indeed, triple-negative breast cancer is known for its invasive potential, poor prognosis, and relapsing potential [24]. In accordance with its ominous behavior, our findings showed most cases (22/29) having invasive ductal subtype with grade III. Ductal carcinoma in situ with high grade (24/29), lymphovascular invasion (22/29), and lymph node metastasis (17/29) were seen in most cases. Other histological subtypes having triple negative profile were invasive lobular, metaplastic carcinoma, and mixed ductal and lobular carcinoma. These findings are in agreement with other studies showing similar histological and pathological profiles for this molecular subtype [25, 26].

Microarray technology has helped classify these triple-negative tumors into basal-like subtype and breast-like subtype, through the interpretation of markers including CK5/6, CK14, CK17, and EGFR [27]. Triple-negative breast cancer poses a significant therapeutic challenge due to its highly invasive behavior and limited responsiveness to treatment, hence currently being under scrutiny of researchers to find alternate treatment modalities for this molecular subtype.

The most frequent molecular subtype in our study was Luminal A (48/161), followed by Luminal B (43/161) type cancer and Her2 enriched (41/161). Most studies in the Asian region have similar results with Luminal A type

cancer being the dominant subtype [11, 15, 28], however, few studies show Luminal B type cancer to have a higher prevalence than Luminal A [29, 30]. In comparative analysis with local studies conducted in Pakistan regarding molecular subtyping of beast carcinoma, two studies had Luminal A subtype predominance, one in Peshawar city and the other in Karachi (Table-IV). However, the majority of studies conducted in Pakistan show Luminal B subtype prevalence in various regions. A single study by Akbar M, *et al*. showed Her 2 enriched carcinoma majority, while studies by Sikandar B, *et al*. and Henna N, *et al*. showed Triple-negative breast cancer predominance in their study sample.

The presentation of 131/161 cases having sizes more than 2 cm and 119/161 cases with grade 2 and 3 cancers in our study sample enlightens the dire need for improvement in cancer diagnostics and breast cancer awareness in Pakistan. Pakistan has the highest incidence rate of breast cancer in Asia, affecting approximately one out of every nine women [31]. Between December 1995 and December 2009, breast cancer accounted for 45.9% of all diagnosed malignancies among adult women in Pakistan, with around 30% of cases being diagnosed at advanced stages (III or IV) [32]. Late diagnosis in Pakistan is primarily attributed to factors such as limited breast health awareness, personal modesty, and religious and cultural factors that contribute to the reluctance to seek medical attention from male doctors [33]. Addressing these challenges is crucial to improve early detection and treatment outcomes for breast cancer in Pakistan.

CONCLUSION

According to our study, the Luminal A subtype was observed in 48 cases (29.9%), followed by Luminal B with 43 cases (26.7%). A significant association between increasing age and breast cancer was found. Further investigations are warranted to explore the clinical implications and therapeutic considerations associated with specific subtypes of breast carcinoma.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Hina Khan: Conceptualization, data curation, validation, Methodology, revisions
Abdul Qadir: Methodology, supervision
Sadia Khan: Data analysis, revisions
Shehla Akbar: Data interpretations, revisions

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021; 71(3): 209-49. DOI: https://doi.org/10.3322/caac.21660
2. Breast cancer statistics in Pakistan 2021 [updated 4th April. Available from: https://gco.iarc.fr/today/data/factsheets/populations/586-pakistan-fact-sheets.pdf.
3. Tsang JYS, Tse GM. Molecular classification of breast cancer. Adv Anat Pathol. 2020; 27(1): 27-35. DOI:https://doi.org/10.1097/pap.0000000000000232
4. Maass SWMC, Boerman LM, Verhaak PFM, Du J, de Bock GH, Berendsen AJ. Long-term psychological distress in breast cancer survivors and their matched controls: A cross-sectional study. Maturitas. 2019; 130: 6-12. DOI: https://doi.org/10.1016/j.maturitas.2019.09.003
5. Özmen V, Özmen T, Doğru V. Breast Cancer in Turkey; An analysis of 20.000 patients with breast cancer. Eur J Breast Health. 2019; 15(3): 141-6. DOI: https://doi.org/10.5152/ejbh.2019.4890
6. Pandit P, Patil R, Palwe V, Gandhe S, Patil R, Nagarkar R. Prevalence of Molecular subtypes of breast cancer: A single institutional experience of 2062 patients. Eur J Breast Health. 2019; 16(1): 39-43. DOI: https://doi.org/10.5152/ejbh.2019.4997
7. Azam M, Aslam M, Basharat J, Mughal MA, Nadeem MS, Anwar F. An empirical study on quality of life and related factors of Pakistani breast cancer survivors. Sci Rep. 2021; 11(1): 24391. DOI: https://doi.org/10.1038/s41598-021-03696-9
8. Shah A, Haider G, Abro N, Bhutto S, Baqai TI, Akhtar S, et al. Correlation between age and hormone receptor status in women with breast cancer. Cureus. 2022; 14(1): e21652. DOI: https://doi.org/10.7759%2Fcureus.21652
9. Ullah Z, Khan MN, Din ZU, Afaq S. Breast cancer awareness and associated factors amongst women in Peshawar, Pakistan: A cross-sectional study.

Breast Cancer (Auckl). 2021 22; 15: 11782234211025346.
DOI: https://doi.org/10.1177/11782234211025346

10. Akbar F, Siddiqui Z, Waheed MT, Ehsan L, Ali SI, Wiqar H, *et al*. Spectrum of germline pathogenic variants using a targeted next generation sequencing panel and genotype-phenotype correlations in patients with suspected hereditary breast cancer at an academic medical centre in Pakistan. Hered Cancer Clin Pract. 2022 16; 20(1): 24. DOI:https://doi.org/10.1186/s13053-022-00232-2
11. Pandit P, Patil R, Palwe V, Gandhe S, Patil R, Nagarkar R. Prevalence of molecular subtypes of breast cancer: A single institutional experience of 2062 patients. Eur J Breast Health. 2019; 16(1): 39-43. DOI: https://doi.org/10.5152/ejbh.2019.4997
12. Widiana IK, Irawan H. Clinical and subtypes of breast cancer in indonesia. Asian Pacific J Cancer Care. 2020; 5(4): 281-5. DOI:https://doi.org/10.22034%2FAPJCP.2018.19.1.161
13. Zhang L, Huang Y, Feng Z, Wang X, Li H, Song F, et al. Comparison of breast cancer risk factors among molecular subtypes: A case-only study. Cancer Med. 2019; 8(4): 1882-92. DOI: https://doi.org/10.1002/cam4.2012
14. Dokcu S, Caparlar MA, Başçeken Sİ, Eroglu A. Distribution anclinicopathological characteristics of breast cancer molecular subtypes in Turkish women. Eur J Clin Med. 2022; 3(6): 14-20. DOI:https://doi.org/10.24018/clinicmed.2022.3.6.220
15. Mthembu JG, Bhuiyan M. Profile of molecular subtyping of breast cancer and clinicopathological features in Mankweng Hospital breast oncology clinic, Limpopo Province, South Africa. S Afr Med J. 2021; 111(11b): 1132-5. DOI: https://doi.org/10.7196/samj.2021.v111i11b.16104
16. Tsoutsou PG, Vozenin MC, Durham AD, Bourhis J. How could breast cancer molecular features contribute to locoregional treatment decision making?. Crit Rev Oncol Hematol. 2017 1; 110: 43-8. DOI:https://doi.org/10.1016/j.critrevonc.2016.12.006
17. Hashmi AA, Aijaz S, Khan SM, Mahboob R, Irfan M, Zafar NI, *et al*. Prognostic parameters of luminal A and luminal B intrinsic breast cancer subtypes of Pakistani patients. World J Surg Oncol. 2018; 16(1): 1-6. DOI: https://doi.org/10.1186/s12957-017-1299-9
18. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, *et al*. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis. 2018; 5(2): 77-106. DOI: https://doi.org/10.1016/j.gendis.2018.05.001
19. Tsang JYS, Tse GM. Molecular classification of breast cancer. Adv Anat Pathol. 2020;27(1):27-35. DOI:https://doi.org/10.1097/pap.0000000000000232
20. Llombart-Cussac A, Cortés J, Paré L, Galván P, Bermejo B, Martínez N, *et al*. HER2-enriched subtype as a predictor of pathological complete response following trastuzumab and lapatinib without chemotherapy in early-stage HER2- positive breast cancer (PAMELA): An open-label, single-group, multicentre, phase 2 trial. Lancet Oncol. 2017; 18(4): 545-54. DOI:http://dx.doi.org/10.1016/S1470-2045(17)30021-9
21. Fragomeni SM, Sciallis A, Jeruss JS. Molecular Subtypes and local-regional control of breast cancer. Surg Oncol Clin N Am. 2018; 27(1): 95-120. DOI:https://dx.doi.org/10.1016%2Fj.soc.2017.08.005
22. Al-Thoubaity FK. Molecular classification of breast cancer: A retrospective cohort study. Ann Med Surg. 2020; 49: 44-8. DOI: https://doi.org/10.1016/j.amsu.2019.11.021
23. Newman LA, Kaljee LM. Health disparities and triple-negative breast cancer in African American women: a review. JAMA Surgery. 2017; 152(5): 485-93. DOi: https://doi.org/10.1001/jamasurg.2017.0005
24. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Res. 2020 Dec;22:1-3. DOI: https://doi.org/10.1186/s13058-020-01296-5
25. Zhao S, Zuo WJ, Shao ZM, Jiang YZ. Molecular subtypes and precision treatment of triple-negative breast cancer. Ann Transl Med. 2020; 8(7): 499. DOI: https://doi.org/10.21037/atm.2020.03.194
26. Fragomeni SM, Sciallis A, Jeruss JS. Molecular subtypes and local-regional control of breast cancer. Surgical Oncol Clin. 2018; 27(1): 95-120. DOI: https://doi.org/10.1016%2Fj.soc.2017.08.**005**
27. Lundgren C, Bendahl PO, Borg Å, Ehinger A, Hegardt C, Larsson C, et al. Agreement between molecular subtyping and surrogate subtype classification: A contemporary population-based study of ER-positive/HER2-negative primary breast cancer. Breast Cancer Res Treat. 2019; 178: 459-67. DOI: https://doi.org/10.1007/s10549-019-05378-7
28. Widiana IK, Irawan H. Clinical and subtypes of breast cancer in Indonesia. Asian Pacific J Cancer Care. 2020; 5(4): 281-5. DOI:https://doi.org/10.31557/apjcc.2020.5.4.281-285
29. Paramita S, Raharjo EN, Niasari M, Azizah F, Hanifah NA. Luminal B is the most common intrinsic molecular subtypes of invasive ductal breast carcinoma patients in East Kalimantan, Indonesia. Asian Pac J Cancer Prev. 2019; 20(8): 2247. DOI: https://doi.org/10.31557/apjcp.2019.20.8.2247
30. Sohail S, Alam SN. Breast cancer in pakistan - awareness and early detection. J Coll Physicians Surg Pak. 2007 Dec;17(12):711-2.
31. Badar F, Faruqui ZS, Uddin N, Trevan EA. Management of breast lesions by breast physicians in a heavily populated South Asian developing country. Asian Pac J Cancer Prev. 2011; 12(3): 827-32.

32. Banning M, Hafeez H. A two-center study of Muslim women's views of breast cancer and breast health practices in Pakistan and the UK. J Cancer Educ. 2010; 25(3): 349-53.
DOI: https://doi.org/10.1007/s13187-010-0051-8

33. Bottorff JL, Grewal SK, Balneaves LG, Naidu P, Johnson JL, Sawhney R. Punjabi women's stories of breast cancer symptoms: Gulti (lumps), bumps, and Darad (pain). Cancer Nurs. 2007; 30(4): E36-45.
DOI:https://doi.org/10.1097/01.ncc.0000281738.15307.d8

Original Article

Microbiological profile of septic arthritis in Pakistani population – A prospective study

Amna Younas, Irim Iftikhar, Karam Rasool

Chughtai Institute of Pathology, Lahore Pakistan

ABSTRACT

Objective: The objective of this study is to observe the positive rate of aspirated synovial fluid culture, bacterial isolation and their antibiotic susceptibility against commonly used drugs in a microbiology laboratory.

Material and Methods: During a period of 6 months, 398 synovial fluid samples were analyzed using VITEK-MS for identification and tested for antibiotic susceptibility following standard recommendations.

Results: Only 22% (89) of the samples showed aerobic bacterial growth while 77.6% (309) were negative. The primary pathogen, *Staphylococcus aureus* (49.4%), exhibited resistance to Cefoxitin (56%), Co-trimoxazole (17.1%), and Clindamycin (13.6%). *Escherichia coli* and *Pseudomonas species* (10% each) were the other significant contributors. Concerningly, gram negative exhibited resistance to Ceftriaxone (68%) and Meropenem (20%), indicating a rise in antimicrobial resistance (AMR) in the community. Other less frequently isolated bacteria included *Coagulase negative staphylococcus, Streptococcus pneumoniae, Streptococcus pyogenes, Burkholderia species*, *Acinetobacter* and *Enterobacter species*. Females (57.3%) in our community were more affected than males (42.6%). Elderly patients, more than 60 years of age, are more affected (25%) than any other age group.

Conclusion: The increasing prevalence of MRSA, ESBL, and CRE poses challenges in treatment, leading to higher mortality and morbidity. Early diagnosis through PCR or culture and sensitivity, targeted or combination therapy, and implementation of an "Antibiotic Stewardship Program" can help reduce morbidity and AMR.

Keywords: Synovial fluid (SF) culture, Septic arthritis (SA), Antibiotic susceptibility in joint fluid, prevalence of septic arthritis, Microbiological profile

This article can be cited as: Younas A, Iftikhar I, Rasool K. Microbiological profile of septic arthritis in Pakistani population – A prospective study. Pak J Pathol. 2024; 35(2): 63-68.

DOI: https://doi.org/10.55629/pakjpathol.v35i2.795

INTRODUCTION

Septic arthritis (SA) is a progressive, destructive condition with potentially irreversible consequences, leading to disability, morbidity, and mortality. Incidence and clinical presentation vary depending on patient comorbidities and demographics. Low-income countries like India reports an incidence ranging between 2 to 20 cases per 100,000 people annually, while Western Europe reports 4-10 cases/ 100,000/ year, and Australia reports 29 cases/ 100,000/year [1-3]. The current prevalence in the Pakistani population remains unknown.

The acute presentation of SA necessitates expedited diagnosis. Predisposing factors include old age, diabetics, rheumatoid arthritis, recent joint surgery, prosthetic joints, and the use of immunosuppressive drugs. Diagnosis involves a combination of radiology, synovial fluid analysis, microbiological culture, and non-specific serum inflammatory markers. Global culture positivity rates are reported to be low, with our study also reporting a 22% positive rate. *Staphylococcus aureus* (*S. aureus*, 49%) emerged as the main causative pathogen in our population. Treatment for SA typically involves a 2-6week regimen, while fastidious bacteria like N. gonorrhoeae or fungal infections require extended antimicrobial courses based on history and serology [4,5,10].

Correspondence: **Dr. Amna Younas, Resident Microbiology, Chughtai Institute of Pathology, Lahore Pakistan**

Email: amnaumer1980@gmail.com

Receiving Date: **29 Oct 2023** Revision Date: **01 Jan 2024** Acceptance Date: **01 Apr 2024**

The objective of this cross-sectional study is to observe the synovial fluid culture positive rate, bacterial isolate frequencies, and antibiotic susceptibility during 6-month period (April - September 2023) at the Microbiology Department of the Chughtai Institute of Pathology, Lahore, Pakistan. Future results will be compared with synovial fluid collected in blood culture bottles to enhance pathogen recovery.

MATERIAL AND METHODS

A cross-sectional, observational study was conducted for 6 months (April to September '23) at the Chughtai Institute of Pathology (CIP) Microbiology BSL-2 laboratory after obtaining the IRB number. The sample size was calculated by using Cochran formula that allowed us to calculate the ideal minimum sample size from unknown population with a desired confidence level (Z), level of precision (e) and estimated proportion of the disease in the population. In this study by reviewing the literature (7) and expert opinion the proportion of *Staphylococcus aureus* the population is 38% with e=5% and 95% confidence level. By using this value in formula, the minimum sample size is 362 was obtained ($\eta_0 = \frac{Z^2 pq}{e^2}$).

Clinical data (Gram stain result, bacterial isolate name, antibiotic susceptibility result) and demographic data (patient age, gender, location) were collected and recorded at Microsoft excel sheet and a unique identification (serial number) was assigned to each sample. No patient identifiable variable (name, contact number etc.) was documented in data to keep confidentiality of patient. Data analysis was performed using Microsoft Excel. Graphs and tables were fabricated considering frequency and/or percentage of categorical data. Antibiotic resistance was calculated by the following formula:

$$Resistance\ percentage = \frac{No.\ of\ resistant\ isolates}{Total\ no\ of\ isolates\ tested} \times 100$$

So, for the current study total of 398 synovial fluid aspirates in a period of 6 months were processed aerobically and anaerobically for culture and sensitivity in both genders and all age groups. Anaerobic chamber (BACTRON300, Sheldon Manufacturing USA) was used to process samples anaerobically. Duplicate samples of the same patient and swabs were excluded. Bacterial isolates after incubation period of 18-24 hours with 5% CO2 at 37°C, were identified using MALDI-TOF. Antimicrobial sensitivities were tested using disc diffusion/minimum inhibitory concentration (MIC) methods on Mueller Hilton Agar (MHA) or SBMHA, following CLSI M100 guidelines, with QC strains *Escherichia coli* (ATCC25922 and ATCC35218), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC25923 and ATCC29213) for testing standardization. Antibiotic breakpoints were species-specific, and results were reported as sensitive (S), resistant (R), or intermediate (I), with 'I' falling between the S and R categories.

