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CONTENTS

ORIGINAL ARTICLES

Original Article

Stratification in lymph node cytology using the novel Sydney classification system: A cross sectional study

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ABSTRACT

Objective: The primary objective of this study is to assess the diagnostic performance of Lymph Node Fine-Needle Aspiration Cytology (LN-FNAC) using the Sydney System in a clinical setting, specifically focusing on patients with suspected lymphoma.

Material and Methods: This study employs a mixed-methods approach, combining both retrospective and prospective analyses. This study was conducted in Combined Military Hospital (CMH), Peshawar, Pakistan. The duration of study was from January 2021 to December 2022. Ethical approval was obtained from the Institutional Ethical Review Board Committee prior to the commencement of the study. LN-FNAC Cases meeting inclusion criteria were identified and corresponding histopathology specimens were included whenever available. Sydney System of lymph node classification was applied to categorize FNAC results in real-time. Histopathology served as the gold standard for diagnosis. Standard statistical tests were applied to calculate diagnostic parameters, including sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of LN-FNAC. Risk of malignancy (ROM) for each Sydney System diagnostic category was also computed.

Results: Most prevalent category according to Sydney classification was Benign, L2 (39.6%). The sensitivity, specificity, positive predictive value and negative predictive value was 98.2%, 84.3%, 93.2% and 95.6% respectively. The ROM was highest for malignant category (98%) and lowest for benign category (4.5%). Discrepancies between FNAC and histopathology were noted, particularly in Hodgkin lymphoma cases.

Conclusion: This study demonstrates the high diagnostic accuracy of Lymph Node Fine-Needle Aspiration Cytology (LN-FNAC) using the Sydney System, especially in the context of suspected lymphoma. The study contributes essential data to the ongoing validation of the Sydney System, emphasizing its role in standardized and effective diagnostic protocols for lymphoma management.

Keywords: Lymph node FNAC, Lymphoma, Risk of malignancy, Sydney system classification

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INTRODUCTION

Lymphadenopathy, characterized by lymph node enlargement, is a common presentation in medical practice. Traditionally, the diagnosis of lymphoma has involved surgical lymph node excision. However, recent years have seen a significant rise in the adoption of less invasive techniques, such as lymph node fine-needle aspiration cytology (LN-FNAC) and core biopsy, for evaluating lymphoma. Despite their growing use, concerns about the diagnostic accuracy of these methods compared to excisional biopsies persist, leading to varying preferences among practitioners [1-3].

Advancements in integrating flow cytometry and immunohistochemistry into LN-FNAC have improved diagnostic precision, resulting in wider acceptance of this technique. To further augment precision, a novel approach encompassing both core biopsy and fine-needle aspiration (FNA) has been advocated, particularly beneficial in cases with constrained tissue samples [4]. Despite these advancements, the lack of a standardized cytopathological diagnostic classification and

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reporting scheme has caused uncertainty in applying LN-FNAC diagnoses to patient management, potentially leading to suboptimal clinical decisions [5].

Addressing this need for a comprehensive and standardized approach, the 20th International Congress of Cytology introduced the Sydney System for reporting LN-FNAC results. This framework includes two diagnostic levels: a classification into five diagnostic categories (inadequate/insufficient, benign, atypical cells of undetermined significance/atypical lymphoid cells of uncertain significance, suspicious, and malignant), and the utilization of ancillary studies for specific subtyping whenever feasible [5].

While these advancements are promising, additional validation of the Sydney System is imperative [6, 7]. To contribute to this validation effort, we conducted a combined retrospective and prospective analysis of the diagnostic performance of LN-FNAC in our institution, focusing on patients with suspected lymphoma. The primary aim of this study is to assess the applicability of the Sydney System to lymph node FNAC, evaluate its diagnostic accuracy, and determine the risk of malignancy associated with each diagnostic category.

The Sydney System's introduction has paved the way for a standardized approach to lymph node cytopathology reporting, offering a coherent diagnostic framework for better communication amongst laboratories and clinicians. However, the underutilization of the system and limited available data in the literature highlights the necessity for further evaluation and validation. Our study aims to bridge this gap by systematically assessing the diagnostic performance of LN-FNAC in our clinical setting using the Sydney System's categories.

The study's findings will contribute crucial insights into the applicability and reliability of the Sydney System in diagnosis of lymph node diseases. By assessing its diagnostic accuracy and evaluating the associated risk of malignancy, we aim to enhance the understanding of this classification system's applicability in guiding clinical management decisions for patients with suspected lymphoma. This research aligns with the global efforts to establish standardized and effective diagnostic protocols, ultimately improving patient care and refining lymphoma management strategies.

MATERIAL AND METHODS

This study was conducted at the Department of Pathology, Combined Military Hospital (CMH), Peshawar, Pakistan, a renowned tertiary care center catering to the population of Khyber Pakhtunkhwa. CMH Peshawar serves as a crucial referral hub for regions including Bannu, Mardan, Nowshera, Risalpur, Landikotal, and Kohat city. The study timeline spanned from January 2021 to December 2022. Ethical approval was acquired from the Institutional Ethical Review Board Committee prior to the commencement of the study. A non-probability consecutive sampling technique was employed to select patients for the study.

Cases involving patients of all ages and genders were considered, ensuring a diverse representation. Pertinent clinical and demographic data were extracted from test request forms to compile a comprehensive profile of each patient. This encompassed clinical workup, radiological investigations, detailed history of the present complaint, and local examination. All case of lymph node enlargement, irrespective of benign or malignant diagnosis were included. Patients with non-lymphoid aspirates, and those with bleeding disorders were excluded. Data bank of our laboratory was researched for lymph node FNAC cases from January 2021 to December 2021 that tailored to our inclusion criteria including percutaneous aspirations and ultrasound guided FNAC. Corresponding histopathology specimens were also searched using patients name, age, hospital MRN number and contact information. Prospective collection of lymph node FNAC cases was done from January 2022 to December 2022 with corresponding histopathology specimens, whenever possible.

FNAC procedure comprised of care for strict aseptic precautions, following written

consent and detailed discussions about the procedure with the patients. A 22-gauge needle was employed for superficial swellings. All deep and non-approachable lymph nodes were aspirated using ultrasound guidance. Aspirated smears were stained using Hemacolor, hematoxylin and eosin and Papanicolaou stains, enabling effective cytological evaluation. To ensure consistency, cytology slides were re-examined by two experienced histopathologists (having at least 5 years' experience in signing out cytopathology cases), using predefined cytological criteria. Blinding of cases was done with discussion related to discordant cases and a final diagnostic category was decided. The diagnostic categories of the Sydney Classification System were meticulously applied: L1 (Inadequate/Non-Diagnostic), L2 (Benign), L3 (Atypical cells of undetermined significance/Atypical lymphoid cells of uncertain significance), L4 (Suspicious), and L5 (Malignant). Histopathology reports for all included patients were gathered and cross-referenced to validate diagnoses whenever possible. Histopathology served as the gold standard for diagnosis.

Diagnostic parameters were calculated for analytical groups, including benign (L2) and malignant (L4, L5) cases. A two by two table was computed for both categories and the Sensitivity (SN), Specificity (SP), Positive predictive value (PPV), Negative predictive value (NPV) and Accuracy of LN-FNAC was calculated using the following formulas and 95% confidence interval, SN= TP/TP+FN, SP= TN/TN+FP, PPV= TP/TP+FP, NPV= TN/TN+FN. (TP-True positive), (FP-False positive), (TN-True negative) (FN-False negative). Additionally, the risk of malignancy (ROM) for each category was computed by dividing histologically verified malignant cases by the total number of patients with available histopathology in each category.