RESULTS

A total of 398 synovial fluid samples were processed in the microbiological laboratory, with 89 samples (22%) showing positive results and 309 (77.6%) were negative. Aerobic and facultative anaerobes were isolated, while no obligate anaerobic bacteria were found. All samples exhibited monobacterial growth, with 66% gram-positive cocci (GPC) and 33% gram-negative rods (GNR). Among GPCs, *Staphylococcus aureus* (49.4%) was the primary pathogen, followed by *Streptococcus viridans group* (4.4%), *Streptococcus pyogenes* (3.3%), and *Streptococcus pneumoniae* (2.2%). *S. aureus* was isolated in 49% of cases and among those 56% were found to be MRSA. Moreover *S. aureus* exhibited resistance to Clindamycin (13.6%) and Trimethoprim-sulfamethoxazole (17.1%) in our population.

Among GNR, *E. coli* and *Pseudomonas species* (10% each) made significant contributions to SA. *Klebsiella, Burkholderia species* (3% each), and *E. cloacae* (2%) were also isolated (chart-1). Resistance to Ceftriaxone (ESBLs) was observed in 68% of GNR, while 20% exhibited resistance to Meropenem (CRE). Ciprofloxacin, a commonly used drug for both GPCs and GNR, showed 77% resistance (Table-I & II). Additionally, 25%

of adults over 60 years, 7.8% of children under 10 years, and 6.8% of young adults aged 31-40 were affected, with females being more affected than males (1.3:1).

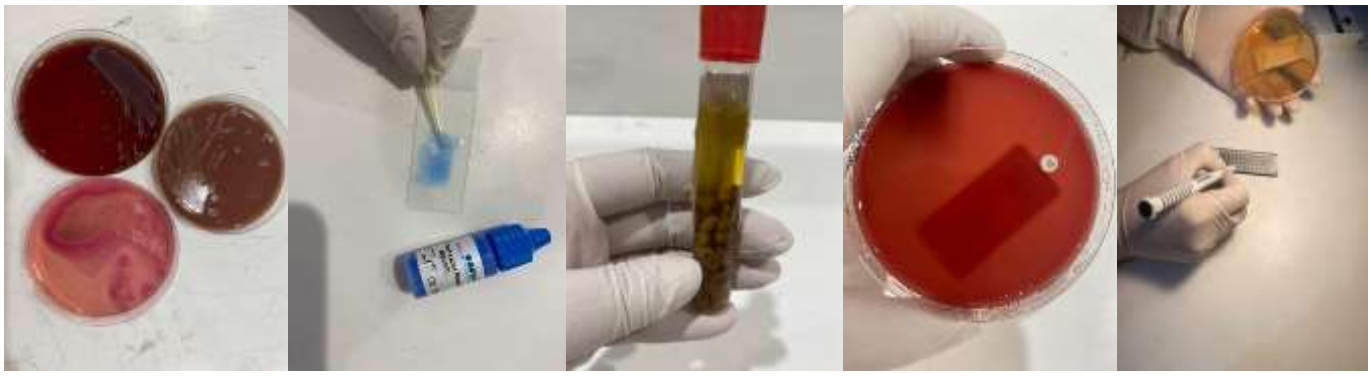

Figure-I: From left to right; growth *of E.coli*, slide coagulase, RCM, plate showing no growth of anaerobe and slide for identification on MALDI-TOF.

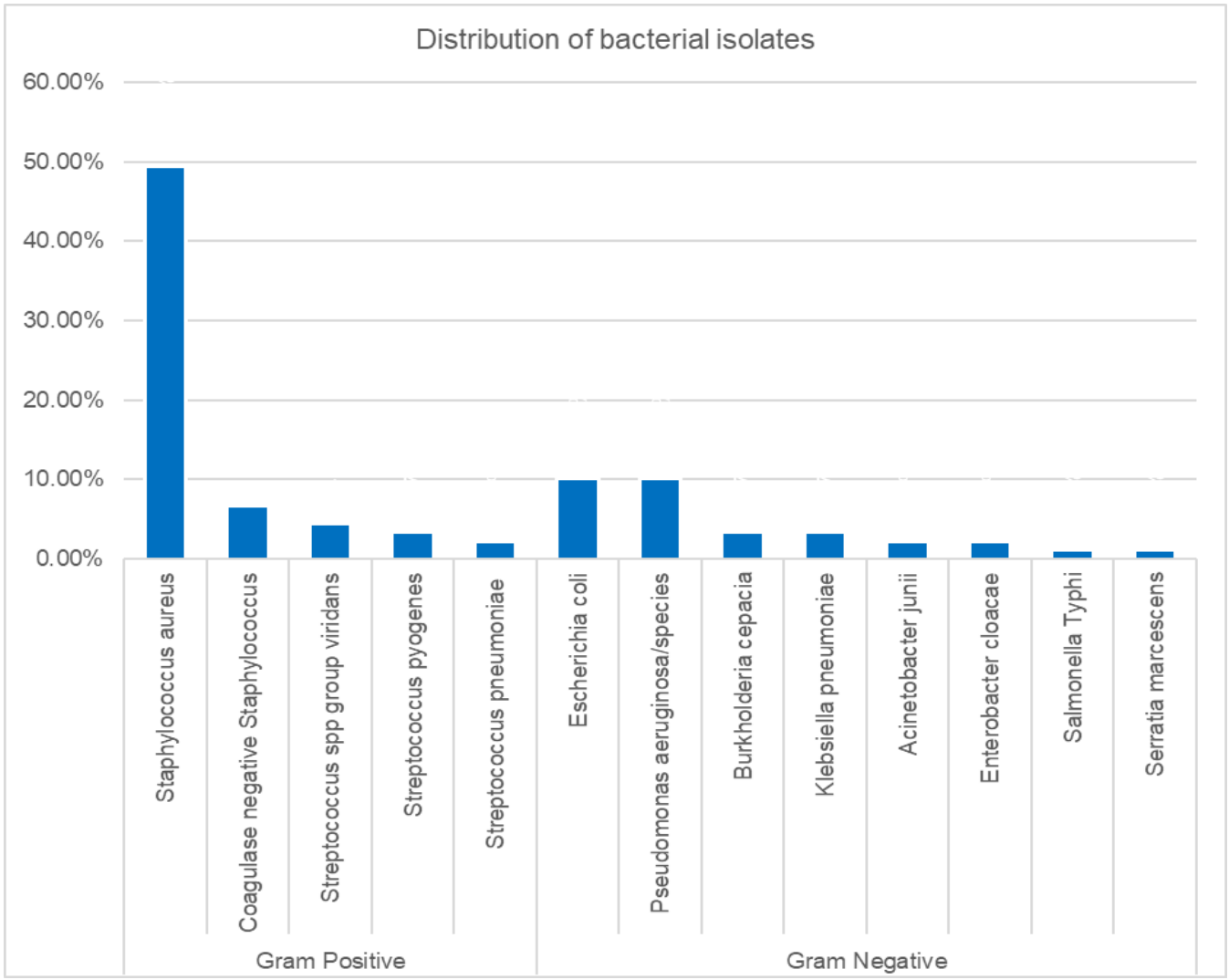

Chart-1: Bacterial isolates in SF culture.

Table-I: Antibiotic resistance profile in Gram positive cocci

Antibiotic resistance profile, Gram positive bacterial isolates	Amikacin	Ampicillin	Ceftriaxone	Ciprofloxacin	Clindamycin	Doxycycline	Erythromycin	Fusidic Acid	Gentamicin	Levofloxacin	Linezolid	Oxacillin	Penicillin	Trime-Sulphamethoxazole	Vancomycin
Staphylococcus aureus	6.7%	NT	NT	88.4%	13.6%	25.0%	79.5%	6.8%	23.3%	NT	0.0%	56.8%	NT	17.1%	0.0%
Coagulase negative Staphylococcus	0.0%	NT	NT	66.7%	0.0%	50.0%	33.3%	16.7%	0.0%	NT	0.0%	0.0%	NT	50.0%	NT
Streptococcus spp group viridans	NT	0.0%	0.0%	NT	0.0%	NT	0.0%	NT	NT	50.0%	NT	NT	NT	NT	0.0%
Streptococcus pyogenes	NT	0.0%	0.0%	NT	33.3%	NT	33.3%	NT	NT	100.0%	NT	NT	NT	NT	0.0%
Streptococcus pneumoniae	NT	NT	0.0%	NT	0.0%	0.0%	0.0%	NT	NT	0.0%	0.0%	NT	0.0%	100.0%	0.0%

Table-II: Antibiotic resistance profile in Gram Negative rods.

Antibiotic resistance profile, Gram negative bacterial isolates	Amikacin	Amoxicillin-Clavulanic acid	Cefepime	Cefixime	Ceftazidime	Ceftriaxone	Ciprofloxacin	Doxycycline	Gentamicin	Imipenem	Levofloxacin	Meropenem	Piperacillin-Tazobactam	Tobramycin	Trime-Sulphamethoxazole
Escherichia coli	11.1%	66.7%	NT	85.7%	NT	88.9%	88.9%	77.8%	44.4%	11.1%	88.9%	11.1%	11.1%	33.3%	44.4%
Pseudomonas aeruginosa/species	22.2%	NT	44.4%	NT	33.3%	NT	44.4%	NT	22.2%	22.2%	44.4%	22.2%	22.2%	22.2%	NT
Burkholderia cepacia	NT	NT	NT	NT	0.0%	NT	NT	NT	NT	NT	33.3%	33.3%	NT	NT	0.0%
Klebsiella pneumoniae	33.3%	66.7%	NT	66.7%	NT	66.7%	66.7%	100.0%	33.3%	66.7%	66.7%	66.7%	66.7%	66.7%	100.0%
Acinetobacter junii	0.0%	NT	0.0%	NT	0.0%	NT	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Enterobacter cloacae	0.0%	NT	NT	100.0%	NT	50.0%	50.0%	100.0%	50.0%	0.0%	50.0%	0.0%	0.0%	50.0%	NT
Salmonella Typhi	NT	NT	NT	100.0%	NT	100.0%	100.0%	NT	NT	NT	NT	0.0%	NT	NT	NT
Serratia marcescens	0.0%	NT	NT	100.0%	NT	100.0%	100.0%	100.0%	100.0%	0.0%	100.0%	0.0%	0.0%	100.0%	0.0%

DISCUSSION

In this prospective study, we have analyzed synovial fluid samples from various regions of Pakistan to identify the bacterial cause of infection and assess antibiotic susceptibility. Septic arthritis diagnosis is based on clinical findings supported by laboratory evidence. Of the 89 (22%) culture-positive synovial fluid (SF) samples, 59 isolates (66%) were gram-positive cocci, and 30 (33%) were gram-negative rods (GNR). *Staphylococcus aureus* was the most frequent (49%) isolate among GPCs. Other studies have also identified *S. aureus* as a major pathogen in septic arthritis, often followed by *Streptococci species*. Abid *et al*, in his research reported polymicrobial and anaerobic co-infections with *E.coli* (1.1%) from SF culture [6]. In contrast, our study noted *E. coli* and *Pseudomonas* species as the second most prevalent pathogens. Notably, the prevalence of MRSA (56%) in our study, as opposed to the reported 11% in a 2020 Indian study, is particularly concerning [7].

There has been limited research on the antibiotic profile in synovial fluid. A study in Nepal in 2023 documented high resistance to Clindamycin (39.6%) and Cotrimoxazole (39.5%) in *S. aureus*, in contrast to our findings of 13.6% (Clindamycin) and 17.1% (Cotrimoxazole) (8). The variation could be due to the difference in sample type and size, as we specifically selected synovial fluid for the research purposes. We have observed 100% sensitivity to Vancomycin and Linezolid; however, an increase in minimum inhibitory concentration (MIC) for vancomycin raises concern. Furthermore, Jin *et al*. in Sweden reported 70% *S. aureus* in SA patients, identifying hematogenous causes, rheumatoid arthritis (RA), as the major predisposing factor for SA, along with recent joint surgery, haemodialysis, HIV, skin infections, intra-joint corticosteroid, and immunosuppression [9-12]. Unfortunately, our study lacks this detailed information.

Another important finding was the 68% resistance to CRO (ESBL), 20% resistance to Meropenem (CRE) and 55% resistance to Levofloxacin in gram negative bacilli. *Pseudomonas aeruginosa/species* exhibited resistance of 33.3% and 22.2% to Ceftazidime and Piperacillin/Tazobactam, respectively. Additionally, we have also observed 3.3% of cases with *Burkholderia species* during 6-months. 33.3% of *Burkholderia cepacia* were resistant to Levofloxacin, possibly due to biofilm formation in prosthetic joint infections (PJI), as described by Mathew *et al* [13-15]. Similarly, Wu *et al* carried out a 10-year retrospective study in China on septic arthritis caused by *Bukrholderia pseudomallei* only. They also documented that secondary infection of *B. pseudomallei* caused either septic arthritis, osteomyelitis or both with high mortality rate of 20%. In the current study we did not rule out it as secondary infection due to various limitations. Our study also illustrated female to male ratio as 1.3:1 which was different to Wu *et al*' s findings (F:M of 1:13.7) [16,17].

CONCLUSION

According to our study, the Luminal A subtype was observed in 48 cases (29.9%), followed by Luminal B with 43 cases (26.7%). A significant association between increasing age and breast cancer was found. Further investigations are warranted to explore the clinical implications and therapeutic considerations associated with specific subtypes of breast carcinoma

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Amna Younas: Substantial contributions to conception of the design, data analysis, data interpretations, revisions, final approval of the work

Irim Iftikhar: Substantial contributions to conception of the design, revisions, final approval of the work

Karam Rasool: Data analysis, data interpretations, revisions

REFERENCES

1. Elsissy JG, Liu JN. Wilton PJ, Ikenna N, Gowd AK, Amin NH. Bacterial septic arthritis of the adult native knee joint: A review. JBJS Rev. 2020; 8(1): pe0059. DOI: https://doi.org/10.2106/jbjs.rvw.19.00059
2. Chiappini E, Mastrolia MV, Galli L, De Martino M, Lazzeri S. Septic arthritis in children in resource limited and non-resource limited countries: An update on diagnosis and treatment. Expert Rev Anti Infect Ther. 2016; 14(11): 1087-96. DOI:https://doi.org/10.1080/14787210.2016.1235973
3. McBride S, Mowbray J, Caughey W, Wong E, Luey C, Siddiqui A, *et al*. Epidemiology, management, and outcomes of large and small native joint septic arthritis in adults. Clin Infect Dis. 2020; 70(2): 271-9. DOI: https://doi.org/10.1093/cid/ciz265
4. Gerena LA, Mabrouk A, DeCastro A. Knee Effusion. [Updated 2024 Feb 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/ NBK532279/
5. Umer M, Hashmi P, Ahmad T, Ahmed M, Umer M. Septic arthritis of the hip in children: Aga Khan University Hospital experience in Pakistan. J Pak Med Assoc. 2003; 53(10): 472.
6. Abid N, Bhatti M, Azharuddin M, Islam M. Septic arthritis in a tertiary care hospital. J Pak Med Assoc. 2006; 56(3): 95-8.
7. George J, Chandy VJ, Premnath J, Hariharan TD, Oommen AT, Balaji V, *et al*. Microbiological profile of septic arthritis in adults: Lessons learnt and treatment strategies. Indian J Med Microbiol. 2019; 37(1): 29–33. DOI: https://doi.org/10.4103/ijmm.ijmm_19_134
8. Adhikari P, Basyal D, Rai JR, Bharati L, Budthapa A, Gharti KP, *et al*. Prevalence, antimicrobial susceptibility pattern and multidrug resistance of methicillin-resistant *Staphylococcus aureus* isolated from clinical samples at a tertiary care teaching hospital: An observational, cross-sectional study from the Himalayan country, Nepal. BMJ open. 2023; 13(5): e067384. DOI: https://doi.org/10.1136/bmjopen-2022-067384
9. Jin T, Mohammad M, Pullerits R, Ali A. Bacteria and host interplay in *Staphylococcus aureus* septic arthritis and sepsis. Pathogens. 2021; 10(2): 158. DOI: https://doi.org/10.3390/pathogens10020158
10. Mathews CJ, Weston VC, Jones A, Field M, Coakley G. Bacterial septic arthritis in adults. The Lancet. 2010; 375(9717): 846-55. DOI: https://doi.org/10.1016/S0140-6736(09)61595-6
11. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler Jr VG. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015; 28(3): 603-61. DOI: https://doi.org/10.1128/cmr.00134-14
12. Geirsson ÁJ, Statkevicius S, Víkingsson A. Septic arthritis in Iceland 1990–2002: Increasing incidence due to iatrogenic infections. Annals Rheumatic Dis. 2008; 67(5): 638-43. DOI: https://doi.org/10.1128/cmr.00134-14
13. Dastgheyb S, Parvizi J, Shapiro IM, Hickok NJ, Otto M. Effect of biofilms on recalcitrance of staphylococcal joint infection to antibiotic treatment. J Infect Dis. 2015; 211(4): 641-50. DOI: https://doi.org/10.1093%2Finfdis%2Fjiu514
14. Dastgheyb SS, Hammoud S, Ketonis C, Liu AY, Fitzgerald K, Parvizi J, *et al*. Staphylococcal persistence due to biofilm formation in synovial fluid containing prophylactic cefazolin. Antimicrob Agents Chemother. 2015; 59(4): 2122-8. DOI: https://doi.org/10.1128/aac.04579-14
15. Alder KD, Lee I, Munger AM, Kwon HK, Morris MT, Cahill SV, *et al*. Intracellular *Staphylococcus aureus* in bone and joint infections: A mechanism of disease recurrence, inflammation, and bone and cartilage destruction. Bone. 2020; 141: 115568. DOI: https://doi.org/10.1016/j.bone.2020.115568
16. Shahpari O, Mousavian A, Elahpour N, Malahias MA, Ebrahimzadeh MH, Moradi A. The use of antibiotic impregnated cement spacers in the treatment of infected total joint replacement: challenges and achievements. Arch Bone Jt Surg. 2020; 8(1): 11-20. DOI: https://doi.org/10.22038/abjs.2019.42018.2141
17. Wu H, Wang X, Zhou X, Chen S, Mai W, Huang H, *et al*. Osteomyelitis and septic arthritis due to Burkholderia pseudomallei: A 10-year retrospective melioidosis study from South China. Front Cell Infect Microbiol. 2021; 11: 654745. DOI: https://doi.org/10.3389/fcimb.2021.654745
18. Spyridakis E, Gerber JS, Schriver E, Grundmeier RW, Porsch EA, St. Geme III JW, *et al*. Clinical features and outcomes of children with culture-negative septic arthritis. J Pediatric Infect Dis Soc. 2019; 8(3): 228-34. DOI: https://doi.org/10.1093/jpids/piy034

Correlation of the capillary and venous blood glucose levels using glucometer with fully automated chemistry analyzer for stress hyperglycemia among critically ill patients

Azooba Fatima[1], Ayesha Hafeez[2], Aamir Ijaz[3], Mehreen Hassan[4]

[1]Islamabad Diagnostic Centre, Jhelum Pakistan
[2]Armed Forces Institute of Cardiology, Rawalpindi Pakistan
[3]NUST School of Health Sciences, Islamabad Pakistan
[4]Pakistan Air Forces Hospital, Islamabad Pakistan

ABSTRACT

Objective: To correlate venous and capillary blood glucose measurements using glucometer with fully automated chemistry analyser in stress hyperglycemia among critically ill patients.