RESULTS

A total of consecutive 368 cases of LN-FNAC were included in this study from January 2021 to December 2022 with 174 males (47.3%) and 194 females (53.7%). The majority of patients fell within the age range of 20-30 years (44.8%), followed by 31-50 years (36.1%). A significant proportion of cases (64.1%) had no relevant medical history. The cervical group was the most common lymph node location (76.1%), with other locations including axillary (6.3%), submandibular (11.7%), inguinal (2.4%), and supraclavicular (3.5%) (Table-I).

Lymph node aspirates were categorized according to the Sydney System, which included five diagnostic levels. The majority of cases fell into the "Benign" category (39.6%), followed by "Suspicious" (20%) and "Malignant" (15.4%) categories. The "Atypical cells of undetermined significance/Atypical lymphoid cells of uncertain significance" and "Inadequate/Non-diagnostic" categories accounted for 9.7% and 14.9% of cases respectively. The "Suspicious" category was further subcategorized into "Suspicious for Hodgkin lymphoma, suspicious for non-Hodgkin lymphoma and metastasis" with a notable number of cases diagnosed as suspicious for Hodgkin lymphoma (11.6%). In the "Malignant" category, both Hodgkin lymphoma and non-Hodgkin lymphoma were identified, making up 7.3% and 4.6% of cases, respectively (Table-II). Table-III elucidates the correlation between the Sydney System Diagnostic Categories and Histological follow-up for the 368 cases. In the L1 category, 55 cases were diagnosed as inadequate or non-diagnostic samples. However, 10 cases were lost to follow-up, leaving 45 cases for analysis. Within this category, histopathological correlation revealed diagnoses of reactive lymphoid hyperplasia (RLH, n=33), Hodgkin lymphoma (HL, n=11), and metastasis (n=1).

The L2 category, denoting benign conditions, encompassed 146 cases diagnosed by FNAC. However, 102 cases were lost to follow-up. Histopathological correlation within this category revealed a diverse diagnoses, including RLH (n=15), dermatopathic lymphadenitis (n=1), Rosai Dorfman disease (n=7), infectious mononucleosis (n=1), T cell-rich B cell lymphoma (n=1), Chronic granulomatous inflammation (Tuberculosis- n=7 ,Fungal infection- n=3 ,Foreign body reaction-n=1, HL- n=1), and abscess (Acute on chronic non-specific inflammation- n=5, Inflamed

Epidermal inclusion cyst- n=1 and Cat scratch disease- n=1)

The L3 category, signifying atypical cells seen, included 36 cases diagnosed by FNAC. Five cases were lost to follow-up. Histopathological correlation within this category revealed diagnoses of toxoplasmosis (n=3), dermatopathic lymphadenitis (n=2), and HL (n=26). The L4 category, suspicious for malignancy, included 74 cases diagnosed by FNAC. Eight cases were lost to follow-up. Histopathological correlation within this category revealed diagnoses of NHL (Follicular lymphoma -n=6, Diffuse large B cell lymphoma - n=9, Small cell lymphoma -n=4), HL (n=33), RLH (n=6), Infectious mononucleosis (n=1) and Squamous cell carcinoma (n=7).

The L5 category, indicating malignancy, comprised 57 cases diagnosed by FNAC. Six cases were lost to follow-up. Histopathological correlation within this category revealed diagnoses of NHL (Diffuse Large B cell lymphoma– n=11, Small cell lymphoma– n=3, Follicular lymphoma– n=2), HL (n=24), RLH (n=1), Anaplastic thyroid carcinoma (n=2), Squamous cell carcinoma (n=5), Melanoma (n=2) and adenocarcinoma (n=1).

Standard statistical tests were applied and sensitivity, specificity, positive predictive value and negative predictive value of FNAC test was calculated using the 2 x 2 table (Table-IV) and keeping confidence interval at 95%. Histopathological diagnosis was considered as gold standard. Sensitivity of FNAC was 98.2% (94.39% to 99.89%), Specificity 84.3% (91.43% to 98.97%), positive predictive value 93.2% (89.44% to 96.28%) and negative predictive value 95.6% (92.60% to 99.71%).

Risk of Malignancy (ROM) was calculated for each Sydney system diagnostic category. For L1 (Inadequate/Nondiagnostic) category the calculated ROM was 26.7% and for L2 (Benign) ROM has lowest value of 4.5%. For category L3 (Atypical cells of undetermined significance/Atypical lymphoid cells of uncertain significance), the ROM was 83.9% and for L4 (Suspicious for lymphoproliferative disorder/ malignancy), ROM came out to be 89.4%. The ROM for the malignant category was the highest, 98%.

Table-I: Demographics (N=368 cases).

Sample characteristics		Frequency	%
Sex	Male	174	47.3%
	Female	194	53.7%
Age	0-20	57	15.4%
	20-30	165	44.8%
	31-50	113	36.1%
	51-70	33	8.9%
Medical History	Previous pathological diagnosis	132	35.9%
	No relevant history	236	64.1%
Location	Cervical group	280	76.1%
	Axillary	23	6.3%
	Submandibular	43	11.7%
	Inguinal	9	2.4%
	Supraclavicular	13	3.5%

Table-II: Sydney system diagnostic categories.

Diagnostic categories		Frequency	%
L1 Inadequate/ non-diagnostic		55	14.9%
L2 Benign		146	39.6%
L3 AUS/ ALUS	Atypical Lymphoid cells seen	15	4.0%
	Atypical cells seen	21	5.7%
	Total	36	9.7%
L4 Suspicious	NHL	22	6.0%
	HL	43	11.6%
	Metastases	9	2.4%
	Total	74	20%
L5 Malignant	NHL	17	4.6%
	HL	27	7.3%

Metastases	13	3.5%
Total	57	15.4%

Table-III: Correlation between Sydney system diagnostic categories and histology/ clinical follow-up.

Diagnostic categories		FNAC diagnosis	Lost to follow up	Histopathological Correlation
L1 (Inadequate/ non-diagnostic)	55	(n=55)	10	RLH (n = 33) HL (n=11) Metastasis (n=1)
L2 Benign	146	Reactive lymphoid hyperplasia (n=63)	38	Reactive lymphoid hyperplasia (n=15) Dermatopathic lymphadenitis (n=1) Rosai Dorfman disease (n=7) Infectious mononucleosis (n=1) T cell rich B cell lymphoma (n=1)
		Chronic granulomatous inflammation (n=64)	52	Tuberculosis (n=7) Fungal Infection (n=3) Foreign body reaction (n=1) Hodgkin lymphoma(n=1)
		Abscess (n=19)	12	Acute on chronic non-specific inflammation (n=5) Inflamed Epidermal inclusion cyst (n=1) Cat scratch disease (n=1)
L3 AUS/ALUS	36	Atypical cell seen (n=36)	5	Toxoplasmosis(n=3) Dermatopathic lymphadenitis (n=2) Hodgkin lymphoma(n=26)
L4 Suspicious	74	NHL (n=22)	3	Follicular lymphoma (n=6) Diffuse large B cell lymphoma(n=9) Small cell lymphoma(n=4)
		HL (n=43)	3	Reactive lymphoid hyperplasia (n=6) Infectious mononucleosis (n=1) Hodgkin lymphoma (n=33)
		Metastasis (n=9)	2	Squamous cell carcinoma(n=7)
L5 Malignant	57	NHL (n=17)	1	Diffuse large B cell lymphoma(n=11) Small cell lymphoma(n=3) Follicular lymphoma(n=2)
		HL (n=27)	2	Hodgkin lymphoma(n=24) Reactive lymphoid hyperplasia(n=1)
		Metastasis (n=13)	3	Anaplastic thyroid carcinoma(n=2) Squamous cell carcinoma(n=5) Melanoma (n=2) Adenocarcinoma (n=1)
Total	**368**	**237**	**131**	

Table-IV: A 2×2 table of sensitivity and specificity.