Material and Methods: This cross-sectional study was conducted at Combined Military Hospital, Rawalpindi from August 2018 to January 2019 and blood specimens were analysed in Department of Chemical Pathology and Endocrinology Armed Forces Institute of Pathology Rawalpindi. Blood samples were collected from thirty-five non-diabetic patients of both genders admitted to Intensive Care Unit (ICU), Coronary Care Unit (CCU) and High Dependency Unit (HDU) of CMH, Rawalpindi. Venous and capillary blood glucose were measured using glucometer. Venous plasma glucose was analysed on fully automated chemistry analyser ADVIA 1800 by spectrophotometric kinetic method using Hexokinase.

Results: Mean (± Standard deviation) of Capillary Blood Glucose (CBG) was 160 (± 34.1) mg/dl, of Venous Blood Glucose (VBG) was 145.4 (± 33.9) mg/dl, and of fully automated chemistry analyser was 121 (± 35.4) mg/dl. Mean values of blood glucose showed significant difference ($p<0.001$) by three methods mentioned above. The CBG and VBG were found significantly correlated ($r=0.91$; $p<0.001$), similarly CBG and blood glucose levels (BGL) measured on automated chemistry analyser were also significantly correlated ($r=0.79$; $p<0.001$) as well as VBG and BGL measured on automated chemistry analyser ($r=0.87$; $p<0.001$)

Conclusion: A significant positive correlation was found between capillary and venous blood glucose measured by glucometers as well as between these two parameters and blood glucose measures on automated chemistry analyser but the means of these three values differ significantly. This warrants cautious use of glucometers for the detection of stress hyperglycaemia.

Keywords: Blood glucose monitoring, Critically ill patients, Glucometer, Stress hyperglycemia

This article can be cited as: Fatima A, Hafeez A, Ijaz A, Hassan M. Correlation of the capillary and venous blood glucose levels using glucometer with fully automated chemistry analyzer for stress hyperglycemia among critically ill patients. Pak J Pathol. 2024; 35(2): 69-75.

DOI: https://doi.org/10.55629/pakjpathol.v35i2.797

INTRODUCTION

Stress hyperglycemia is a transient or temporary increase in blood glucose during acute physiological or mental stress in the absence of glucose homeostasis dysfunction [1]. According to the guidelines of The American Diabetes Association (ADA) stress hyperglycemia is defined as having a random glucose level > 140 mg/dL at any given time in hospitalized patients [2]. Stress hyperglycemia occurs commonly among patients suffering with critical illness and trauma [3]. Multiple causes of stress hyperglycemia are present but mostly proposed include excessive counter-regulatory hormones (corticosteroid, growth hormone, catecholamines, glucagon) and release of cytokines interleukin (IL)-1 and tumour necrosis factor (TNF)-alpha [4]. In critical illness, intricate interactions between cytokines and counter-regulatory hormones cause excessive production of glucose [5]. These hormones such

Correspondence: **Dr. Azooba Fatima, Pathologist, Islamabad Diagnostic Centre, Jhelum Pakistan**

Email: azoobafatima@gmail.com

Receiving Date: **23 Nov 2023**
Revision Date: **01 Jan 2024**
Acceptance Date: **01 May 2024**

as cortisol causes elevation in blood glucose through stimulation of gluconeogenesis and reduction in glucose utilization because of impaired insulin release and action, resulting in stress hyperglycaemia [6]. Pro-inflammatory cytokines that are released in response to acute stress increases insulin resistance by interfering with insulin signaling. Exogenous factors, such as parenteral and enteral nutrition, vasopressors, dextrose, and corticosteroids, further aggravate this hyperglycemia [7].

Prevalence of stress hyperglycemia has been variedly reported from 16.8% to 79.8% in critically ill patients e.g. 16.8% by Khalfallah *et al* [8],16.9% in children admitted with febrile seizures as demonstrated in study by Costea *et al* [9] and a frequency of 18% was reported by Satti *et al* at Combined Military Hospital Quetta in patients admitted in Medical Intensive Care Unit [10]. Effective glycemic control in critically ill patients has been shown to result in marked improvements in clinical outcome.

Measured glucose level depends on the kind of sample used for analysis (plasma vs blood), the site of blood (capillary, venous or arterial) and chemical analysis used for the test. General rule of glucose concentration level from high to low according to sampling site is artery, capillary, and then venous blood [11]. There is a higher glucose concentration in the plasma than whole blood. The reason behind this is that there is higher water content in plasma resulting in increased glucose concentration. Laboratory blood glucose measurement using plasma is said to be more accurate and reliable than the point of care glucose measurement using glucometers [12]. In a critically ill patient, various stresses such as fasting and a hypermetabolic state, results in significant variation between glucose values [13]. There is also concern regarding accuracy and reproducibility of results using capillary samples due to hypotension and oedema giving inaccurate results in critically ill patients [14].

Despite these limitations, point of care testing using glucometer in critically ill patients is a routine practice and limited local data was available regarding use of an appropriate sample and method used for the detection of stress hyperglycaemia. Present study has, therefore, been designed to determine the difference in glucose values by glucometer which is point of care testing and the main clinical laboratory for ICU patients having stress hyperglycemia and whether the site of blood sampling had a significant impact on glucose values.

MATERIAL AND METHODS

This Cross-sectional study was conducted at Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology from August 2018 to January 2019 after approval from Institutional Review Board (IRB) of AFIP Rawalpindi (FC-CHP15-6/READ-IRB/17/315) Sample size was calculated according to following formula

$$N = [(Z\alpha+Z\beta)/C]2 + 3 \text{ [15]}$$

Correlation coefficient " r= 0.93" from a regional study [16] was used to find sample size of our study. Sample size calculated was 7 which was too small to conduct a study. As sample size larger than 30 is appropriate for most research, we used sample size 35 for our study. Sample size calculation was done with the help of statistician. Using non-probability consecutive sampling technique, a total of 31 non-diabetic patients admitted in ICU, CCU and HDU of Combined Military Hospital Rawalpindi were included in the study. Patients with diabetes and those who have received pre-sampling intravenous dextrose solution or glucocorticoids were excluded. HbA1c was used to exclude patients with pre-existing diabetes. Stress hyperglycemia is considered as random plasma glucose concentration of >7.8 mmol/L (140 mg/dl) in the hospital setting in first 24 to 48 hours after admission, therefore blood samples were collected in first 24 hours of admission. Venous blood was collected in EDTA and sodium fluoride tubes for Glycated Haemoglobin (HbA1c) and glucose analysis respectively. Plasma was then separated within 45 minutes of collection by centrifugation at 3000 Revolution

per minute (RPM) for 3 minutes. Capillary blood samples were obtained with finger prick. Venous blood glucose (VBG) was measured on glucometer as well as by fully automated Clinical Chemistry Auto-analyser ADVIA 1800® (SIEMENS Germany) by Hexokinase method. Capillary blood glucose (CBG) was measured using glucometer. HbA1c was measured on fully automated chemistry analyser ADVIA 1800® (SIEMENS Germany) by immunoturbidimetric method. Quality control was maintained utilizing 2 levels of controls (Roche) in each run with inter-assay and intra assay CV (Coefficient of Variation) of 3.4%. During the study period Proficiency Testing (PT) was carried by External Quality Assessment Scheme (EQAS BioRad) was run monthly and it was within acceptable Z value (2.0) for study glucose. Aim was to ensure accuracy and authenticity of data generated for the study being carried out. Descriptive statistics were used to analyse qualitative and quantitative variables. Qualitative variables like gender and disease were expressed in frequency and percentage. Quantitative variables like age, blood pressure, pulse, capillary blood glucose, venous blood glucose measured by glucometer and venous blood glucose measured in laboratory were expressed in mean and SD. Statistical analysis was done using paired t test, One-way analysis of variance (ANOVA) and Pearson's correlation analysis.

RESULTS

Thirty-five patients were included in the study, 23 (65.7%) were males and 12 (34.3%) were females. Mean age was 56.2 ± 13.5 years, range 18-70 years). Mean age of the females and males were 54.74 ± 14.95 and 59 ± 10.2 years, respectively. There was no significant difference between the age of two genders p = 0.328). It was observed that 48% of the patients having stress hyperglycemia had cardiovascular disease. In Table-I mean, SD and range of blood pressure, pulse and HbA_{1C} of all patients are shown.

Based on the obtained results, mean of capillary blood glucose, venous blood glucose measured by glucometer and venous blood glucose measured on automated analyser are 160.67 ± 34.1, 145.37 ± 33.9 and 121.04± 35.4 respectively. Performing paired t test and Pearson correlation on the obtained data showed significant difference (p< 0.001) and positive correlation as given in Table-II.

There was a good correlation between CBG and VBG (r=0.912; (p < 0.001) (Figure-I). Correlation between CBG and BGL on automated chemistry analyzer was also quite significant (r=0.796;p <0.001) (Figure-II). The correlation rate between VBG and BGL on automated chemistry analyzer was also statistically significant (r=0.83; p < 0.001) (Figure-III).

One way ANOVA test also showed significant difference in the mean of blood glucose level measured by glucometer and lab testing (p=0.036).

Table-I: Values for selected non-study variables in 35 critically ill patients.

Variables	Mean± SD	Range
SBP mmHg	134.4 ±29.1	90-196
DBP mmHg	82.3±13.4	46-100
PULSE /min	79.3±13.4	52-131
HbA1C %	5.8±0.44	4.9-6.5

SBP= Systolic blood pressure, DBP= Diastolic blood pressure

Table-II: Comparison of different glucose estimation methods.

	Paired differences and Correlation			
	Mean ± SD	p value	r	p-value
GCBG vs GVBG	160.77 ± 34.06 vs 145. 37 ± 33.97	0.000	0.912	0.000
GCBG vs BGL	160.77 ± 34.06 vs 121.04 ± 35.37	0.000	0.796	0.000
GVBG vs BGL	145.37 ± 33.97 vs 121.04 ± 35.37	0.000	0.838	0.000

*P < 0.05 was considered significant. BGL, blood glucose laboratory; GCBG, glucometric capillary blood glucose; GVBG, glucometric venous blood glucose

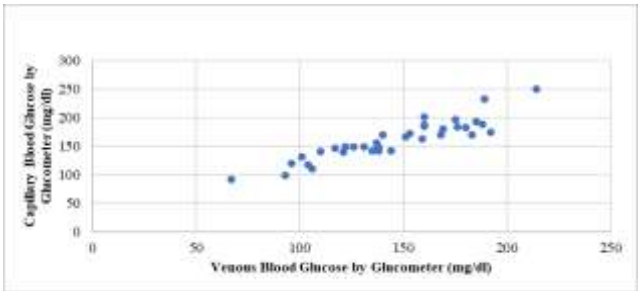

Figure-I: Correlation between capillary blood glucose and venous blood glucose by glucometer mg/dl (r = 0.912).

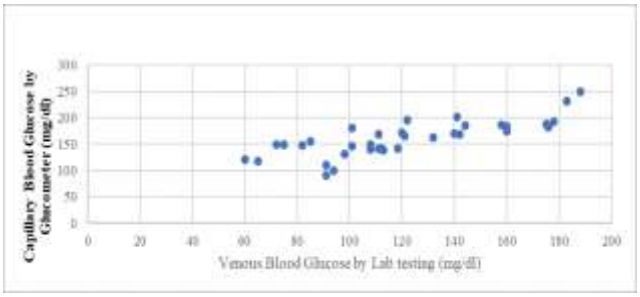

Figure-II: Correlation between capillary blood glucose and laboratory venous blood glucose mg/dl (r = 0.796).

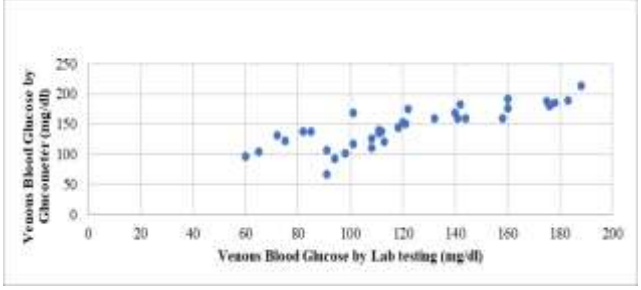

Figure-III: Correlation between venous blood glucose by glucometer and laboratory venous blood glucose mg/dl (r = 0.83).

DISCUSSION

Use of capillary blood glucose estimation using glucometer and treatment decisions on its basis is routine practice in critical care setting now-a-days. We estimated BGL with three different types of samples; CBG, VBG and venous plasma on automated analyser in the lab. The reason of conducting the current study was to compare these three types of samples and to correlate the results of glucometer with laboratory estimated values on clinical chemistry analyser. In comparison to the laboratory, we established that our glucometers yielded higher glucose levels in capillary and venous samples. These results are comparable to the observations stated by Boyd *et al* in 2005 and Critchell *et al* in 2007 [17]. According to the results obtained from our study, the mean of CBG, VBG and BGL on automated chemistry analyser had significant difference in both methods. In Boyd *et al*.'s study [18], samples of venous and capillary blood were taken from 20 patients in the emergency room and the glucose levels in both samples were checked by a glucometer and in the laboratory. Significant difference was obtained. Similar to our study, Patel *et al* showed that venous plasma glucose measured in laboratory is lower than mean capillary blood glucose analysed by glucometer. Adnan *et al* suggested that there was a significant inter method mean difference. This difference was not significant at normal glucose values but increases gradually with a rise in blood glucose levels and was significant at higher glucose levels. Our study results were in contrast to the study conducted by Lacara *et al* [19] which indicated no significant difference between glucose values of laboratory and point of care testing (POCT) glucometer values. Mean laboratory glucose level was 135 (SEM 5.3, range 58–265) mg/dL. In point-of-care testing, bias ± precision and root-mean-square differences were 2.1 ± 12.3 and 12.35, respectively, for fingerstick blood and 0.6 ± 10.6 and 10.46 for catheter blood. In a study conducted by Sharma *et al* [20], strong correlation (r=0.93) was observed between capillary blood glucose measured by glucometer and venous blood glucose measured in laboratory in Neurosurgical patients. Yarghai *et al* [15] also showed that no significant difference was present in between venous blood glucose and capillary blood glucose measured by POC glucometer. We found a strong correlation between CBG and VBG (r=0.92) while in Yarghai *et al* also showed a similar strong correlation (r= 0.93). Our observed correlation between CBG and BGL on automated chemistry analyser was somewhat less strong (r=0.796), similar to Yarghai *et al* who found a correlation coefficient of 0.78. The strength of correlation between the VBG and BGL on automated chemistry analyzer (r=0.83) was quite similar to

that found by Yarghai *et al* (r= 0.81). Thus, if laboratory measured venous blood glucose was considered as the reference standard, the level of VBG and CBG greatly differ to it and so glucometer should be used very cautiously in critically ill patients with stress hyperglycemia. In another study on 97 healthy volunteers conducted by Funk *et al* [21] capillary and venous blood samples were taken simultaneously from individuals and the blood glucose level of the two samples was measured by a glucometer. A weak correlation was obtained between the levels of venous and capillary blood glucose. Petersen *et al* [22] compared venous, arterial and capillary blood glucose levels using blood gas instrument, glucometer and main clinical laboratory instruments and suggested that all methods (blood gas, POCT, and central laboratory) were highly correlated to each other and to the reference method except for glucose meter testing using capillary sampling which had significantly weaker correlations similar to our study. In a study conducted by Dubose *et al* [23], capillary and venous blood glucose levels of patients with and without shock were correlated, and a slight difference was observed between both groups. In 2010, Fekih Hassen [24] studied 43 hyperglycemic patients older than 18 years admitted to the intensive care unit. There was difference of venous and capillary blood glucose levels in these patients and capillary sampling was not recommended to determine blood glucose level. The major difference between our study and some of the previous studies could be attributed to the difference in clinical setting and the types of population studies. For example, in the study by Yarghai *et al*, blood glucose level of poisoned patients in coma was measured by these methods while in Funk *et al*, only healthy population was studied. In Matthew *et al* and Adnan *et al* [25], only patients with diabetes were studied. The subjects selected in the study by Patel *et al* [26] were all adults, who came for checkup in Out Patient Department (OPD) of a tertiary care level hospital. Furthermore, we observed that 48% of the patients having stress hyperglycemia had cardiovascular disease. As hyperglycemia is a risk factor for adverse outcomes during acute illness and is related to increased mortality and morbidity [27], this warrants stringent glucose level monitoring in critically ill patients by a suitable methodology.