Cytology	Histopathology		Total
	Malignant	Benign	
Malignant	TP (109)	FP (8)	117
Benign	FN (2)	TN (42)	44
Total	111	50	161

Table-V: Stratification of ROM in the Sydney system diagnostic categories (N=237 cases).

Sydney System Diagnostic Category	Histological or Clinical Follow-Up	Confirmed by Histopathology		Risk of Malignancy (ROM)
		Benign Lesions	Malignant Lesions	
L1 Inadequate/ non-diagnostic	45	33	12	26.7%
L2 Benign	44	42	2	4.5%
L3 AUS/ALUS	31	5	26	83.9%
L4 Suspicious	66	7	59	89.4%
L5 Malignant	51	1	50	98.0%

DISCUSSION

Persistent lymphadenopathy, particularly prolonged enlargement of lymph nodes 1 cm, is a source of considerable apprehension for patients. FNAC is generally recognized as the accepted minimally invasive procedure for

assessing lymphadenopathy. The Sydney System, an innovative classification framework for LN-FNAC, seeks to standardize result reporting by employing five diagnostic categories. It also advocates for specific subtyping through ancillary studies, when necessary, thereby enriching the diagnostic approach to lymph node conditions.

A total of 368 cases of LN-FNAC were included in this study. Most of the patients were in age range 20-50 years, with cervical lymph node being the most common lymph node location. Similar findings were found in a study by Elena Vigliar *et al* [8]. Majority of the patients presenting to our unit (64.1%) did not have any prior medical history or relevant investigations done. This is a major source of difficulty in reaching a sound diagnosis in FNAC samples as serological and radiological evidences serve as important adjuncts in FNAC diagnosis [9]. Currently a number of authors are suggesting the usefulness of a combined approach including flow cytometry and immunohistochemistry with LN-FNAC [10].

Within our study, 55 cases were categorized under L1 (Inadequate/non-diagnostic). Limitations inherent in lymph node aspiration encompass sampling errors arising from deep-seated or diminutive lymph nodes, extensive necrosis or inflammation and nodal fibrosis. Moreover, inadequacies in the obtained specimens contribute to diagnostic complexities, primarily due to the loss of architectural or vascular patterns and the partial involvement of the lesion within the respective lymph node [11, 12].

Majority of our study cases were diagnosed as benign entities (39.6%), comprising diagnoses including reactive lymphoid hyperplasia, chronic granulomatous inflammation and abscess. Apart from persistently enlarged, painful lymph nodes or lymph nodes not regressing after treatment, most of lymph nodes after a benign LN-FNAC diagnosis are not excised. Prevalence of a high tuberculosis disease burden in Pakistan leads to commencement of anti-tubercular treatment after LN-FNAC diagnosis of chronic granulomatous inflammation, serological tests and gene expert test [13,14]. Two cases in benign category showed discrepancy with Histopathological correlation. One case being Hodgkin lymphoma (Figure-I, II) and other T cell rich/ B cell lymphoma (Figure-III, IV).

Suspicious category L4 showed discrepancy in a total of 7 cases diagnosed as “Suspicious for Hodgkin lymphoma”. Subsequent resection of lymph node showed reactive lymphoid hyperplasia (Figure V, VI) in 6 cases and one case diagnosed as Infectious mononucleosis (Figure-VII, VIII).

L5-Malignant category had one case showing conflicting diagnosis. A case cytologically diagnosed as “Lympho-proliferative disorder- Hodgkin lymphoma” (Figure-IX), on resection showed Reactive lymphoid hyperplasia (Figure-X). This case was immunohistochemically confirmed with CD 3 and CD 20 stains.

Figure-I

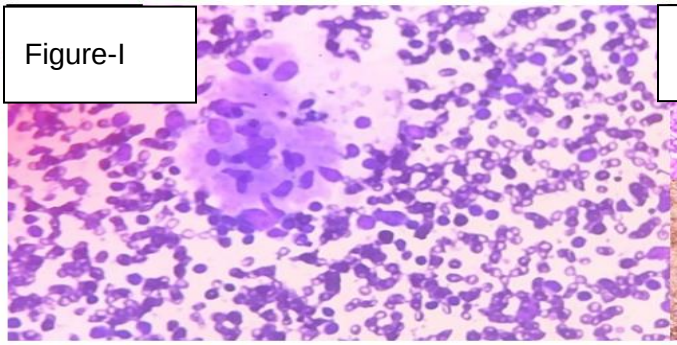

Figure-I: Case cytologically diagnosed as Chronic Granulomatous Inflammation (False negative).

Figure-II

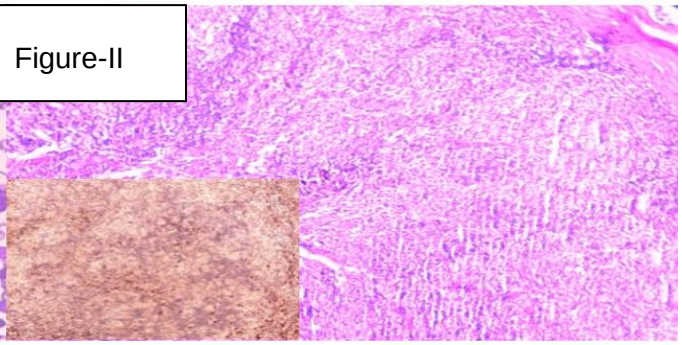

Figure-II: Same case histologically diagnosed as Hodgkin lymphoma. Inset shows CD 15 Positive RS cells.

Figure-III

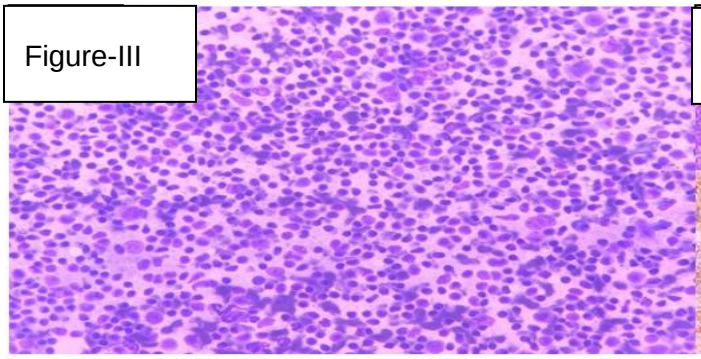

Figure-III: Case cytologically diagnosed as reactive lymphoid hyperplasia (False negative).

Figure-IV

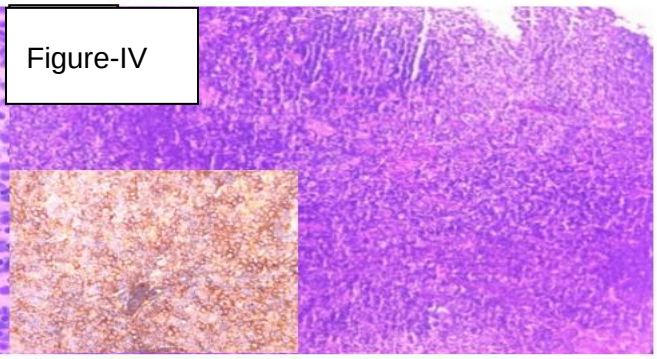

Figure-IV: Same case histologically diagnosed as T cell rich B cell lymphoma. Inset shows CD 3 positive T cells with scattered atypical B cells.

Figure-V

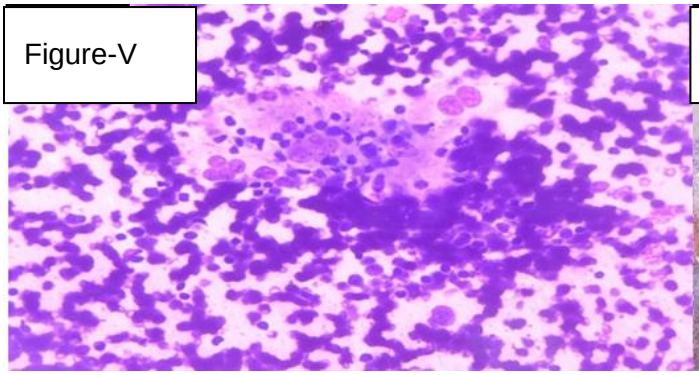

Figure-V: Case cytologically diagnosed as "Suspicious for lympho-proliferative disorder-Hodgkin lymphoma" (False positive).

Figure-VI

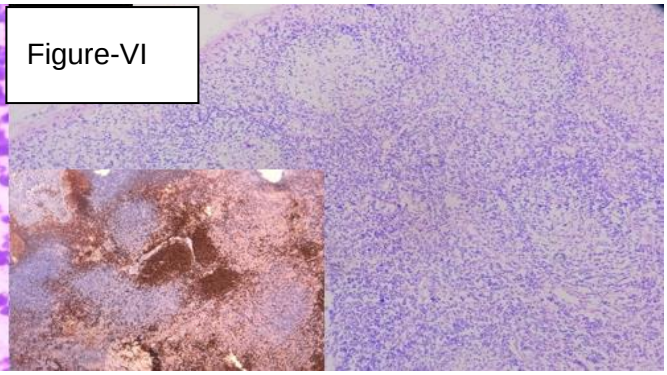

Figure-VI: Same case histologically diagnosed as Reactive lymphoid hyperplasia. Inset shows CD3 positive T cell zones.

Figure-VII

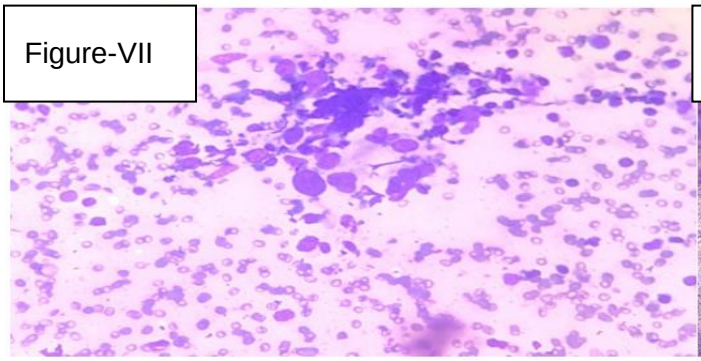

Figure-VII: Case cytologically diagnosed as "Suspicious for lympho-proliferative disorder-Hodgkin lymphoma" (False positive).

Figure-VIII

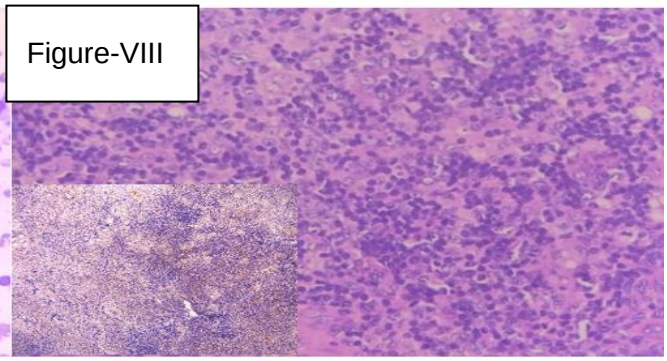

Figure-VIII: Same case histologically diagnosed as "Suggestive of Infectious mononucleosis". Numerous immunoblasts are seen. Inset shows CD30 negative cells.

Figure-IX

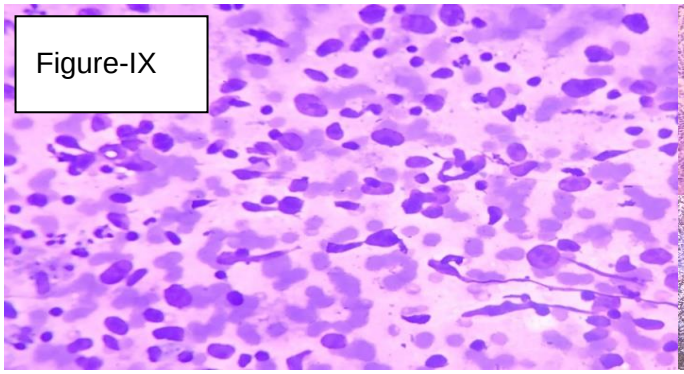

Figure-IX: Case cytologically diagnosed as "Lymphoproliferative disorder – Hodgkin lymphoma" (False positive).

Figure-X

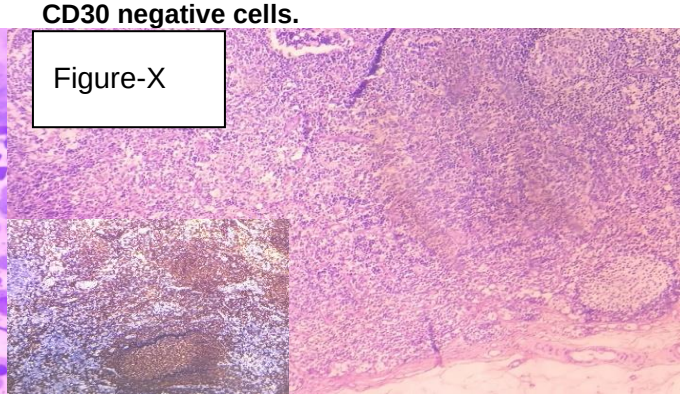

Figure-X: Same case histologically diagnosed as Reactive Lymphoid hyperplasia. Inset shows reactive pattern of staining (CD 20).

Majority of discrepant cases between cytology and histopathology were of Hodgkin lymphoma. Causes of misdiagnosis included sclerosis, a ` lymphoid cell population, vague collections of epithelioid cells and mononuclear RSLIKE cells. Indeed, reactive lymphoid hyperplasia and lymphomas with polymorphous population are difficult to diagnose on cytology alone. Many researchers suggest the use of additional diagnostic techniques (Immuno-cytochemistry and flow cytometry) with LN-FNAC for diagnosis of lymphoma on FNAC [15, 16].

According to our study, the sensitivity of FNAC was high (98.2 %), signifying that there were few false negative tests according to FNAC. However, specificity came out to be comparatively lower (84.3%), meaning a slightly greater number of false positive results. Our study showed a high positive and negative predictive value, 93.2 % and 95.6% respectively. A number of studies show similar findings [2,17-18]. In contrast, a study by Gupta *et al*. showed a lower sensitivity and higher specificity of 79.9% and 98.7% respectively [6]

The risk of malignancy (ROM) was lowest for L2 (Benign) category in our study with a value of 4.5%, similar to findings of a study by Torres Rivas *et al*. [19]. Conversely, studies by Saradva N *et al*. and Gupta *et al*. showed a higher ROM for L2 category of lymph node FNAC, according to Sydney classification. The ROM for L4 category was much lower in study by Saradva N *et al*. (50%) as compared to our study (89.4%). Comparable to our findings of high ROM in L4 and L5 categories are studies by Caputa A *et al*, Robert AS *et al* and few others [20-23].