According to the results obtained in our study, level of blood glucose measured by glucometer is significantly different from blood glucose measured by laboratory. So in critical settings, there is a substantial difference in blood glucose values by laboratory and from venous blood glucose determined by glucometer. So, venous blood glucose estimation by glucometer is not recommended for use in such settings.

CONCLUSION

Glucometer estimations in critically ill patients can differ significantly from venous blood specimen measured on automated chemistry analyser in the laboratory. Measuring the glucose level in venous blood sample by laboratory is an acceptable and recommended method. Glucose measurement in capillary blood sample using glucometer should be done cautiously in critically ill patients with periodic venous blood testing.

LIMITATIONS OF THE STUDY

Sample size of this study was very small. Only 35 subjects were included in our study, so a larger study is essential for validation of the conclusion drawn in this study.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Azooba Fatima: Manuscript writing, literature search, study design, data analysis

Ayesha Hafeez: Conception of work, draft, final approval

Aamir Ijaz: Conception of work, data analysis, drafting

Mehreen Hassan: Data collection

REFERENCES

1. Scheen M, Giraud R, Bendjelid K. Stress hyperglycemia, cardiac glucotoxicity, and critically ill patient outcomes current clinical and pathophysiological evidence. Physiol Rep. 2021; 9(2): e14713. DOI: https://doi.org/10.14814/phy2.14713
2. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes 2019. Diabet Care 2019; 42: S13–S28.
3. Li L, Zhao M, Zhang Z, Zhou L, Zhang Z, Xiong Y. *et al*. Prognostic significance of the stress hyperglycemia ratio in critically ill patients. Cardiovasc Diabetol. 2023; 22: 275. DOI: https://doi.org/10.1186%2Fs12933-023-02005-0
4. Alhatemi G, Aldiwani H, Alhatemi R, Hussein M, Mahdai S, Seyoum B. Glycemic control in the critically ill: Less is more. Cleve Clin J Med. 2022; 89(4):191-9. DOI: https://doi.org/10.3949/ccjm.89a.20171
5. Angelousi A, Margioris AN, Tsatsanis C. ACTH action on the adrenals. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dungan K, editors. Endotext [Internet]. MDText.com, Inc.; South Dartmouth (MA): Jun 13, 2020.
6. Thau L, Gandhi J, Sharma S. Physiology, Cortisol. [Updated 2023 Aug 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538239
7. Vedantam D, Poman D S, Motwani L, Asif N, Patel A, Anne KK*, et al*. Stress-induced hyperglycemia: Consequences and management. Cureus 2022. 14(7): e26714. DOI: https://doi.org/10.3949/ccjm.89a.20171
8. Khalfallah M, Abdelmageed R, Elgendy E, Hafez YM. Incidence, predictors and outcomes of stress hyperglycemia in patients with ST elevation myocardial infarction undergoing primary percutaneous coronary intervention. Diab Vasc Dis Res. 2020; 17(1): 1479164119883983. DOI: https://doi.org/10.1177/1479164119883983
9. Costea RM, Maniu I, Dobrota L, Neamtu B. Stress hyperglycemia as predictive factor of recurrence in children with febrile seizures. Brain Sci. 2020; 10(3): 131. DOI: https://doi.org/10.3390/brainsci10030131
10. Satti SA, Khattak AL, Tariq AM, Majoka SM, Naeem A, Din RU. The frequency of stress hyperglycemia and mortality in patients with hyperglycemia in medical intensive care unit. Pak Armed Forces Med J. 2021; 71(3): 753–56. DOI: https://doi.org/10.51253/pafmj.v71i3.3298
11. Yang C, Chang C, Lin J. A comparison between venous and finger-prick blood sampling on values of blood glucose. Int Proceedings Chemical, Biologic Environment Engineering. 2012; 39: 206-10.
12. Sirohi R, Singh RP, Chauhan K. A comparative study of venous and capillary blood glucose in a tertiary care hospital. Indian J Pub Health Res Development. 2020; 11(7): 673-7.
13. Sharma K, Mogensen KM, Robinson MK. Pathophysiology of critical illness and role of nutrition. Nutr Clin Pract. 2019; 34(1): 12-22. DOI: https://doi.org/10.1002/ncp.10232
14. Finfer S, Wernerman J, Preiser JC, Cass T, Desaive T, Hovorka R, et al. Clinical review: Consensus recommendations on measurement of blood glucose and reporting glycemic control in critically ill adults. Crit Care. 2013; 17: 229. DOI: https://doi.org/10.1186/cc12537
15. Hulley SB, Cummings SR, Browner WS, Grady D, Newman TB. Designing clinical research: an epidemiologic approach. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2013. Appendix 6C, page 79.
16. Yaraghi A, Mood NE, Dolatabadiali LK. Comparison of capillary and venous blood glucose levels using glucometer and laboratory blood glucose level in poisoned patients being in coma. Adv Biomed Res. 2015; 4: 247. DOI: https://doi.org/10.4103/2277-9175.170242
17. Critchell CD, Savarese V, Callahan A, Aboud C, Jabbour S, Marik P. Accuracy of bedside capillary blood glucose measurements in critically ill patients. Intensive Care Med. 2007; 33(12): 2079-84. DOI: https://doi.org/10.1007/s00134-007-0835-4
18. Boyd R, Leigh B, Stuart P. Capillary versus venous bedside blood glucose estimation. Emerg Med. 2005;22:177–9. DOI: https://doi.org/10.1136/emj.2003.011619
19. Lacara T, Domagtoy C, Lickliter D, Quattrocchi K, Snipes L, Kuszaj J, *et al*. Comparison of point-of-care and laboratory glucose analysis in critically ill patients. Am J Crit Care. 2007; 16(4): 336-46.
20. Jyoti S, Renu B Singh, Seema. Correlation of venous blood sugar measured by lab method and capillary blood sugar measured by glucometer in neurosurgical patients receiving dexamethasone. Asian J Neurosurg. 2023; 19 (1); 21-25. DOI: https://doi.org/10.1055/s-0043-1775569
21. Funk DL, Chan L, Lutz N, Verdile VP. Comparison of capillary and venous glucose measurements in healthy volunteers. Prehosp Emerg Care. 2001; 5: 275–7. DOI: https://doi.org/10.1080/10903120190939788
22. Petersen JR, Graves DF, Tacker DH, Okorodudu AO, Mohammad AA, Cardenas VJ Jr. Comparison of POCT and central laboratory blood glucose results using arterial, capillary, and venous samples from MICU patients on a tight glycemic protocol. Clin Chim Acta. 2008; 396:10-3. DOI: https://doi.org/10.1016/j.cca.2008.06.010
23. Du Bose JJ, Inaba K, Branco BC, Barmparas G, Lam L, Teixeira PG, *et al*. Discrepancies between capillary glucose measurements and traditional

laboratory assessments in both shock and nonshock states after trauma. J Surg Res. 2012; 178: 820–6. DOI: https://doi.org/10.1016/j.jss.2012.04.003

24. FekihHassen M, Ayed S, Gharbi R, Ben Sik Ali H, S. Marghli S, Elatrous S. Bedside capillary blood glucose measurements in critically ill patients: Influence of catecholamine therapy. Diabetes Res Clin Pract. 2010; 87: 87–91. DOI: https://doi.org/10.1016/j.diabres.2009.09.018
25. Adnan M, Imamb F, Shabbira I, Alia Z, Rahata T. Correlation between capillary and venous blood glucose levels in diabetic patients. Asian Biomed. 2015 Mar 23;9(1):55-9. DOI: https://doi.org/10.5372/1905-7415.0901.368
26. Patel N, Patel K. A comparative study of venous and capillary blood glucose levels by different methods. GCSMC J Med Sci. 2015;4(1):53-6.
27. Bar-Or D, Rael L T, Madayag RM, Banton K.L, Tanner A, Acuna DL, *et al*. Stress Hyperglycemia in Critically Ill Patients: Insight Into Possible Molecular Pathways. Front Med 2019; 6: 54. DOI: https://doi.org/10.3389/fmed.2019.00054

Reference values of serum osteocalcin in the healthy population: A potential biomarker for bone turnover

Tayyaba Rashid[1], Muhammad Dilawar Khan[1], Hijab Batool[1], Masood Afzal[1], Muhammad Hashir Nazir[2], Muhammad Ahmad[2]

[1]Chughtai Institute of Pathology, Lahore Pakistan
[2]King Edward Medical University, Lahore Pakistan

ABSTRACT

Objective: To analyze the serum concentrations of Osteocalcin in healthy subjects to establish the reference intervals in the Pakistani population.

Material and Methods: This Cross-sectional, observational study was conducted at the Department of Clinical Chemistry and Immunology, Chughtai Lab Lahore from October 2022 to March 2023. Serum samples from 240 healthy subjects (120 males and 120 premenopausal females) were collected according to CLSI recommendations after taking informed consent. The samples were analyzed for the quantitative determination of Osteocalcin by sandwich electrochemiluminescence immunoassay. Shapiro Wilk test was applied to check normality. A P-value of < 0.05 was considered significant. The formulas used for calculating the 2.5th and 97.5th percentiles were 0.025 (n+1) and 0.0975 (n+1) respectively.

Results: The histogram revealed a non-parametric distribution of the data. The established reference intervals by the rank-based method for males were 10.16 ng/mL and 43.33 ng/mL and for females were 5.25 ng/mL and 33.25 ng/mL corresponding to 2.5th and 97.5th percentiles respectively.

Conclusion: Ethnic and geographic variation affects the trends of reference intervals of every parameter. This is the need of the hour each laboratory should establish its assay and population-specific reference intervals for accurate clinical decisions.

Keywords: Osteocalcin, Reference values, Bone density, Osteoporosis

This article can be cited as: Rashid T, Khan MD, Batool H, Afzal M, Nazir MH, Ahmad M. Reference values of serum osteocalcin in the healthy population: A potential biomarker for bone turnover. Pak J Pathol. 2024; 35(2): 76-80.

DOI: https://doi.org/ 10.55629/pakjpathol.v35i2.812

INTRODUCTION

Bone is a dynamic tissue that undergoes constant remodeling. Bone mass in healthy adults is maintained by the coordination between bone formation and resorption [1,2]. Bone turnover markers are the biochemical products that indicate bone metabolic activity and are classified into two major groups: Bone formation and resorption [3,4]. Osteocalcin (OC), also known as bone gamma-carboxy glutamic acid-containing protein (BGLAP), is a non-collagenous vitamin K-dependent bone-specific protein produced primarily during bone formation predominantly by osteoblasts [1]. It binds to hydroxyapatite and accumulates in the bone matrix [5].

Measurement of Serum OC is a convenient way to study bone metabolism, as the levels represent the turnover rate of bone metabolism; therefore, it can be used in monitoring disorders that affect bone health, such as osteoporosis, hyperparathyroidism, renal osteodystrophy, Paget's disease, and bone metastasis [6]. The concentration of circulating OC varies according to age and gender [7]. Serum OC levels in young males are higher than in females of the same age group because they have longer and broader bones and reach peak bone mineral density (BMD) later in life. After peak BMD is achieved a drop in concentration of OC is observed in both males and females. In women transitioning to menopause, serum OC levels increase significantly [1].

Reference values of biological parameters significantly from one laboratory to another based on the population, methodology, and selection criteria for the reference group. (8). Under ideal conditions, a laboratory should conduct its reference intervals (RIs) study to

Correspondence: **Dr. Tayyaba Rashid, Resident Pathologist, Chughtai Institute of Pathology, Lahore Pakistan**

Email: rtayyaba50@gmail.com

Receiving Date: **24 Jan 2024**
Revision Date: **12 Feb 2024**
Acceptance Date: **07 May 2024**

determine the RIs specific to its method and population. However, establishing RIs is frequently beyond the capacity of an individual laboratory because it is a complex, costly, and time-consuming procedure [9].

To our knowledge, no study has been conducted to establish the RIs of serum OC in the Pakistani population. The RIs for biochemical parameters in developing nations are obtained from textbooks containing data from developed countries or the literature inserts of reagent kits [10]. To establish RIs, the reference population is sampled according to predefined criteria, and then reference ranges are computed using a direct approach. The majority of RIs in use are described by the central 95% of the reference population used in the study [11]. The Clinical and Laboratory Standards Institute (CLSI) guidelines recommend selecting a statistically significant group with at least 120 healthy reference subjects to establish RIs [12]. According to the standard guidelines, if a laboratory cannot perform its RI study, due to financial constraints or some other reasons, the guidelines emphasize on at least verifying the transferred RIs, which requires samples from as few as 20 reference samples [13]. To establish the reference intervals of serum Osteocalcin in healthy Pakistani population.

MATERIAL AND METHODS

A cross-sectional study was carried out at the Department of Chemical Pathology, Chughtai Laboratory Lahore, from October 2022 to March 2023. Serum samples from 240 healthy subjects (120 males and 120 premenopausal females) were collected according to CLSI recommendations [14]. The CLSI-recommended number of subjects for the establishment of RIs is 120 healthy subjects. As we have established the RIs for both males and females we took a total of 240 individuals. Subjects with underlying bone disorders, fractures, diabetes mellitus, and other chronic diseases like thyroid disorders, malignancies, etc. were excluded from the study. Subjects with a history of drug intake such as Vitamin D, multivitamins, and steroids were also excluded from the study. A purposive, non-probability sampling technique was used. Informed consent was taken from study participants. The samples were analyzed for the quantitative determination of OC by sandwich electrochemiluminescence immunoassay (ECLIA) on a fully automated chemistry analyzer [Cobas 6000 (e601)]. Hemolyzed, lipemic, and icteric samples were rejected. Two levels of quality control were run with each batch and validated by Westgard rules. The Shapiro-Wilk test was applied to assess the distribution of osteocalcin values. Values were arranged in ascending order, followed by a ranking of the data so the corresponding value of Osteocalcin can be taken. The rank numbers were calculated using the formulas 0.025 (n+1) and 0.0975 (n+1) for the percentile values that correspond to the rank no 3 and 118 respectively.

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 27:00. The data was assessed for normality by applying the Shapiro-Wilk test. A CI of 90% was used to establish RIs using non-parametric statistical methodology. A P-value of < 0.05 was considered significant. The lower and upper reference limits correspond to the 2.5th percentile and 97.5th percentile of the distribution of test results for the reference population, respectively (15,16). Values (2.5th and 97.5th percentiles) were computed using the formula 0.025 (n+1) and 0.0975 (n+1) respectively.

RESULTS

Of the 240 subjects, 120 were males and 120 were females. The mean age of male subjects was 26.65 years, and female subjects was 32.09 years. The mean serum OC levels in males and females were 20.85 ng/mL and 16.49 ng/mL, respectively (Table-I). The histogram revealed a non-Gaussian distribution for both males and females. (Figure-I & II respectively). Non-parametric statistics were applied, and the reference intervals based on the 2.5th and 97.5th percentiles (corresponding to rank number 3 and 118, respectively) established were 10.16 ng/mL and 43.33 ng/mL for males and 5.25 ng/mL and 33.25 ng/mL for females (Table-II).

Table-I: Descriptive statistics of Serum OC (ng/mL) in healthy Pakistani population (n=240).

	Minimum	Maximum	Median	Mean	Standard Deviation
Male (n=120)	8.54	45.80	18.78	20.85	7.83
Female (n=120)	4.31	38.87	15.13	16.49	7.11

Table-II: Percentile Details of Serum Osteocalcin in healthy Pakistani population (n=240).

Percentile	Corresponding OC Levels in Males (ng/mL)	Corresponding OC Levels in Females (ng/mL)
2.5	**10.16**	**5.25**
5	11.46	6.55
10	12.58	8.63
25	15.30	11.82
50	18.78	15.13
75	24.45	20.52
90	33.46	27.47
95	35.92	31.06
97.5	**43.62**	**33.20**

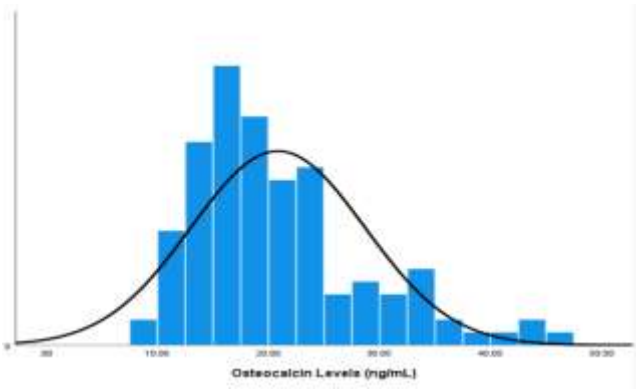

Figure-I: Histogram showing the non-parametric distribution of OC Levels (ng/mL) in males (n=120).