CONCLUSION

The application of the Sydney System is advocated to achieve consistency and reproducibility in lymph node FNAC diagnoses, aiding in risk-stratification. The study's limitations include its partial retrospective nature, potential selection bias due to consecutive sampling, and the single-center setting, which may impact the generalizability of the findings. This study concludes that FNAC and adherence to the Sydney System, proves to be an accurate and a minimally invasive tool for evaluating lymphadenopathy

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, accountable for all aspects of the work

Abdul Qadir: Methodology, supervision, accountable for all aspects of the work

Sadia Khan: Data analysis, revisions, accountable for all aspects of the work

Shehla Akbar: Data interpretations, revisions, accountable for all aspects of the work

REFERENCES

1. Salim DN, Obinah MP, Ternov NK, McCullagh MJ, Larsen MS, Hendel HW, *et al*. Fine needle and core needle ultrasound guided biopsies for assessing suspected melanoma metastasis in lymph nodes and subcutaneous tissue. J Surg Oncol. 2022; 126(6): 1058-66.
   DOI: https://doi.org/10.1002/jso.26998
2. Kulkarni SS, Murchite S, Patil A. A comparative study between fine needle aspiration cytology and core needle biopsy in the diagnosis of palpable breast lumps. J Surg Res. 2022; 5(2): 221-8.
3. Pillai AV, Ramachandran R, Borkar PV, Bhaskaran R. Effectiveness of fine needle aspiration cytology versus excisional biopsy in cervical lymphadenopathy. Formosan J Surg. 2021 May 1;54(3):79-84.
   DOI: https://doi.org/10.4103/fjs.fjs_104_20
4. Zeppa P, Cozzolino I, Caraway NP, Al-Abbadi MA, Barroca H, Bode-Lesniewska B, *et al*. Announcement: The international system for reporting lymph node cytopathology. Acta Cytologica. 2020; 64(4): 299-305.
   DOI: https://doi.org/10.1159/000507413
5. Al-Abbadi MA, Barroca H, Bode-Lesniewska B, Calaminici M, Caraway NP, Chhieng DF, *et al*. A proposal for the performance, classification, and reporting of lymph node fine-needle aspiration cytopathology: The Sydney system. Acta Cytologica. 2020; 64(4): 306-22.
   Doi: https://doi.org/10.1159/000506497
6. Gupta P, Gupta N, Kumar P, Bhardwaj S, Srinivasan R, Dey P, *et al*. Assessment of risk of malignancy by application of the proposed Sydney system for

classification and reporting lymph node cytopathology. Cancer Cytopathol. 2021; 129(9): 701-18. DOI: https://doi.org/10.1002/cncy.22432

7. Caputo A, Fraggetta F, Cretella P, Cozzolino I, Eccher A, Girolami I, *et al*. Digital examination of lymph node cytopathology using the Sydney system (DELYCYUS). An international, multi-institutional study. Cancer Cytopathol. 2023; 131(11): 679-92. DOI: https://doi.org/10.1002/cncy.22741
8. Vigliar E, Acanfora G, Iaccarino A, Mascolo M, Russo D, Scalia G, *et al*. A novel approach to classification and reporting of lymph node fine-needle cytology: application of the proposed Sydney system. Diagnostics. 2021; 11(8): 1314. DOI:https://doi.org/10.3390%2Fdiagnostics11081314
9. Ronchi A, Caputo A, Pagliuca F, Montella M, Marino FZ, Zeppa P, *et al*. Lymph node fine needle aspiration cytology (FNAC) in paediatric patients: Why not? Diagnostic accuracy of FNAC in a series of heterogeneous paediatric lymphadenopathies. Pathology-Research and Practice. 2021 Jan 1;217:153294. DOI: https://doi.org/10.1016/j.prp.2020.153294
10. Barroca H, Marques C. A basic approach to lymph node and flow cytometry fine-needle cytology. Acta Cytol. 2016; 60(4): 284-301. DOI: https://doi.org/10.1159/000448679
11. Ha HJ, Lee J, Kim DY, Kim JS, Shin MS, Noh I, *et al*. Utility and limitations of fine-needle aspiration cytology in the diagnosis of lymphadenopathy. Diagnostics. 2023; 13(4): 728. DOI:https://doi.org/10.3390%2Fdiagnostics13040728
12. Aragon-Sanchez S, Oliver-Perez MR, Madariaga A, Tabuenca MJ, Martinez M, Galindo A, *et al*. Accuracy and limitations of sentinel lymph node biopsy after neoadjuvant chemotherapy in breast cancer patients with positive nodes. Breast J. 2022; 2022. 1507881 DOI: https://doi.org/10.1155/2022/1507881
13. Tahseen S, Khanzada FM, Baloch AQ, Abbas Q, Bhutto MM, Alizai AW, *et al*. Extrapulmonary tuberculosis in Pakistan-A nation-wide multicenter retrospective study. PloS One. 2020; 15(4): e0232134. DOI: https://doi.org/10.1371/journal.pone.0232134
14. Ali S, Khan MT, Khan AS, Mohammad N, Khan MM, Ahmad S, et al. Prevalence of multi-drug resistant Mycobacterium tuberculosis in Khyber Pakhtunkhwa–a high tuberculosis endemic area of Pakistan. Polish J Microbiol. 2020; 69(2): 133-7. DOI: https://doi.org/10.33073/pjm-2020-005
15. Ha HJ, Lee J, Kim DY, Kim JS, Shin MS, Noh I, *et al*. Utility and limitations of fine-needle aspiration cytology in the diagnosis of lymphadenopathy. Diagnostics. 2023; 13(4): 728. DOI:https://doi.org/10.3390%2Fdiagnostics13040728
16. Chong Y, Park G, Cha HJ, Kim HJ, Kang CS, Abdul-Ghafar J, *et al*. A stepwise approach to fine needle aspiration cytology of lymph nodes. J Pathol Transl Med. 2023; 57(4): 196-207. DOI: https://doi.org/10.4132%2Fjptm.2023.06.12
17. Rammeh S, Romdhane E, Sassi A, Belhajkacem L, Blel A, Ksentini M, *et al*. Accuracy of fine-needle aspiration cytology of head and neck masses. Diagn Cytopathol. 2019; 47(5): 394-9. DOI: https://doi.org/10.1002/dc.24120
18. Ahuja S, Malviya A. Categorisation of lymph node aspirates using the proposed Sydney system with assessment of risk of malignancy and diagnostic accuracy. Cytopathology. 2022l; 33(4): 430-8. DOI: https://doi.org/10.1111/cyt.13094
19. Rivas HET, Zarra KV, Pabón LAP, Gutierréz MDG, Ortiz NZ, Fernández MDO, *et al*. Ultrasound-guided fine-needle aspiration of superficial lymphadenopathy performed by interventional pathologists: The applicability of the Sydney system from 2 years of experience and 363 cases. Acta Cytol. 2021; 65(6): 453-62. DOI: https://doi.org/10.1159/000517314
20. Saradva N, Trivedi DP. Lymph node fine needle aspiration cytology reporting using Sydney system at tertiary care center. Int J Clin Diagn Pathol. 2022; 5(4): 109-13 DOI: https://doi.org/10.33545/pathol.2022.v5.i4b.501
21. Caputo A, Ciliberti V, D'Antonio A, D'Ardia A, Fumo R, Giudice V, *et al*. Real-world experience with the Sydney system on 1458 cases of lymph node fine needle aspiration cytology. Cytopathology. 2022; 33(2): 166-75. DOI: https://doi.org/10.1111/cyt.13079
22. Robert AS, Saldanha C. An approach to classification and reporting lymph node cytopathology using Sydney system and evaluating the likelihood of malignancy. Biomedicine. 2023; 43(02): 701-5. DOI: http://dx.doi.org/10.51248/.v43i02.2517
23. Sreelekshmi JR, Joseph TP. Structured reporting of lymph node cytopathology using the 2020 Sydney system guidelines-A retrospective study. Nat J Lab Med. 2023; 12(2): PO39-PO44. DOI:https://doi.org/10.7860/NJLM/2023/61109.2722