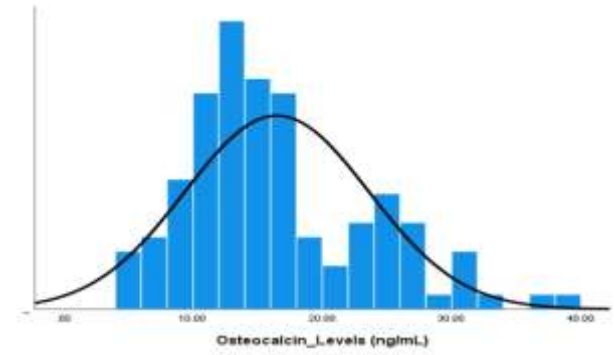

Figure-II: Histogram showing the non-parametric distribution of OC Levels (ng/mL) in females (n=120).

DISCUSSION

The diagnostic utility of the laboratory results depends on their interpretation, which helps clinicians to differentiate between health and disease states [17]. Each laboratory should establish its RIs for each parameter according to the standard recommendations specific to the testing methodology used and the population covered by that particular laboratory [18]. Establishing the RIs is expensive, complex, and time-consuming, and many laboratories cannot determine their RIs due to these constraints [17,18].

This study establishes the RIs of Serum OC in the healthy adult Pakistani population. The RIs are established as the difference between two threshold values, the 2.5th and 97.5th percentiles of the distribution of the data, which account for 95% of observations from healthy subjects [1,2]. The idea of establishing RIs and their application seems relatively simple, but the accurate and reliable procedure for their determination is somewhat complex. Problems are frequently encountered by a lack of samples from healthy populations, moral issues, and disparities such as age- and sex-specific variations in physical characteristics, immunological response, and metabolism [10].

For quite some time, there has been an increased interest in quantifying markers of bone metabolism in clinical practices. They might offer a dynamic, momentary assessment of skeletal health that is not just reflected in the physical characteristics of bones [2]. Thus far, no single parameter has matched all the requirements needed to be the perfect indicator of bone turnover [13]. High intraindividual variation, lack of specificity for bone tissue, release during distinct anabolic and catabolic processes, and the effect of non-skeletal activities on circulating

levels all pose challenges to the therapeutic efficacy of bone markers. Markers such as OC indicate both bone formation and resorption simultaneously and can be used to assess bone turnover [3].

A study was conducted by Hannemann A *et al*. in Pomerania in 2013 to establish the RIs for OC. The established RIs for adult males using 2.5th and 97.5th percentiles were 6.5 and 36.2 ng/mL, and for adult premenopausal females, the established reference values were 7.6 and 39.5 ng/mL, respectively [1]. These values differ slightly from the RIs established in our study. The testing methodology in our research study was electrochemiluminescence immunoassay, and the instrument used was Cobas 6000. While in the Pomeranian study, the analytical technique used was chemilumine-scence, and the instrument used for the OC analysis was the ids-Immunodiagnostic system.

The comparison with other studies shows that RIs are affected by factors such as analytical assays, ethnic origins, living styles, population, and geographic differences [19]. It is the need of the hour each laboratory should establish its RIs for the population being covered based on the specific testing method used at that particular laboratory.

CONCLUSION

The established reference intervals by the rank-based method for males were 10.16 ng/mL and 43.33 ng/mL, and for females, they were 5.25 ng/mL and 33.25 ng/mL, corresponding to 2.5th and 97.5th percentiles, respectively. It was concluded that RIs are affected by ethnic and geographic variation. It is recommended that every laboratory should establish its reference intervals.

LIMITATIONS OF THE STUDY

Our study covered a small population; there is a dire need for more extensive studies to establish RIs for the effective and timely management of patients.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Tayyaba Rashid: Paper write-up, literature search, data collection and analysis
Muhammad Dilawar Khan: Study design, proofreading, finalization of study
Hijab Batool: Statistical analysis, paper write-up, proofreading
Masood Afzal: Data analysis, discussion
Muhammad Hashir Nazir and Muhammad Ahmad: Sample collection, paper write-up

REFERENCES

1. Hannemann A, Friedrich N, Spielhagen C, Rettig R, Ittermann T, Nauck M, *et al*. Reference intervals for serum osteocalcin concentrations in adult men and women from the study of health in Pomerania. BMC Endocr Disord. 2013;13(1);11. DOI:: https://doi.org/10.1186%2F1472-6823-13-11
2. Niedźwiedzki T, Filipowska J. Bone remodeling in the context of cellular and systemic regulation: The role of osteocytes and the nervous system. J Mol Endocrinol. 2015; 55(2): 23-36. DOI: https://doi.org/10.1530/jme-15-0067
3. Monjardino T, Silva P, Amaro J, Carvalho O, Guimarães JT, Santos AC, *et al*. Bone formation and resorption markers at 7 years of age: Relations with growth and bone mineralization. PLoS One. 2019; 14(8): e0219423. DOI:https://doi.org/10.1371%2Fjournal.pone.0219423
4. Kojima N, Sakata S, Nakamura S, Nagai K, Takuno H, Ogawa T, *et al*. Serum concentrations of osteocalcin in patients with hyperthyroidism, hypothyroidism and subacute thyroiditis. J Endocrinol Invest. 1992; 15(7): 491-6. DOI: https://doi.org/10.1007/bf03348786
5. Lin X, Patil S, Gao YG, Qian A. The bone extracellular matrix in bone formation and regeneration. Front Pharmacol. 2020 May 26; 11: 757. DOI: https://doi.org/10.3389/fphar.2020.00757
6. Greenblatt MB, Tsai JN, Wein MN. Bone turnover markers in the diagnosis and monitoring of metabolic bone disease. Clin Chem. 2017; 63(2): 464–74. DOI: https://doi.org/10.1373/clinchem.2016.259085
7. Li W, Wang Y, Dong J, Di R, Liu X, Liu S. Age- and sex-specific differences in the association of serum osteocalcin and cardiometabolic risk factors in type 2 diabetes. Diabetol Metabo Syndr. 2022; 15: 48. DOI: https://doi.org/10.1186/s13098-023-01021-0

8. Iqbal S, Ghani F, Siddiqi I, Khan AH. Verification and determination of the thyroid peroxidase antibody reference interval: Insight into the CLSI guideline. Lab Med. 2013; 44(2): e37-e43. DOI: https://doi.org/10.1309/lmj2j52mpmfshdgx
9. Ozarda Y, Higgins V, Adeli K. Verification of reference intervals in routine clinical laboratories: practical challenges and recommendations. Clin Chem Lab Med. 2018; 57(1): 30-37. DOI: https://doi.org/10.1515/cclm-2018-0059
10. Abebe M, Melku M, Enawgaw B, Birhan W, Deressa T, Terefe B, *et al*. Reference intervals of routine clinical chemistry parameters among apparently healthy young adults in Amhara National Regional State, Ethiopia. PLoS One. 2018; 13(8): e0201782. DOI:https://doi.org/10.1371%2Fjournal.pone.0201782
11. Saeed M, Waheed U, Wazeer A, Saba N. Do we need pakistan-specific reference ranges in laboratory medicine? J Lab Physicians. 2023; 15(2): 324-5. DOI: https://doi.org/10.1055%2Fs-0042-1760669
12. Geffré A, Friedrichs K, Harr K, Concordet D, Trumel C, Braun JP. Reference values: A review. Vet Clin Pathol. 2009; 38(3): 288-98. DOI: https://doi.org/10.1111/j.1939-165x.2009.00_179.x
13. Araújo PA, Thomas D, Sadeghieh T, Bevilacqua V, Chan MK, Chen Y, *et al*. CLSI-based transference of the CALIPER database of pediatric reference intervals to Beckman Coulter DxC biochemical assays. Clin Biochem. 2015; 48(13-14): 870-80. DOI: https://doi.org/10.1016/j.clinbiochem.2015.06.002
14. Henny J. The IFCC recommendations for determining reference intervals: Strengths and limitations J Lab Med. 2009; 33(2): 45–51. DOI: https://doi.org/10.1515/JLM.2009.016
15. Ozarda Y. Reference intervals: Current status, recent developments and future considerations. Biochemia Medica. 2016; 5-16. DOI: https://doi.org/10.11613/bm.2016.001
16. Płaczkowska S, Terpińska M, Piwowar A. The importance of establishing reference intervals - is it still a current problem for laboratory and doctors? Clin Lab. 2020; 66. DOI: https://doi.org/10.7754/clin.lab.2020.191120
17. Ozarda Y, Higgins V, Adeli K. Verification of reference intervals in routine clinical laboratories: Practical challenges and recommendations. Clin Chem Lab Med. 2018; 57(1): 30–7. DOI: https://doi.org/10.1515/cclm-2018-0059
18. Zhou Q, Li X, Jia Y, Guo W, Guan B, Xu J. Establishment and verification of sex- and age-specific serum electrolyte reference intervals in healthy han children in Changchun, Northeastern China. Biomed Res Int. 2019; 2019: 8282910.
19. Muneer S, Siddiqui I, Majid H, Jafri L, Humayun KN, Ahmed S, *et al*. Establishing reference interval for thyroid-stimulating hormone in children below two-year ages in Pakistani population. Ann Med Surg. 2021; 68: 102601. DOI:https://doi.org/10.1016%2Fj.amsu.2021.102601

Original Article

Comparison of classification of anemia based on mean corpuscular volume by hematology analyzer and peripheral smear examination

Sarah Farrukh[1], Qurat Ul Ain Ayaz[2], Farhan Ali Khanzada[3], Huma Sheikh[4], Ambreen Anwar[5], Soubia Cheema[5]

[1]Teshil Headquarter Hospital, Ferozewala Sheikhupura Pakistan
[2]Combined Military Hospital, Multan Pakistan
[3]Shaikh Zayed Medical College, Rahim Yar Khan Pakistan
[4]King Edward Medical University, Lahore Pakistan
[5]Punjab Institute of Cardiology, Lahore Pakistan

ABSTRACT

Objective: This study was conducted to identify different morphological patterns of anemia based on mean corpuscular volume determined by a hematology analyzer and comparing it with peripheral smear examination.

Material and Methods: A total of 94 anemic patients were studied at Punjab Institute of Cardiology. Anemia was characterized by a decrease in hemoglobin (Hb) concentration below normal limit i.e <12g/dl in women and <13.0 g/dl in men using an automated analyzer. Morphological classification was done based on peripheral smear examination findings and mean corpuscular volume (MCV). SPSS version 26 was used for data analysis. Frequencies were calculated for gender and subtypes of anemia and its severity was calculated into percentages. Age was calculated as mean and SD. Post stratification Chi-square test was applied to compare PSE and automated analyser taking p value of more 0.0001 as significant.

Results: The mean age of included patients was 34.88± 15.25 years with minimum and maximum age 7 months old and 85 years. Females were more commonly affected than males with male to female ratio 1:2. Majority, i.e. 53% of patients suffered from moderate degree of anemia while 39% participants had hypochromic microcytic pattern of anemia. Post stratification Chi- square test was applied to compare peripheral smear examination and automated analyzer which gave a significant p value of 0.0002.

Conclusion: This study emphasizes the role of PSE in comparison with automated hematology analyzer for the diagnosis and subtyping of various forms of anemias.

Keywords: Anemia, Microcytic hypochromic, Normocytic normochromic

This article can be cited as: Farrukh S, Ayaz QUA, Khanzada FA, Sheikh H, Anwar A, Cheema S. Comparison of classification of anemia based on mean corpuscular volume (MCV) by hematology analyzer and peripheral smear examination. Pak J Pathol. 2024; 35(2): 81-86.

DOI: https://doi.org/10.55629/pakjpathol.v35i2.793

INTRODUCTION

Anemia is functionally defined as an insufficient RBC mass to supply oxygen to peripheral organs. Hematocrit, red blood cell (RBC) count and RBC indices also play an important role in classification of anemia. Proper management of patients relies on identification of anemia and its subtypes [1]. Hemoglobin (Hb) is reflected by underlying nutritional status. The reduction in the Hb concentration of the peripheral blood below the normal limit or the reduction in hematocrit below the lower limit of the 95% reference interval in relation to age and gender is called anemia. It is an expression or sign of an underlying disorder [3]. By WHO criteria, an Hb of < 13 gm/ dl in males and <12g/dl in females is classified as anemia [4]. However, age and pregnancy have different reference intervals. There are numerous etiologies for different categories of anemia such as nutritional, hemolytic, aplastic, hemorrhagic, sideroblastic anemias, and anemia of chronic disease [8].

Patients suffering from anemia may present with all kinds of different symptoms

Correspondence: **Dr. Sarah Farrukh, Consultant Pathologist,** Tehsil **Headquarter Hospital, Ferozewala Sheikhupura Pakistan**

Email: sarah.farrukh.javaid@gmail.com

Receiving Date: **27 Oct 2024** Revision Date: **28 Feb 2024** Acceptance Date: **27 May 2024**

depending upon their underlying disease status such as lethargy, light headedness, fainting, poor appetite, palpitations and poor weight gain [7]. Anemia generally affects elderly patients more commonly than the younger ones, adversely affecting the overall health [5]. In Pakistan, however, younger population especially females suffer from anemia to a greater extent. The most common subtype encountered are nutritional anemias especially iron deficiency and it is regarded as one of the most important factors leading to various health related complications such as obstetrical issues [6]. Nutritional anemias are also directly related to educational status of individual patients. Illiterate or less educated females are more commonly affected than the ones who had basic education or access to schools. Personal habits such as smoking also contributes to anemia [2].

Morphological appearance of RBCs studied on a stained blood smear is the most convenient, cost-effective and quickest way of classification of anemia into normocytic, microcytic and macrocytic anemia by determining the size of RBCs. By visualizing the Hb content of individual RBCs on peripheral smear examination (PSE), hypochromic or normochromic nature of anemia can also be determined. However, the use of peripheral smear does carry a risk of observer bias. Automated hematology analyzer is another tool used for diagnosis of anemias. This study will focus on the comparison of peripheral smear examination with automated hematology analyzer for diagnosis and subtyping of anemia while also focusing on the different morphological patterns of anemias encountered

MATERIAL AND METHODS

A total of 100 anemic patients were studied for morphological pattern of anemia based on red cell indices and PSE. Prior approval was obtained from IEC and informed consent was obtained from all patients who participated in the study. Anemia was graded into mild, moderate and severe according to WHO criteria [3] (Table-I). Patients with anemia, characterized by decrease in hemoglobin concentration below normal limit i.e. <12.0 g/dl in women and <13.0 g/dl in men [3]. Patients with known systemic illness, hematological disorders and neoplastic disease who had taken radiotherapy or chemotherapy were excluded to avoid selection bias.

By definition, microcytic anemia was defined as mean corpuscular volume (MCV) below 80 fl, MCV between 80 and 100 fl as normocytic and MCV above 100 fl as macrocytic [9]. Mixed deficiency anemia was characterized as normal MCV with raised red cell distribution width (RDW). Whole blood was taken into EDTA vacutainer and analysed using automated cell counter (Mindray BC-6000 cell counter 5 part). Microscopy (Peripheral smear examination) was performed on slides stained with Geimsa stains for categorization of anemia into various morphological subtypes. 2 slides were prepared for each specimen and examined by two different hematologists to minimize chances of observer bias.

Statistical Package for the Social Sciences (SPSS) version 26 was used for entry of data and analysis. Qualitative variables such as gender was calculated into frequencies, while subtypes of anemia and its severity was calculated in to percentages. Continuous variables like age was calculated as mean and SD. Data was stratified for age, gender and morphological subtypes based on microscopy and automated analyser. Post stratification Chi-square test was applied which equalled to 22.404 with p value of 0.0002 which was highly significant.

RESULTS

Amongst 94 patients enrolled in the study, the youngest was seven months old infant and eldest one being 85 years old. The mean age was 34.88± 15.25 years. Young patients of 20-29 years were the most affected age group. (Figure-III) Females were more commonly affected than males. There were 63 females and 31 males with male to female ratio 1:2 (Figure-I).

Severity of anemia was categorized into mild, moderate and severe (Figure-II) Morphological typing of anemia was done based on PSE findings. The analysis revealed 39 (39%) participants had hypochromic microcytic pattern, followed by 28 participants (28%) with a

normocytic normochromic pattern, 19 participants had mixed deficiency picture showing both hypochromic microcytic as well as macrocytic pattern of anemia whereas 09 participants (09%) had macrocytic anemia and 5 (5%) patients suffered from hemolytic anemia with presence of schistocytes and nucleated red blood cells (Table-III). The findings of PSE and automated analyzer were compared and post stratification Chi Square test results revealed p-value of 0.0002 which was highly significant.

Table-I: Severity of anemia.

Severity	Hemoglobin concentration (g/dL)
Mild	Men-11-12.9
	Women 11-11.9
Moderate	8-10.9
Severe	< 8

Table-II: Distribution of morphological patterns of anemia.

Type of anemia	No of patients (%)		Total
	Male	Female	
Normocytic normochromic anemia	11 (11.7)	16 (17)	27
Hypochromic microcytic anemia	12 (12.7)	24 (25.5)	36
Macrocytic anemia	4 (4.2)	5 (5.3)	09
Mixed deficiency anemia	5 (5.3)	12 (12.7)	17
Hemolytic	1 (1)	4 (4.2)	5
Total	**33**	**61**	**94**

Table-III: Comparison between peripheral smear and auto analyzer interpretation in cases of different morphological anemia.