Original Article

Comparison of demographic variables and clinical findings with immune biomarkers in type1 diabetes

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ABSTRACT

Objective: To explore how demographic variables and clinical findings relate to immune biomarkers, assess their impact on glycemic control, and identify the most relevant immune biomarker for the Pakistani population with Type 1 Diabetes.

Material and Methods: This cross-sectional analytical study was conducted at Chughtai Institute of Pathology, from April 2021 to March 2022. We enrolled 130 male and female diagnosed cases of Type 1 Diabetes of age below18 years in this study. A total of 100 cases were included in the study as per defined criteria and 30 were excluded. Relevant details of demographic variables & clinical findings were noted on a predesigned proforma. 5ml whole blood was taken from each subject. All samples were analyzed for Plasma Glucose, HbA1c%, C-peptide, Anti GAD65, Anti IA2 and Anti IAA. SPSS 25.0 was used for statistical analysis.

Results: Mean age of the Demographic details of study participants was 14.2±3.6years. Majority of the study participants were male (57%). Mean height was 4.89±0.69feet, mean weight of the participants was 57.8±18.0 Kilograms, mean BMI was 27.0±7.7kg/m2and mean Fasting blood glucose level was 213.3 ±128.2 mg/dL. Majority of the participants (57%) belonged to middle socioeconomic class, had normal BMI with a poor glycemic control. When means were compared, it was found that there was a significant difference in the mean anti-GAD level, where group with poor glycemic control having higher values.

Conclusion: Anti-GAD65 is the most prevalent immune biomarker in the Pakistani population, with elevated levels linked to poor glycemic control. While low socioeconomic status correlates with worse glycemic outcomes. A targeted approach for high-risk populations may enhance clinical outcomes and alleviate financial and mental burdens for patients.

Keywords: Type 1 diabetes mellitus, GAD65, IA-2, IAA, Biomarker

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INTRODUCTION

Globally, the incidence of diabetes mellitus is rapidly on the rise. People having diabetes are expected to increase from 425 million in 2017 to 629 million in 2045, and in low- and middle-income countries like Sudan, Pakistan, India, Sri Lanka, Azerbaijan and Armenia, 79% rise is estimated [1]. After China and India, Pakistan is ranked third in the prevalence of diabetes where around 33 million people are enduring with diabetes [2]. With an enhancing incidence of 2-5% annually worldwide, Type 1 diabetes is affecting a large number of individuals mostly targeting people of under 19 years of age. A wide variety is seen worldwide as some portions of the world have a very high incidence than others. One description of it can be a correlation between genetic and environmental factors [3, 4]. Type 1 diabetes is observed as one of the most frequent dreadful childhood diseases. It develops mostly in children and adolescent populations, although any age group can present with type 1 diabetes [2].

Interactions between multiple genes, environmental factors &the immune system of

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body results in autoimmunity which pave the way for the destruction of the insulin producing β cells of the pancreas that cause Type 1 diabetes. Child becomes symptomatic after going through two asymptomatic stages. Up to 70-80% of the pancreatic cells have already been damaged at this specific stage. Hypoglycemia and diabetic ketoacidosis are the most frequent acute complications and long term micro- and macro-vascular complications results from bad control which remarkably affects quality of life and public health care costs. Future results can be predicted and prevented by intervening in lengthy latent (asymptomatic) phase [2,4,5,6].

To mark & differentiate between diverse types of diabetes sometimes numerous tests may be required. To diagnose Type 1 Diabetes specially in the asymptomatic phase the role of autoantibodies has been well accepted [3,7]. Most of the time these autoantibodies are not able to cope with the diverseness inherent to type 1 diabetes progression [8]. For bulk of the people with type 1 diabetes, access to superior treatment options is difficult in the developing countries like Pakistan [9]. Therefore, these persons are sensitive to acute and chronic complications of T1D influencing their quality of life [10].

No study has been conducted in our population to look for the differences in immune biomarkers among various groups based on factors like age and gender. This study is designed to explore whether there are variations in immune biomarker levels among these groups and if categorizing into groups can help pinpoint those at a higher risk.

Inspite of the fact that we are dealing a huge number of patients with Type 1 Diabetes (T1D) in Pakistan, but sadly the documentation of local data about the role of immune biomarkers in early diagnosis of Type 1 diabetes with the impact of demographic variables on disease is not yet available. Categorizing individuals by their age, gender, and other traits and examining how these factors affect the lab tests can aid in early diagnosis. Early management can prevent the later severe complications. Moreover, tailoring treatment plans for various age groups and socio-economic classes can lead to more effective disease management. This study focused to find association between different demographic variables and immune biomarkers in T1D so that disease can be detected at an early stage if persons are classified properly.

MATERIAL AND METHODS

This study was conducted at the Chughtai Institute of Pathology in a duration of one year (from 1st April 2021 to 31st March 2022). The research work was initiated after approval from the Institutional Ethical Review Board (IRB # CIP / IRB / 1064 B, approval date: 24-02-2021). It was a cross-sectional analytical study in which both male and female subjects < 18 years of age were included. Participants were included after explaining the nature of the study to their parents/guardians and informed consent was taken. We enrolled 130 male and female diagnosed cases of T1D of age below18 years in this study. A total of 100 cases were included in the study as per defined criteria and 30 were excluded [11]. 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 data and findings of other authors, the proportion of type-1 diabetes in the population is 10 % with e = 5% and 90% confidence level. By using this value in formula, the minimum sample size is 98 was obtained. Therefore, in this study 100 patients easily fulfilled our criteria of sample size. Individuals taking anti hyperlipidemic treatment, individuals with acute infections, undergoing surgery or admitted in intensive care units/high dependency units were excluded from the study. Also, the individuals whose parents/guardians did not give enough information about the disease history and demographic variables also excluded from the study. Relevant details of all cases i.e., age, gender, BMI, socio-economic status and medical history were noted on a predesigned proforma. After noting down the

required information, 5ml whole blood was taken from each subject and centrifuged at 1500 g for 5 minutes.