Type of anemia	Auto analyzer	PBS
Normocytic normochromic (%)	33 (35.1%)	26 (27.6%)
Microcytic hypochromic (%)	49 (52.1%)	37 (39.3%)
Macrocytic (%)	8 (8.5%)	09 (9.5%)
Dimorphic (%)	2 (2.1%)	18 (19.1%)
Hemolytic (%)	01 (01%)	04 (4.2%)
Total	**94**	**94**

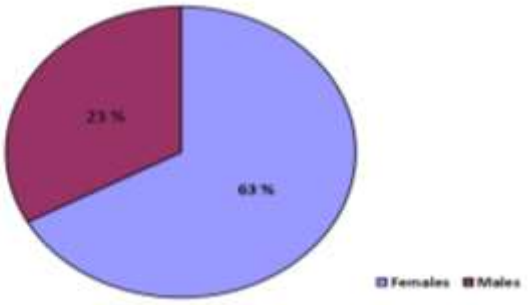

Figure-I: Gender wise distribution of patients.

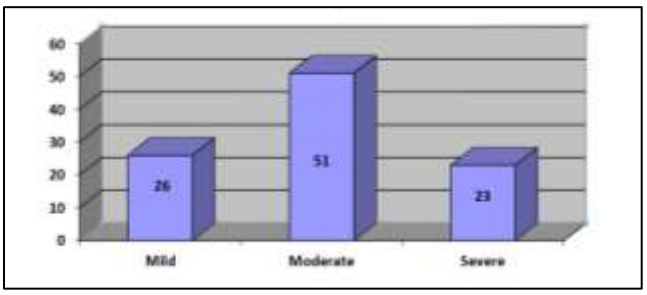

Figure-II: Distribution of patients based on severity of anemia.

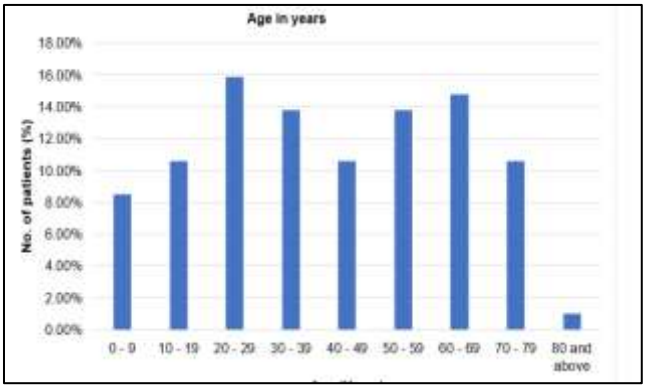

Figure-III: Age distribution of patients.

DISCUSSION

Developing countries like Pakistan face one of the biggest challenges of curtailing anemia. Poverty, lack of awareness and education along with limited access to hospitals with majority of the population living in rural areas are contributory elements in these areas [3]. In this study, patients of both genders were observed which is in contrast to other studies where special groups such as pregnant females and children were targeted. This has given us a valuable insight into various patterns of anemia but also helped in determining that if it is done on a larger scale, can be extremely valuable in defining reference ranges for the same.

Diagnosis of anemia and appropriate management of patients rests on correct categorization of morphological subtype of anemia. It can be done using automated technology which has improved precision, accuracy and reduces the subjective error and is efficient [10]. Peripheral smear is employed as crucial diagnostic technique for anemia, leukemia and other hematological disorders as an adjunct to automated counts. It carries the advantage of being cost-effective and can be carried out in any setup with minimal equipment needs [11].

Anemic patients observed in this study belonged to a wide range of age groups. The youngest patient was a seven months old infant and eldest one was an 85-year-old individual. In another study conducted by Chand FM *et al*. the mean age was 54.36±8.21 years, however this study was conducted on patients suffering from myocardial infarction [13]. The most affected group was 20-29 years old with a female predominance. Increased nutritional needs accompanied with regular blood loss and obstetrical phenomenon can explain the possible relationship of this age group [12]. Male to female ratio in this study was 1:2. This is similar to a study conducted by Solomon D *et al* where male to female ratio of 1:1.745 was present [14].

Microcytic hypochromic anemia was the most common morphological category of anemia in our study, encountered in 39% of the patients. This was in accordance with an observation by Ongole AP *et al* who found 47.8% patients with same morphological subtype in their study and Patel S *et al* whose results showed 46% patients with similar subtype [15]. In a study done by Ashraf FF *et al* the most common pattern of anemia was normocytic normochromic 38% whereas hypochromic microcytic anemia was found in 29% of patients [16].

Most of the patients i.e. 51% suffered from moderate degree of anemia 51% followed by mild and severe anemia i.e. 23% and 26% respectively. It is in contrast to another study conducted by Chaudhry *et al*. which showed prevalence of 39% of mild and moderate anemia [17]. While the significance of PSE in determining the subtype of anemia is an established fact, this study was conducted with the purpose of encouraging the use of PSE in resource limited settings and peripheral healthcare centers where automated equipment is not routinely available. PSE, while being cost-effective and time-saving, when correctly carried out, can aid in proper and timely management of patients. It can used as a guiding tool to carry out further tests in light of morphological subtype of anemia. Automated analyzer is also an effective and swift tool to obtain complete blood counts in routine practice. While PSE carries the risk of observer bias and directly relates to experience of the examining physician, these risks are eliminated by the use of automated analyzers.

It was observed that certain hematological findings become evident on PSE only as revealed in comparative analysis where few differences were seen in cases of hemolytic anemia and hypochromic microcytic anemia. Normocytic normochromic anemia was found in 34% cases by automated analyzer whereas PSE revealed 28% cases. Which is explainable by the fact that normochromic, normocytic cells may appear normal looking on peripheral smear making the anemia challenging to diagnose. Moderate difference was seen in microcytic hypochromic anemia. Automated analyzer and PSE examination revealed 52% and 39% cases respectively. Giant platelets or platelet clumps and schistocytes in hemolytic anemia can be counted as microcytes and contribute in difference of result by automated analyzer. The findings of macrocytic anemia were more or less the same in two methods. In cases of dimorphic anemias, 3% cases were diagnosed by automated analyser while results of PSE revealed 19% cases. Dimorphic anemia can be mistyped as macrocytic, microcytic and normocytic on basis of predominant RBC population by automated analyzer. Dual population of RBC in dimorphic anemias are better picked on PSE. Most of these findings are in concordance with similar studies conducted by Garg M *et al* and Chavda J *et al*. [18] BJ Bain reviewed PSE in the age of automation in 2005 and found that the blood smear remains an important tool to diagnose anemia and further added that sophisticated latest investigations of hematologic disorders should be analysed keeping in view the findings of peripheral blood features as well as the clinical background [19].

Our study has highlighted that despite minor differences in the diagnosis of different subtypes of anemias by the two methods, the importance of peripheral smear in delineating the underlying cause of anemia cannot be ignored. When carried correctly and with proper expertise, it is a very useful aid in timely diagnosis and proper management of patients in light of morphological subtype of anemia. We encourage

the use of PSE where automated analyzers are not available for the benefit of patients.

CONCLUSION

This study emphasizes the role of PSE in comparison with automated hematology analyzer for the diagnosis and subtyping of various forms of anemias. While encountering minor differences in few subtypes of anemias, the results of both methods have been comparable with a significant p-value of 0.002. This scientifically proves that PSE carries as much importance as automated analyzer for diagnosis of anemia while being easily available, cost-effective, low maintenance and time-saving. It is encouraged to use both methods simultaneously before classifying an anemia as hematology analyzer may miss findings of mixed deficiency and hemolytic anemias.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Sarah Farrukh: Study concept, literature search, data analysis, questionnaire design, final approval to be published

Qurat Ul Ain Ayaz: Conception / design of work, data interpretation, data analysis, final approval to be published

Farhan Ali Khanzada: Literature search, data interpretation, data collection

Huma Sheikh: Data analysis, data interpretation, Drafting

Ambreen Anwar: Literature search, data collection, data interpretation

Soubia Cheema: Literature search, Study concept, questionnaire design

REFERENCES

1. Cappellini MD, Musallam KM, Taher AT. Iron deficiency anaemia revisited. J Intern Med. 2020; 287(2):153-70. DOI: https://doi.org/10.1111/joim.13004
2. Saleh MA Haleis ER, Elferjani AAK, Beltamer NM, Saleh MA. Outcomes of teenage pregnancy at Benghazi Medical Center 2019-2020. Int J Sci Acad Res. 2022; 3(3): 3588-602.
3. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann N Y Acad Sci. 2019; 1450(1): 15-31. DOI: https://doi.org/10.1111/nyas.14092
4. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. World Health Organization; 2011.
5. Guralnik J, Ershler W, Artz A, Lazo-Langner A, Walston J, Pahor M, *et al*. Unexplained anemia of aging: Etiology, health consequences, and diagnostic criteria. J Am Geriatr Soc. 2022; 70(3): 891-9. DOI: https://doi.org/10.1111/jgs.17565
6. Khan RS, Ain HB, Tufail T, Imran M, Imran S, Siddique R, *et al*. Undernutrition with special reference to iron-deficiency anemia in reproductive age group females in Pakistan: Iron-deficiency anemia in reproductive age group females. Pak BioMed J. 2022; 5(5): 21-8. DOI: https://doi.org/10.54393/pbmj.v5i5.412
7. Bhadra P, Deb A. A review on nutritional anemia. Indian J Natural Sci. 2020; 10(59): 18674-81.
8. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology and etiology in low-and middle-income countries. Ann NY Acad Sci. 2019; 1450(1): 15-31. DOI: https://doi.org/10.1111%2Fnyas.14092
9. Van Hove L, Schisano T, Brace L. Anemia diagnosis, classification, and monitoring using Cell-Dyn technology reviewed for the new millennium. Lab Hematol. 2000; 6: 93-108.
10. Sharma D, Meenai FJ, Patil MC. To study automated histogram patterns with morphological features noticed on peripheral smear at CMCH Bhopal. J Cardiovasc Dis Res. 2023; 14(2): 1763-8.
11. Garg M, Gitika G, Sangwan K. Comparison of automated analyzer generated red blood cell parameters and histogram with peripheral smear in the diagnosis of anaemia. Int J Contemp Med Res. 2019; 6(8): 1–6. DOI: http://dx.doi.org/10.21276/ijcmr.2019.6.8.4
12. Chatterjee M. Nutritional deficiencies in young people: Causes, consequences and strategies. J Nat Med. 2020; 1.
13. Lal FM, Chand FM, Ahmed R, Ali A, Mal V, Memon GR. Frequency of anemia among the patients presented with myocardial infarction at a Tertiary Care Hospital of Karachi, Pakistan. Pak J Med Health Sci. 2022; 16(10): 311. DOI: https://doi.org/10.53350/pjmhs221610311
14. Solomon D, Bekele K, Atlaw D, Mamo A, Gezahegn H, Regasa T, *et al*. Prevalence of anemia and associated factors among adult diabetic patients attending Bale zone hospitals, South-East Ethiopia. PlosOne. 2022; 17(2): e0264007.

DOI: https://doi.org/10.1371/journal.pone.0264007
15. Patel S, Shah M, Patel J, Kumar N. Iron deficiency anemia in moderate to severely anaemia patients. Guj Med J. 2009; 64(2): 15-17.
16. Ahmed R, Afsar HH, Afsar M, Mazhar S, Chaudhry S, Ashraf A, *et al*. Frequency of anaemia in patients presenting to a Tertiary Care Hospital in Lahore, Pakistan. Pak J Med Health Sci. 2018; 12(3): 1297-9.
17. Shuchismita, Jamal I, Raman RB, Sharan S, Choudhary MK, Choudhary V, *et al*. Clinico hematological profile of anemia in adolescent age group: A retrospective study from Eastern India. Eur J Mol Clin Med. 2022; 9(3): 1672-8.
18. Garg M, Gitika G, Sangwan K. Comparison of automated analyzer generated red blood cell parameters and histogram with peripheral smear in the diagnosis of anaemia. Int J Contemp Med Res. 2019; 6(8): 1–6.
DOI: http://dx.doi.org/10.21276/ijcmr.2019.6.8.4
19. Bain BJ. Diagnosis from the blood smear. New Engl J Med. 2005; 353: 498-507.
DOI: https://doi.org/10.1056/nejmra043442

Frequency of different uro-pathogens causing asymptomatic bacteriuria or bacteriuria without pyuria

Naila Iqbal[1], Muhammad Zeeshan Khalid[2], Abdul Rehman[2], Amber Jamil Siddiqi[1], Humera Javed[3], Saira Salim[4]

[1]Izzat Ali Shah Hospital, Wah Cantt Taxila, Rawalpindi Pakistan
[2]Tehsil Headquarters Hospital Pindigheb, Attock Pakistan
[3]Tehsil Headquarters Hospital Pindigheb, Attock Pakistan
[4]Islamabad Diagnostic Center, Wah Cantt Taxila, Rawalpindi Pakistan

ABSTRACT

Objective: This study determines the frequency of different isolates identified in urine culture, which were identified as asymptomatic bacteriuria on urine RE.

Material and Methods: This descriptive cross-sectional study was conducted at the Department of Microbiology at Izzat Ali Shah Hospital, Wah Cantt from August 2022 to August 2023. A total of 275 urine specimens were included in the study. Mid-stream urine specimens were obtained and routine examination was performed on a fully automated FUS-2000 urinalysis system. Urine culture was performed by inoculating the specimen on CLED and Blood agar plates. The plates were incubated at 37ºC in ambient air for 24-48 hours. Growth was observed and identified based on gram stain and biochemical tests.

Results: Out of 275 specimens, growth was observed in 100 specimens. 175 specimens did not show any growth. Out of the 100 positive urine culture specimens, majority of the isolates belonged to Enterobacterales group. Out of these, 40% were *Escherichia coli* while 25% were *Klebsiella pneumoniae*. Growth of *Staphylococcus saprophyticus* was seen in 12% of specimens. Growth of *Pseudomonas aeruginosa, Enterococcus faecalis, and Streptococcus agalactae was seen in 5% of specimens each,* followed by *Enterobacter cloacae, Enterobacter aerogenes, and Citrobacter koseri* in 2% of specimens each. Growth of *Proteus mirabilis* and *Serratia marcescens* was seen in 1% of specimens each.

Conclusion: In conclusion, among the positive urine cultures, Enterobacterales group dominated followed by Escherichia coli and klebsiella pneumoniae as major isolates. Additionally, Staphylococcus saprophyticus was also identified in a few specimens. These findings highlight the importance of accurate differentiation between urinary tract infection and asymptomatic bacteriuria by correlating urine culture results with routine examination.

Keywords: Asymptomatic bacteriuria, Pyuria, Uropathogens

This article can be cited as: Iqbal N, Khalid MZ, Rehman A, Siddiqi AJ, Javed H, Salim S. Frequency of different uro-pathogens causing asymptomatic bacteriuria or bacteriuria without pyuria. Pak J Pathol. 2024; 35(2): 87-91.

DOI: https://doi.org/10.55629/pakjpathol.v35i2.799

INTRODUCTION

A urinary tract infection (UTI) presents as bacteriuria with or without any symptoms [1,2]. Routine screening and treatment for asymptomatic bacteriuria in non-pregnant individuals is not recommended due to its low prevalence, lack of adverse effects, and limited benefit from antibiotic therapy. Some patients, such as those who are pregnant, have just undergone kidney transplant surgery, or are undergoing urological procedures where mucosal bleeding is possible, should be checked for and treated for asymptomatic bacteriuria [1]. The presence of a single morphotype of bacteria in mid-stream voided urine cultures in an individual without symptoms, with 10 [3-5] colony-forming units (CFU) of the bacteria per milliliter of urine, is known as asymptomatic bacteriuria [2]. Females are considered as being more prone to develop asymptomatic bacteriuria due to multiple factors such as shorter urethra, and non-hygienic or non-sterile sample collection techniques [3]. As women age, the prevalence of asymptomatic bacteriuria rises, rising from approximately 1% in schoolgirls to over 20% in older women [4]. Apart

Correspondence: **Dr. Saira Salim, Consultant Pathologist, Islamabad Diagnostic Center, Wah Cantt Taxila, Rawalpindi Pakistan**

Email: sairasalim2010@hotmail.com

Receiving Date: **07 Dec 2023** Revision Date: **08 May 2024** Acceptance Date: **27 Jun 2024**

from symptoms like pain in the suprapubic area, blood in the urine, contractions in the uterus, pain and burning during micturition can be seen in people with symptomatic UTIs [4,5]. Use of urine catheters for longer durations not only leads to the development of bacteriuria without pyuria but may also be the cause of resistance against antibiotics by bacteria [6,7]. To differentiate urethral contamination from bladder bacteriuria, a quantitative bacteriuria threshold has been devised. The following are the most prevalent uropathogens *Enterococcus spp, coagulase-negative Staphylococcus,* and *Staphylococcus saprophyticus* amongst gram-positive isolates. *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Citrobacter koseri, Proteus mirabilis*, and *Serratia marcescens* are examples of most frequently isolated gram-negative pathogens [2,8]. Studies indicate lower socio-economic states to have higher rates of asymptomatic bacteriuria which are also associated with higher rates of antibiotic resistance as well [8,9,10]. This study aimed to map out the different bacterial isolates causing asymptomatic bacteriuria which may help in establishing a guide to any empirical treatments that may need to be given to any patient experiencing it.

MATERIAL AND METHODS

This descriptive cross-sectional study was carried out at the Department of Microbiology, Izzat Ali Shah Hospital, Wah Cantt, from August 2022 to August 2023 for a period of one year. A sample size of 275 was calculated by keeping a margin of error at 5%, confidence interval at 95%, and prevalence of UTI at 23.3% [11]. Approval from ethical review committee was taken before initiating the study. Written informed consent was obtained from all patients before enrolling them in the study.

A total of 275 mid-stream urine samples from patients of both sexes, ranging in age from 18 to 75, that were being evaluated for asymptomatic bacteriuria were included in the study.