All samples were analyzed for following parameters: GAD 65 Ab: (Sandwich Chemilumine-scence Immunoassay), IAA: (Sandwich Chemiluminescence Immunoassay) and IA-2 Ab:(Sandwich Chemiluminescence Immunoassay) All above mentioned tests were performed on fully automated immunoassay analyzer (Maglumi- Snibe). Data was analyzed via SPSS version 23.0. Frequencies and percentages were calculated for demographic variables such as gender, socioeconomic status, BMI categories, glycemic control, blood pressure, symptoms, and family history. Mean and standard deviation were calculated for continuous variables including age, height, weight, BMI, and fasting blood glucose level. Normality of data was assessed using the Shapiro-Wilk test. One-way ANOVA was used to compare means of immune biomarkers (Anti GAD, AntiIA2, IAA) across different groups (gender, socioeconomic status, BMI categories, glycemic control, blood pressure, symptoms, family history). Independent sample t-test used to compare means of immune biomarkers between two groups (e.g., gender). The Mann-Whitney U test was used for comparing means between two groups when data did not follow a normal distribution, while the Kruskal-Wallis test was used as a non-parametric alternative to ANOVA for more than two groups. Both tests were employed when data did not meet the assumptions for parametric testing, with a p-value of less than 0.05 considered statistically significant.

RESULTS

Mean age of the Demographic details of study participants was 14.2 ±3.6 years. Majority of the study participants were male (57%). Mean height was 4.89±0.69 feet, mean weight of the participants was 57.8±18.0 Kilograms, mean BMI was 27.0±7.7kg/m2and mean Fating blood glucose level was 213.3 ±128.2 mg/dL. Mean Values of immune biomarkers in the study is given in Table-I.

Distribution of cases in different study groups is given in table-II. This distribution shows that majority of the participants (57%) belonged to middle socioeconomic class, had normal BMI with a poor glycemic control. The details are given below.

Mean values of all the chemical biomarkers were analyzed in each group separately to the means were compared to see if any significant difference was present among the groups or not. When means of biomarker levels were compared on the basis of glycemic control, it was found that there was a significant difference in the mean anti-GAD level, where group with poor glycemic control having higher values. There was no significant difference in the immune biomarker levels when values among the groups were compared using ANOVA and independent sample T-test. Non-parametric tests like Mann-Whitney and Kruskal-Wallis were used for data that was not normal. Mean and IQR were used to depict non-parametric data. Variable wise distribution on immune biomarkers along with the p values are given in Table-III.

Table-I: Mean values of immune biomarkers.

Immune Biomarker	Mean	Interquartile range
Anti GAD	64.19	73
AntiIA2	23.05	9
IAA	12.14	10

Table-II: Distribution of participants in different study groups.

Group	Frequency (Percentage)
Gender	
Male	57%
Female	43%
Socioeconomic Status	
Lower	11%
Middle	57%
Upper	32%
BMI	
Underweight	8%
Normal weight	41%
Over weight	22%
Obese	29%
Glycemic Control	
Poor control	84%
Average control	12%
Good control	4%
Blood Pressure	
Low BP	64%
Normal BP	36%

Table-III: Comparison of mean values of Chemical Biomarkers analyzed in the study among all groups.

Variable	Anti GAD	AntiIA2	IAA
Gender			
Male	75.8	18.9	11.2
Female	48.8	28.4	13.2
p-value	0.129	0.38	0.383
Socioeconomic Status			
Lower	68.2	9.5	10.1
Middle	48.8	32.03	11.7
Upper	89.7	11.5	13.4
p-value	0.106	0.15	0.665
BMI			
Underweight	32.8	42.3	16.8
Normal weight	83.8	17.2	11.01
Overweight	58.6	19.9	10.3
Obese	49.6	28.4	13.7
p-value	0.27	0.594	0.416
Glycemic control			
Good control	7.6	11.5	12.7
Average control	16.7	32.29	8.7
Poor control	73.8	22.3	12.5
p-value	0.04	0.752	0.569
Blood Pressure			
Low	64.1	19.9	12.8
Normal	64.3	28.4	10.9
p-value	0.99	0.45	0.437
Symptoms			
No	68.5	20.7	11.5
Yes	50.7	30	14
p-value	0.116	0.173	0.203
Family History			
Negative	64.49	20.8	12.39
Positive	63.8	25.2	11.8
p-value	0.05	0.412	0.316

Interpretation:

- Significant differences in Anti GAD levels were found across groups categorized by glycemic control ($p = 0.04$), indicating higher levels in those with poor control.
- No significant differences were observed in AntiIA2 and IAA levels across any of the groups analyzed (all $p > 0.05$).

DISCUSSION

In type 1 diabetes, there is a loss of beta cells due to autoimmune processes. Destruction of these cells results in loss of endogenous insulin production which is reversible, demanding the daily administration of insulin from outside of the body. Infection or environmental factors stimulate the immune system of those people who already have genetic susceptibility. The role of both humoral and cellular is let autoimmunity has been established [1].

Autoantibodies in type 1 diabetes have been broadly accepted as the hallmark of the disease by the scientific society. The combination of all of these autoantibodies would be a stronger and confident diagnostic measure for the patients having type 1 diabetes. Their role has been established as biomarkers of the pre-symptomatic stage of the disease [1,2].

This can be seen in our study that autoantibodies like Anti-GAD65 antibodies, Anti- IA2 antibodies, and Insulin autoantibodies are positive in almost all of the cases of type 1diabetes. This shows a strong relationship between these immune biomarkers and type1diabetes.One can confidently go for these kinds of immune biomarkers as a screening tool for the initial workup of suspected cases. In fact, it is highly encouraging practice to ask for these tests for the early intervention to minimize the disease progression and related complications. So far, the Pakistani data was not available for such type of relationship between the immune biomarkers and the type 1 diabetes. Our study has been managed to fill the gap in this particular area and successfully established the role of these immune biomarkers in diseased cases as we commonly see in other races across the globe.

The age of autoantibody development can also be used to stratify individuals with regard to the likelihood of quick progression to clinical diabetes, with more rapid disease progression being observed in children who develop islet autoantibodies early. Children who develop autoimmunity in the second decade of life or later mostly present with GAD auto antibodies earlier than any other immune biomarker [2].

In our study, the male population had a predominance over female cases. Although this is not a prevalence study, it has been noted that diabetes prevalence differs by gender depending on the study setting and the study population. There is no confident data available on gender difference in type 1 diabetes mellitus. This disease affects males more frequently than females but this may be change in different communities [3]. So, in our study no specific correlation has been found between gender difference and status of immune biomarkers. Patients of type 1 diabetes from low socioeconomic class, specifically those with low wages and less education, were more expectedly to suffer from type 1 diabetes related complications and comorbidities [4]. In our study no such type of correlation has been found with immune biomarkers. Autoantibodies are positive in every socioeconomic class of patients. Although persons of the upper class could manage their disease effectively to avoid future complications.

In the context of type 1 diabetes, several research have looked at the association between auto-antibodies and BMI. According to several studies, having a higher BMI may increase your likelihood of getting auto-antibodies and ultimately type 1 diabetes. To demonstrate a clear and consistent association between these parameters, more research is required as the results so far have been contradictory [5]. In our study there is no such kind of a relationship found and the patients even with normal BMI shows positive results for autoantibodies.

The presence of diabetes auto antibodies affects the HbA1c level and the total number of insulin units used per day by the patients; the more diabetes autoantibodies are present, the higher the HbA1c level, the more insulin units that patients need to control their blood glucose levels [6]. In our study, it is found that almost all of the patients with positive GAD65 autoantibodies have poor glycemic control meaning by that they have high values of HbA1c and Fasting Blood Sugar levels. HbA1c in our study was a marker of glycemic control whereas Fasting Blood Sugar is strongly correlated with HbA1c.