Duplicate samples and samples collected by non-sterile methods or received from outdoor departments were excluded from the study. Urine samples were first analyzed by automated urine analyzer FUS 2000 (DIURI) to find out the physical and biochemical parameters of the sample. Presence of cells was also determined. A significant finding of ≥10 pus cells per high power field (HPF) in the urine sample is considered pathognomonic of a urinary tract infection (UTI).

For culture, all samples were inoculated onto CLED (Oxoid, UK) and Blood (Oxoid, UK) agar plates in quantitative as well as semi-quantitative techniques. In the quantitative technique, a calibrated loop delivering 0.001 mL of urine was used to streak the agar plates to estimate the number of colony-forming units (CFUs) per milliliter of urine. In the semi-quantitative technique, first 10µl of urine is spread vertically across half of plate with a calibrated loop followed by dilution smear with the same loop from top to bottom cross streaking technique to provide an approximation of bacterial growth. The plates were then incubated at 37ºC in ambient air for 24-48 hours. Any pure growth observed was then identified by gram staining and biochemical testing. Catalase and coagulase tests were used to identify gram-positive isolates. Enterococcus spp were then further identified by Lancefield grouping (12) and arabinose fermentation. *Staphylococcus saprophyticus* was identified by using novobiocin diagnostic disc in Muller Hinton agar plates. Gram-negative isolates were identified further by using API 20E and 20NE on basis of positive or negative oxidase test.

The data was analyzed by using Statistical Package for Social Sciences, SPSS version 23. Frequency and percentages were calculated for categorical variables while Mean ± SD were calculated for continuous variables. A p-value of <0.05 was taken as significant

RESULTS

A total of 275 mid-stream urine samples were included in the study. Out of these, 165 samples were from female patients while 110 samples were from male patients. Gender distribution of patients in urinary isolates had shown in Table-I. Of the 275 samples, 100 samples yielded positive bacterial growth of a single morphotype of bacteria. 175 samples did not yield any growth even after 48 hours of incubation. Frequency of different bacterial isolates identified from cultures is shown in Table-II. All of the 275 isolates were analyzed on the

automated FUS 2000 urine analyzer for the presence of pus cells. Among the 275 isolates, 125 exhibited numerous pus cells, while 150 showed no pus cells. 38 samples showed presence of numerous pus cells but no growth on cultures and hence were labeled as false negative, whereas 13 samples were culture positive without any pus cells and were labeled as false positive. 87 samples showed positive cultures positive as well as presence of pus cells and were labeled as true positive. 137 samples did not show any growth or any pus cells and hence were labeled as true negatives. The sensitivity of uro-pathogen was 69.60%, specificity was 91.33, PPV 87.0%, NPV was 78.29% and diagnostic accuracy was 81.45% as shown by following Table-III

Table-I: Gender distribution (n=275).

Gender	N (%)
Females	165(60%)
Males	110(40%)
Total	**275**

Table-II: Bacterial isolates from positive cultures (n=100).

Urinary isolates	N (%)
Escherichia coli	40 (40)
klebsiella pneumoniae	25 (25)
Staphylococcus saprophyticus	12 (12)
Pseudomonas aeruginosa	5 (5)
Enterococcus fecalis	5 (5)
Streptococcus agalactiae	5 (5)
Enterobacter cloacae	2 (2)
Enterobacter aerogenes	2 (2)
Citrobacter koseri	2 (2)
Proteus mirabilus	1 (1)
Serratia marcescens	1 (1)
Total	100

Table-III: Odds ratio and diagnostic accuracy of urinary isolates.

Culture	Urine Pus Cells		Total	p-value
	Positive	Negative		
Positive	87 (TP)	13 (FP)	100	0.001
Negative	38 (FN)	137 (TN)	175	
Total	**125**	**150**	**275**	

Sensitivity= TP/(TP+FN) = 87/(87+38)*100=69.60 %
Specificity= TN/(TN+FP) = 137/(137+13)*100=91.33%
Positive Predictive Value= TP/(TP+FP)* 100= 87/(87+13)= 87.0%
Negative Predictive Value= TN/(TN+FN)*100=137/(137+38)= 78.29%
Diagnostic Accuracy=(TP+TN)/All patients*100 = (87+137)/275=81.45%

DISCUSSION

If pyuria is not seen on urine analysis, asymptomatic bacteriuria (ASB) is established on urine culture when a bacterial count of ≥10^5 colony-forming units [CFU]/mL) is found in the urine. ASB is a common observation in female populations and in many women or men with genitourinary tract disorders that either introduce a foreign body in the urinary tract or hinder voiding [6,7]. According to the above-mentioned criterion, a specimen from a mid-stream urine (MSU) of asymptomatic women with consistently high levels of bacteriuria as much as 10^5 CFU/mL pointed towards a UTI, whereas lower colony counts indicated bacterial contamination [8,9].

Pathogenesis of asymptomatic bacteriuria is attributed to multiple reasons which includes host factors such as urinary catheter usage, surgeries of the urinary tract or non-sterile sample collection. Pathogen factors such as microbiota attachment via fimbriae adhesions is also an important cause for the persistence of symptomatic or asymptomatic infection [13-16].
The findings of the current study align with those of a comparable study conducted in the United States in 2021 where about 45% patients showed positive cultures in the absence of any symptoms. Another similarity to this study was the isolation of *E. coli* as the most frequent pathogen [17] and a similar research study conducted in Denmark in 2020 revealed a positive culture percentage of 42% with *E. coli* as the most common isolated organism [18].

The urine samples were initially assessed for leukocyte counts using a fully automated FUS-2000 urinalysis hybrid based on flow cytometry principles before undergoing culture and sensitivity testing. The sensitivity of uro-pathogen detection was determined to be 69.60%, with a specificity of 91.33%, a positive predictive value (PPV) of 87.0%, a negative predictive value (NPV) of 78.29%, and a diagnostic accuracy (DA) of 81.45%. These results were compared with those of a research study conducted in India. Out of total 216 pregnant female subjects, 36 tested positives for ASB. And similar to the current study, *Escherichia coli* (61.1%, n=22) was the dominating isolate followed by Cons (16.7%, n=6) and *S. aureus* (8.3%, n=3) [19].

This research aimed to comprehensively identify and characterize the bacterial isolates associated with asymptomatic bacteriuria, a condition characterized by the presence of bacteria in the urine without accompanying symptoms of urinary tract infection. By mapping out the diverse range of bacterial species involved in asymptomatic bacteriuria, the study sought to shed light on the microbial landscape of this condition.

CONCLUSION

In conclusion, among the positive urine cultures, Enterobacterales group dominated followed by Escherichia coli and klebsiella pneumoniae as major isolates. Additionally, Staphylococcus saprophyticus was also identified in a few specimens. These findings highlight the importance of accurate differentiation between urinary tract infection and asymptomatic bacteriuria by correlating urine culture results with routine examination.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Naila Iqbal: Main idea and concept
Muhammad Zeeshan Khalid: Data analysis
Abdul Rehman: Result writing
Amber Jamil Siddiqi: Data collection
Humera Javed: Critical review
Saira Salim: Proofreading and revisions

REFERENCES

1. Nicolle LE, Gupta K, Bradley SF, Colgan R, DeMuri GP, Drekonja D, *et al*. Clinical Practice Guideline for the Management of Asymptomatic Bacteriuria: 2019 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2019;68(10):e83-e110. DOI: https://doi.org/10.1093/cid/ciy1121
2. Cheng B, Zaman M, Cox W. Correlation of pyuria and bacteriuria in acute care. Am J Med. 2022;135(9): e353-e8. DOI: https://doi.org/10.1016/j.amjmed.2022.04.022
3. Givler DN, Givler A. Asymptomatic Bacteriuria. [Updated 2023 Jul 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441848/
4. Hooton TM, Scholes D, Stapleton AE, Roberts PL, Winter C, Gupta K, *et al*. A prospective study of asymptomatic bacteriuria in sexually active young women. N Engl J Med. 2000; 343(14): 992-7. DOI:https://doi.org/10.1056/nejm200010053431402
5. Fonseca J, Mavrides D, Graham P, McHugh T. Results of urinary bacterial cultures and antibiotic susceptibility testing of dogs and cats in the UK. J Small Anim Pract. 2021; 62(12): 1085-91. DOI: https://doi.org/10.1111/jsap.13406
6. Cash MC, Hile G, Johnson J, Stone T, Smith J, Ohl C, *et al*. Sustained impact of an antibiotic stewardship initiative targeting asymptomatic bacteriuria and pyuria in the emergency department. Antimicrob Steward Healthc Epidemiol. 2022;2(1): e148. DOI: https://doi.org/10.1017%2Fash.2022.289
7. Colgan R, Jaffe GA, Nicolle LE. Asymptomatic bacteriuria. Am Fam Physician. 2020; 102(2): 99-104.
8. Norden CW, Kass E. Bacteriuria of pregnancy--a critical appraisal. Annual Review of Medicine. 1968;19(1):431-70. DOI: https://doi.org/10.1146/annurev.me.19.020168.002243
9. Luu T, Albarillo FS. Asymptomatic bacteriuria: Prevalence, diagnosis, management, and current antimicrobial stewardship implementations. The Am J Med. 2022. 135(8): 236-44. DOI: https://doi.org/10.1016/j.amjmed.2022.03.015
10. Yasmin H, Niaz WA, Zahoor S, Shamsher S, Shaikh M. Prevalence of urinary tract infection with asymptomatic bacteriuria among gravid females: A Pakistani multicenter cross-sectional study. Professional Med J. 2024;31(01):107-12.
11. Szasz M, Lehotkai N, Kristóf K, Szabó D, Nagy K. Prevalence and antimicrobial resistance of uropathogens in different inpatient wards. Acta Microbiol Immunol Hung. 2009; 56(4): 375-87. DOI: https://doi.org/10.1556/amicr.56.2009.4.7
12. Hamzah AM, Kadim HK. Isolation and identification of Enterococcus faecalis from cow milk samples and vaginal swab from human. J Entomol Zoology Studies. 2018; 6: 218-22.
13. Roos V, Ulett GC, Schembri MA, Klemm P. The asymptomatic bacteriuria Escherichia coli strain 83972 outcompetes uropathogenic E. coli strains in human urine. Infect Immun. 2006; 74(1): 615-24. DOI: https://doi.org/10.1128/iai.74.1.615-624.2006
14. Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic Escherichia coli. EcoSal Plus. 2018;8(1). DOI: https://doi.org/10.1128/ecosalplus.esp-0004-2017

15. Tamma PD, Avdic E, Li DX, Dzintars K, Cosgrove SE. Association of adverse events with antibiotic use in hospitalized patients. JAMA Intern Med. 2017; 177(9): 1308-15.
16. Gołębiewska JE, Krawczyk B, Wysocka M, Dudziak A, Dębska-Ślizień A. Asymptomatic bacteriuria in kidney transplant recipients-a narrative review. Medicina (Kaunas). 2023; 59(2): 198.
DOI: https://doi.org/10.3390/medicina59020198
17. Hooton TM, Roberts PL, Stapleton AE. Asymptomatic bacteriuria and pyuria in premenopausal women. Clin Infect Dis. 2021;72(8):1332-8.
DOI: https://doi.org/10.1093/cid/ciaa274
18. Greve VH, Greve T, Helmig RB. Bacteriuria in pregnancy in a Danish contemporary cohort of women. Infect Dis Obstet Gynecol. 2020; 2020. 8398537.
DOI: https://doi.org/10.1155/2020/8398537
19. Sonkar N, Banerjee M, Gupta S, Ahmad A. Asymptomatic bacteriuria among pregnant women attending tertiary care hospital in Lucknow, India. Dubai Med J. 2021; 4(1): 18-25.
DOI: https://doi.org/10.1159/000513626

Leukocyte adhesion deficiency type 1 with normal expression of CD 11a, CD11b and CD11c

Muhammad Hussain, Mustajab Alam, Muhammad Zain Arshad, Muhammad Aftab Hussain, Maryam Bibi, Hina Mushtaq

Armed Forces Institute of Pathology (National University of Medical Sciences), Rawalpindi Pakistan

ABSTRACT

Leukocyte Adhesion Deficiency Type I (LAD-I) is a rare autosomal recessive disorder resulting from ITGB2 gene mutations on long arm of chromosome 21. This mutation disrupts leukocyte migration, causing impaired wound healing, recurrent infections, periodontitis, delayed umbilical cord separation and neutrophilic leukocytosis. Diagnosis involves flow cytometry to assess CD18, CD11a, CD11b, and CD11c surface expression, along with gene mutation analysis. Early detection and management are crucial for those with LAD-I. Here, we present a case of 11 years old male child with recurrent skin infections and diagnosed with rare phenotype of LAD-1 with normal expression of CD11a, CD11b and CD11c. This case improved our understanding of the mild and delayed presentation of Leukocyte Adhesion Deficiency Type 1 (LAD-I) with variable CD marker expression. It highlights the value of using flow cytometry methods to diagnose inborn errors of immunity, highlighting the need for continued study and increased awareness in this area. Increased knowledge of the various phenotypic expressions of LAD-I among medical professionals and researchers could facilitate prompt diagnosis and treatment, ultimately leading to better outcome and improved quality of life in patients.

Keywords: Adhesion molecules, Flow-cytometric analysis, Integrins, Leukocyte adhesion deficiency, Molecular analysis

This case report can be cited as: Hussain M, Alam M, Arshad MZ, Hussain MA, Bibi M, Mushtaq H. Leukocyte adhesion deficiency type 1 with normal expression of CD 11a, CD11b and CD11c. Pak J Pathol. 2024; 35(2): 92-94.

DOI: https://doi.org/10.55629/pakjpathol.v35i2.817

INTRODUCTION

Leukocyte adhesion deficiency Type I (LAD-I) is a rare inborn error of immunodeficiency resulting from mutations in ITGB2 genes located on long arm of chromosome 21, specifically coding for CD18. It follows an autosomal recessive inheritance pattern [1].

Patients with LAD-1 have very high mortality, about 75% of the patient die before the age of 2 years and both genders are affected equally [2]. LAD-I results from the absence of CD18 expression, a shared subunit in beta 2 integrins: CD11a/CD18 (LFA-1), CD11b/CD18 (Mac 1 or CR3), and CD11c/CD18 (CR4).

Individuals with LAD-I face heightened vulnerability to recurrent bacterial infections, non-purulent abscesses, impaired wound healing, neutrophilic leukocytosis and potentially death if left untreated. Neutrophils release from the bone marrow is normal but their migration from blood to infection sites is compromised. Three distinct leukocyte adhesion deficiency types have been recognized. Diagnosis primarily relies on flow cytometric analysis of neutrophils for surface expression of CD18 and CD11. Prophylactic antibiotics and interferon gamma have shown less response to the disease. Bone marrow transplantation is the treatment of choice in leukocyte adhesion defect (LAD) with a very high success rate. Gene therapy with insertion of the CD18 subunit is currently under trial [3].

CASE REPORT

A 11 years old male child, the second offspring of consanguineous parents living in

Correspondence: **Dr. Muhammad Hussain, Consultant Immunologist, Department of Immunology, Armed Forces Institute of Pathology (National University of Medical Sciences), Rawalpindi Pakistan**

Email: capthussainkk@yahoo.com

Receiving Date: **03 Apr 2024** Revision Date: **20 May 2024** Acceptance Date: **29 Jun 2024**

Rawalpindi, Punjab, sought evaluation for immunodeficiency at the Department of Immunology, Armed Forces Institute of Pathology due to recurrent skin and chest infections. The infant, weighing 2.8 kg at full term and delivered by cesarean section, his umbilical cord was separated on 9th day of postnatal life. He received immunizations under the expanded program of immunization (EPI) in Pakistan. Recurrent infection started at the age of six months, with a serious gastrointestinal infection and progressed to milder skin and chest infections. Around 7 years of age, the frequency of skin infections increased. The older sibling of the child likewise suffered from severe chest and skin illnesses, eventually passed away at the age of 11 years from sepsis.

On general physical examination, the child revealed that there were no oral or cutaneous ulcerations, pallor or cyanosis. The spleen and liver were not enlarged. A blood complete picture showed that the platelet count was 504 x 10^9/L, the hemoglobin level was 9.9 g/L, and the total leukocyte count was 17,100/µL (comprising 60% neutrophils). Neutrophils analysis on flow cytometric analysis showed the complete absence of CD18 but normal expression of the surface markers CD11a, CD11b, and CD11c (Figure-I). Because of the unique nature of this phenotype at our center, additional verification was obtained via DNA testing by sending samples at a private laboratory in Germany. A homozygous pathogenic variation, c.1777C>T (p.Arg593Cys), in the ITGB2 gene was identified by whole exome sequencing, supporting the diagnosis of leukocyte adhesion deficiency type I (LAD-I).

This LAD-I patient presented with milder infections, normal umbilical cord separation and blood complete picture also not showing the typical features of neutrophilia leukocytosis and hence missed early diagnosis. Presently, the patient is receiving prophylactic antibiotics, and the process of HLA matching for bone marrow transplantation is underway.

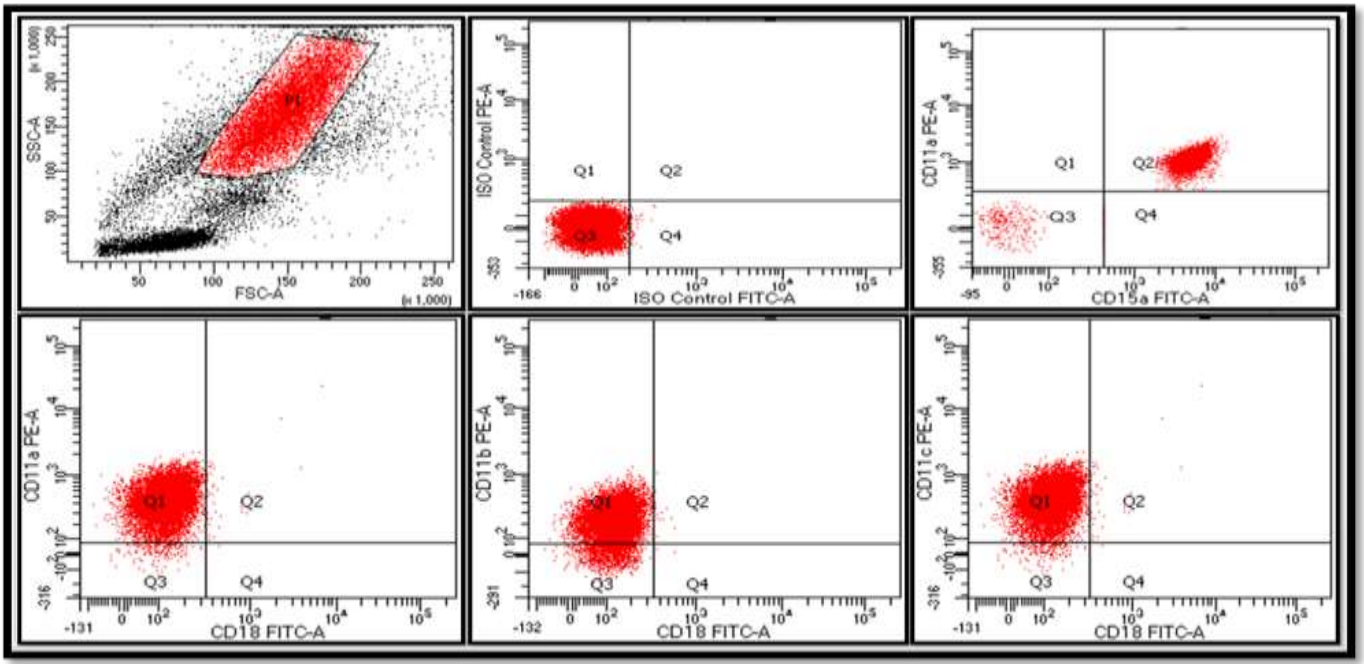

Figure-I: Flow cytometric analysis for Leucocyte Adhesion Deficiency Type I.

DISCUSSION

Leukocyte adhesion deficiencies constitute a group of autosomal recessive disorders characterized by defective leukocyte and endothelial adhesion molecules, leading to inability of leukocytes especially neutrophils to efficiently migrate to site of infection. This deficiency results in recurrent infections in early life. The three types of adhesion molecule deficiencies are categorized as LAD-I, LAD-II, and LAD-III [4].

In LAD-I, patients fail to express the beta chain of beta-2 integrins, encoded by the CD18 gene on chromosome 21's long arm. Notable

beta-2 integrins include leukocyte function associated antigen 1 (LFA-1 or CD11a/CD18), Mac-1 (CD11b/CD18), and P150, 95 (CD11c/CD18) [5]. A case report already published in 2019 in Pakistan which showed absence of CD18, CD11c with low expression of CD11b [6]. A multi-center study conducted in India and published in 2019 showed that CD18 expression varied from absent to normal but CD11a expression was absent in all tested 127 patients [7]. Another multi-center study published by Wolach B *et al* in 2016 also showed CD11a was near absent in all cohort of the study [8] whereas this rare case of leukocyte adhesion defect showed normal expression of CD11a, CD11b and CD11c.

This case highlighted the variety of clinical manifestations and diagnostic challenges associated with Leukocyte Adhesion Deficiency Type I (LAD-I). Unlike traditional cases, which are distinguished by severe skin and chest infections, delayed umbilical cord separation and distinctive laboratory characteristics like neutrophilic leukocytosis but our patient had a milder illness profile, normal umbilical cord separation and no neutrophilic leukocytosis, which made diagnosis difficult and delayed the identification of an underlying immunodeficiency. At our tertiary care center, total 17 cases of LAD have been reported in last 3 years but this case is a very rare variant of leukocyte adhesion deficiency with normal umbilical cord separation, milder skin infection with complete absence of CD18 and normal expression of CD11a, CD11b and 11c and is being reported for the first time as per known literature.

CONCLUSION

This case serves as an important clue for pediatrician about typical sign, symptoms and laboratory investigations may vary in some immunodeficiency variants. However, to gain a deeper understanding of its underlying mechanisms, disease progression and prognosis, further collaborative research across multiple medical centers is essential.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

PATIENT'S CONSENT:

Written informed consent was taken from patient and parents to publish this case report.

AUTHORS CONTRIBUTION

Muhammad Hussain: Conception of the work, drafting and final approval

Mustajab Alam: Critical revision, interpretation of data

Muhammad Zain Arshad, Muhammad Aftab Hussain: Drafting, critical revision

Hina Mushtaq, Maryam Bibi: Interpretation of data

REFERENCES

1. Novoa EA, Kasbekar S, Thrasher AJ, Kohn DB, Sevilla J, Nguyen T, *et al*. Leukocyte adhesion deficiency-I: A comprehensive review of all published cases. J Allergy Clin Immuno Pract. 2018; 6(4): 1418-20. DOI: https://doi.org/10.1016/j.jaip.2017.12.008
2. van de Vijver E, van den Berg TK, Kuijpers TW. Leukocyte adhesion deficiencies. Hematol Clin. 2013; 27(1): 101–16.
3. Geroldinger-Simić M, Lehner K, Klein G, Sepp N, Jabkowski J. An adult with severe leukocyte adhesion deficiency type 1. JAAD Case Rep. 2022; 19: 1-3. DOI: https://doi.org/10.1016%2Fj.jdcr.2021.10.031
4. Das J, Sharma A, Jindal A, Aggarwal V, Rawat A. Leukocyte adhesion defect: Where do we stand circa 2019? Genes Dis. 2020; 7(1): 107-14. DOI: https://doi.org/10.1016/j.gendis.2019.07.012
5. Vandendriessche S, Cambier S, Proost P, Marques PE. Complement receptors and their role in leukocyte recruitment and phagocytosis. Front Cell Dev Biol. 2021; 9: 624025. DOI: https://doi.org/10.3389/fcell.2021.624025
6. Bashir MM, Hussain M, Ahmad D, Tipu HN. Leukocyte adhesion deficiency type 1 with low expression of cd 11b. J Coll Physicians Surg Pak. 2018; 28(6): S87-s8. DOI: https://doi.org/10.29271/jcpsp.2018.06.s87
7. Kambli PM, Bargir UA, Yadav RM, Gupta MR, Dalvi AD, Hule G, *et al*. Clinical and genetic spectrum of a large cohort of patients with leukocyte adhesion deficiency type 1 and 3: a multicentric study from India. Front Immunol. 2020; 11: 612703. DOI: https://doi.org/10.3389/fimmu.2020.612703
8. Wolach B, Gavrieli R, Wolach O, Stauber T, Abuzaitoun O, Kuperman A, *et al*. Leucocyte adhesion deficiency* - A multicentre national experience. Eur J Clin Invest. 2019; 49(2): e13047. DOI: https://doi.org/10.1111/eci.13047

GUIDLINES FOR AUTHORS

Pakistan Journal of Pathology (PAK J PATHOL) is the official journal of Pakistan association of Pathologists. Acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership.

1. Editorial Policy

The Journal will publish research material of interest to the researchers, scientists and medical practitioners. The Journal publishes peer-reviewed original papers, reviews, case reports, and editorials concerned with clinical practice and research in the fields of pathology. Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors'. In preparing manuscripts, authors should follow the "Uniform Requirements for Manuscript Submitted to Biomedical Journals updated October 2008, available at www.icmje.org" and specific author instructions detailed below.

2. Manuscript Submission

(1) Go to the Pak J Pathol official website: **https://www.pakjpath.com/**

(2) Click **'make a submission'** tab on Pak J Pathol website.

(3) Please read checklist and make sure that no point in the checklist is missing

(4) Click **'register'** if you are submitting to Pak J Pathol for the first time. If you are already registered with Pak J Pathol then click 'login'.

(5) A new page will open once you click 'register'. Enter the required information in the given fields. Pakistan Journal of Pathology requests all its authors **to register themselves with Pak J Pathol as reviewer** by clicking 'Yes, request the reviewer role' available at the bottom of 'registration' page. After clicking on 'register' a new page will open.

(6) Please click **'make a new submission'** on this page

(7) A new page will open, with 5 tabs on the top.

(i) Start,

(ii) Upload submission,

(iii) Enter metadata,

(iv) Confirmation,

(v) Next steps. You will be in 'start' tab.

(8) In 'Section' dropdown list, select the appropriate option. Please make sure that all the 'submission requirements' are fulfilled. Tick the checkbox against each requirement. **Note:** if any of the requirement is not fulfilled, and you put a tick mark against it, you still will be able to proceed and complete your submission process but the submission will be declined automatically by the system. In 'comments for the editor' area you can write your comments, however, it is optional. Under 'Acknowledge the copyright statement' tick the checkboxes against both the options. Click 'save and continue'

(9) Now you will be in 'upload submission' tab. A dialogue box will open and here you can upload all your files **(article text, title page, ERC/IRB approval letter, undertaking, processing fee)** one by one. Click 'save and continue'.

(10) Now you will be in 'enter metadata' tab. You may leave 'prefix' field blank. Write title of the article in 'title' field. Write running/short title in 'subtitle' field (you may leave it blank). Copy/paste abstract in abstract area. In 'list of contributors' sections, click 'add contributor' (if you have other authors with you). A new dialogue box will open. Please fill in details of each contributor (author). Please do give **affiliation** of each contributor. Additional details about the contributor can be given in the text box below. For 'contributor's role' please click 'author'. Please tick the check box 'Principal contact for editorial correspondence' for corresponding author only. Lower checkbox 'Include this contributor in browse lists?' will remain ticked for all the authors. Click 'save' and repeat the same process for all the authors one by one. Please **add all the authors** as per the pre-decided

sequence. Please fill the 'languages', 'subjects', 'discipline' and 'key words' fields appropriately. These fields are essential. Each key word **MUST be added SEPARATELY** one by one instead of 'copy/paste' all the key words together. Please click 'save and continue'.

(11) You are now in 'confirmation' tab. Please click 'finish submission'. Congratulations, you have successfully completed your submission to Pak J Pathol.

(12) You can track the status/progress of your article through editorial process any time by logging into your account.

3. Undertaking

The undertaking must be signed by all authors and must contain following statements

(1) The material submitted for publication is, original and has not been submitted for publication elsewhere.

(2) Co-authors qualify authorship and share the responsibility of contents in manuscript.

(3) The research has been approved by ethical committee, and there is no conflict of interest.

4. Format requirements of original article

A. Title page

The title page of the manuscript should include:

(1) Title: Should be concise and self-descriptive.

(2) Authors: First, middle and last name of each author in sequence of authorship merit.

(3) Affiliation for each author, with the name of department and institution.

(4) Corresponding address, cell number, and e mail address of any of first three authors.

(5) A short running head or foot line of no more than 40 characters.

B. Abstract: Abstract should not be of more than 250 words, it must be structured in headings as objective, material & methods, results, and conclusion. Also include 3-5 key words relating to the article subject as abbreviated in the Index -Medicus.

C. Material and Methods: Describe the type, duration & place of study. Summarize the demographic data of the subjects. Provide brief descriptions for methods along-with references. Identify all drugs and chemicals used, including generic name, dose, and route of administration. Reports of randomized clinical trials should present information on all major study elements and protocols. Describe statistical methods used, and specify computer program employed for this purpose.

D. Results: Present results in logical sequence in the text, tables, and illustrations. Do not repeat text data in the tables or illustrations.

E. Discussion: Justify result findings sequentially one by one. While justifying the findings, cite references that support or negate the findings of your study in a logical way. Compare your findings with other similar studies carried out locally and internationally. In this section limitation of study, recommendations, and conclusions drawn must be clearly mentioned.

F. References: References should be cited in consecutive numerical order at first mentioned in the text and designated by the reference number in parenthesis. The references must be written in Vancouver style. A few examples are given below:

(1) For journal articles, list the first six authors only and add, *et al* for the others. For example: Guilarde AO, Turchi MD, Siqueira JB Jr, Mochan DP, Tersi NL, Mouchan L, *et al*. Dengue hemorrhagic fever in adults: clinical outcome related to the serotypes. J Infect Dis. 2012; 197: 817-24.

(2) For books and book chapters, follow as: DeGroot IJ. Evaluation of adrenal function and adrenal disease. In: DeGroot L, Stanbury J B, eds. The thyroid and its diseases. 5th ed. New York: Wiley, 1985, pp 199-258.

(3) Publications in press follow as: Benner AI. Molecular mechanisms of alcohol in addiction. N Engl J Med. In press 1999.

(4) Journal article in electronic form, follow as: Cosby AI. Factors in the - emergence of communicable diseases. Emerg Infect Dis [serial online] 1995 Jan-Mar [cited 2000 July 5], Computerized Educational Systems, 2000.

(5) Monograph in electronic form, follow as**:** CDI, clinical dermatology illustrated [monographs on CD-ROM] Reeves JRT, Maibach H. CMEA Multimedia group, producers 2nd ed. Version 2.0. San Diego: CMEA, 1995.

(6) Conference proceedings follow as: Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EKG and Clinical Neurophysiology, 2000 Oct 19-25, Kyoto, Japan. Amsterdam: Elsevier, 1996.

(7) Dissertation, follow as: Webster SJ. Post-hospital general health care the elderly's access and utilization [Thesis]. St. Louis (MO): Washington Univ., 1995.

(8) Volume with supplement, follow as: Lolti HM, Chong AR. Risk assessment of Codmium carcinogenicity and occupational skin cancer. Global Health Perspect, 1999; 102 Suppl 1:274-94.

G. **Tables:** 3–5 figures and or tables are allowed (each table, complete with legends and footnotes, should be merged in the manuscript). Tables and figures should be embedded and not supplied separately. Table/figure should be written with a capital T and capital F with a hyphen between the word and number e.g., Table-1.
Note: Kindly design table/graph on MS Word/MS Excel. Do not attach table/graph in the form of pictures

H. **Illustrations and legends:** Illustrations should clarify and augment the text. Submit two complete sets of glossy illustrations. No hand drawn art will be accepted. Each illustration must be numbered and cited in consecutive order in the text. Legends for illustrations should be concise and should not repeat the text. Legends should be typed double-spaced. Each figure should be cited in consecutive numerical order in the text. Give the figures a number following the word Figure. Use letters to designate parts of illustrations (e.g, A, B, C) and describe each part clearly in the legend. Any letter designations or arrows appearing on the illustration should be identified and described fully.

I. **Letter from Institutional Review Board / Ethical Review Committee:** Authors are required to send approval letter from Institutional Review Board / Ethical Review Committee along with Original articles and Case reports.

J. **Permission Letter from Med Dte GHQ (Only for Army Serving Doctors)**
Med Dte GHQ permission letter is mandatory for all army serving doctors at the time of article/ case report submission.

K. **Processing / publication fee**
The processing fee of **Rs. 3000/-** (non-refundable) is to be paid at the time of submission of the article through demand/ bank draft payable in the favor of **Commandant AFIP** account. It is further intimated that authors` will have to pay **Rs. 5,000/-** as publication charges/ fee, if the article is accepted for publication. The publication charges for case report and short communication will be half of the above charges. Research Protocol will be published with the same publication charges as that of case report. (Payable before issuance of acceptance letter)

L. **Subscription Fee**

- Single Issue: Rs. 500/-
- Annual Subscription Rs. 2000/-

UNDERTAKING & COPYRIGHT AGREEMENT

Pakistan Journal of Pathology (Pak J Pathol)

All manuscripts must be submitted along with **"Undertaking & Copyright Agreement"** form, as given below, and signed by the Principal author and co-author(s).

It is certified that the following Editorial/ Original/ Review article titled "_____
_____
_____"
has been submitted to Pakistan Journal of Pathology (PAK J PATHOL) for publication. The undersigned stipulate that manuscript has been seen and approved by all authors involved and is neither published nor being considered for publication elsewhere. All copyrights ownership for the article are transferred to the Pak J Pathol.

Name	Institute / Affiliation	Qualification	Contact no	Email	Signature

Authors' Contributions:

Please insert below the detailed contributions made by each author. Examples may include literature search, study design and concept, questionnaire design, data collection, data analysis, data interpretation, drafting, etc.

The current ICMJE authorship criteria, of which all four criteria must be met for authorship, are as follows:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work;
2. Drafting the work or revising it critically for important intellectual content;
3. Final approval of the version to be published;

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

INFORMATION FOR SUBSCRIBERS

SUBSCRIPTION RATES

Pakistan Journal of Pathology is published quarterly by AFIP on behalf of Pakistan Association of Pathologists (PAP).

Annual Subscription

Annual Subscription : Rs. 2000 (Post-paid)

Single copy : Rs. 500 (Post-paid)

Please make demand/ bank draft payable in the favor of **Commandant AFIP** account.

EDITORIAL SUBSCRIPTION AND ADVERTISING INQUIRIES SHOULD BE ADDRESSED TO:

EDITORIAL OFFICE:

Pakistan Journal of Pathology
Armed Forces Institute of Pathology (AFIP)
Rawalpindi – Pakistan
Phone: 0344-55164440

Email: pakjpathol@hotmail.com
Website: www.pakjpath.com

The editorial committee acknowledge the assistance of Computer Expert / Civil Clerk **Muhammad Baqir Zar** for manuscript typing composing and graphic analysis of this Journal.