A statistically convincing relationship has been found between positive immune biomarkers and osmotic symptoms of type 1 diabetes. But this relationship is weak in childhood and more pronounced in adulthood. We studied the demographic and clinical features in terms of biomarkers in patients with T1DM.In our study, which includes patients of less than 18 year of age, no significant clinical findings had been located in type 1 diabetes cases. Even most of the cases with positive autoantibodies had no clinical presentation at that time [7].

International literature supports the fact that children who were autoantibody positive and progressed to type 1 diabetes had at least one relative with type 1 diabetes [8]. In our study population, no such relation established and results are evenly distributed among patients with positive family history and those with no such history. Overall anti- GAD65 positivity was more than any other type of biomarker.

CONCLUSION

Anti-GAD65 is identified as the most prevalent immune biomarker in the Pakistani population. Elevated levels of immune biomarkers are associated with poor glycemic control. Although low socioeconomic status correlates with worse glycemic control, no significant differences are found in Anti-IA2 and IAA levels across groups. Implementing a targeted approach for high-risk populations may improve

clinical outcomes and reduce both financial and mental burdens for patients.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

AUTHORS CONTRIBUTION

Hafiz Muhammad Bilal: Conceptualization, manuscript writing, study design, data collection, accountable for all aspects of the work

Muhammad Dilawar Khan: Overall supervision, final approval of the work, accountable for all aspects of the work

Hijab Batool: Data collection, accountable for all aspects of the work

Akhtar Sohail Chughtai: Critical review, accountable for all aspects of the work

Omer Rashid Chughtai: Revisions, proofread, accountable for all aspects of the work

Shakeel Ashraf: Data analysis, accountable for all aspects of the work

REFERENCES

1. Bavuma CM, Musafiri S, Rutayisire PC, Ng'ang'a LM, McQuillan R, Wild SH. Socio-demographic and clinical characteristics of diabetes mellitus in rural Rwanda: time tocontextualize the interventions? A cross-sectional study. BMC Endocrine Disorders. 2020; 20: 1-0. DOI: https://doi.org/10.1186/s12902-020-00660-y
2. Magliano DJ, Boyko EJ; IDF Diabetes Atlas 10th edition scientific committee . IDF DIABETES ATLAS [Internet]. 10th edition. Brussels: International Diabetes Federation; 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK581934/
3. Mobasseri M, Shirmohammadi M, Amiri T, Vahed N, Fard HH, Ghojazadeh M. Prevalence and incidence of type 1 diabetes in the world: a systematic review andmeta-analysis. Health Promot Perspect. 2020; 10(2):98. DOI: https://doi.org/10.34172%2Fhpp.2020.18
4. Giwa AM, Ahmed R, Omidian Z, Majety N, Karakus KE, Omer SM, *et al.* Current understandings of the pathogenesis of type 1 diabetes: Genetics to environment. World J Diabetes. 2020; 11(1): 13-25. DOI: https://doi.org/10.4239/wjd.v11.i1.13
5. Purohit S, She JX. Biomarkers for type 1 diabetes. Int J Clin Exp Med. 2008;1(2): 98.
6. Primavera M, Giannini C, Chiarelli F. Prediction and prevention of type 1 diabetes. Front Endocrinol. 2020; 11: 248. DOI: https://doi.org/10.3389/fendo.2020.00248
7. Yi L, Swensen AC, Qian WJ. Serum biomarkers for diagnosis and prediction of type 1 diabetes. Transl Res. 2018; 201: 13-25. DOI: https://doi.org/10.1016/j.trsl.2018.07.009
8. Mathieu C, Lahesmaa R, Bonifacio E, Achenbach P, Tree T. Immunologicalbiomarkers for the development and progression of type 1 diabetes. Diabetologia. 2018; 61: 2252-8. DOI: https://doi.org/10.1007/s00125-018-4726-8
9. Global report on diabetes [Internet]. World Health Organization. World HealthOrganization; [cited 202 3 Mar 31]. Available at: https://www.who.int/publications-detail-redirect/9789241565257
10. Fawwad A, Ahmedani MY, Basit A. Integrated and comprehensive care of peoplewith type 1 diabetes in a resource poor environment-"Insulin My Life (IML)" project. Int J Adv Res. 2017; 5(4): 1339-42. DOI: http://dx.doi.org/10.21474/IJAR01/3952
11. Hamadi GM. Immunological markers in type 1 diabetes mellitus in Thi-Qar province, southern Iraq. J Med Life. 2022; 15(10): 1234-9. DOI: https://doi.org/10.25122/jml-2021-0387
12. Mathieu C, Lahesmaa R, Bonifacio E, Achenbach P, Tree T. Immunologicalbiomarkers for the development and progression of type 1 diabetes. Diabetologia. 2018; 61: 2252-8. DOI: https://doi.org/10.1007/s00125-018-4726-8
13. Tatti P, Pavandeep S. Gender difference in type 1 diabetes: An underevaluated dimension of the disease. Diabetol.2022; 3(2): 364-8. DOI: https://doi.org/10.3390/diabetology3020027
14. Talbo MK, Katz A, Dostie M, Legault L, Brazeau AS. Associations between socioeconomic status and patient experience with type 1 diabetes management and complications: Cross-sectional analysis of a cohort from Québec, Canada. Can J Diabetes. 2022; 46(6): 569-77. DOI: https://doi.org/10.1016/j.jcjd.2022.02.008
15. Martin C, Boudaoud AA, Poghosyan T, Zhu J, Larger E, Greenfield JR, *et al*. Prevalence of anti-GAD and IA2 autoantibodies in a French cohort of patients with diabetes eligible for bariatric surgery. Diabetes Metabol. 2020; 46(5): 407-9. DOI: https://doi.org/10.1016/j.diabet.2019.12.004
16. Hamed MS, Samy M, Mahmoud H, Yehia N. Study of the difficult glycemic control inrelation to the presence of diabetes-autoantibodies in a sample of

Egyptians with type 1 diabetes. Diabetes Res Clin Pract. 2019; 152: 53-7.
DOI: https://doi.org/10.1016/j.diabres.2019.04.026

17. Bravis V, Kaur A, Walkey HC, Godsland IF, Misra S, Bingley PJ, *et al*. Relationship between isletautoantibody status and the clinical characteristics of children and adults with incident type 1 diabetes in a UK cohort. BMJ Open. 2018; 8(4): e020904.
DOI: https://doi.org/10.1136/bmjopen-2017-020904

18. Kuusela S, Keskinen P, Pokka T, Knip M, Ilonen J, Vähäsalo P, *et al*. Extendedfamily history of type 1 diabetes in HLA-predisposed children with and without islet autoantibodies. Pediatr Diabetes. 2020; 21(8): 1447-56.
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Comparison of disease activity by disease activity score-28 C-reactive protein and disease activity score-28 erythrocyte sedimentation rate in established rheumatoid arthritis patients – A comparative study

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ABSTRACT

Objective. To compare disease activity in patients of established Rheumatoid Arthritis estimated by Disease Activity Score-28 (DAS-28) C-reactive protein (CRP) and Disease Activity Score-28 Erythrocyte Sedimentation Rate (ESR).

Material and Methods: This cross-sectional comparative study was conducted at Department of Rheumatology, Pak Emirates Military Hospital Rawalpindi, May 2022 to Oct 2022. Using consecutive non probability sampling, patients of Rheumatoid Arthritis (RA) of 30 to 65 years age of either gender were selected who had the disease for at least 1 year, never received biologics Disease modifying anti-rheumatic drugs (DMARDs) or immunosuppressive therapy and no signs of active infective etiology. DAS28 score was calculated using ESR and CRP to assess disease severity. Sensitivity, specificity, and agreement comparison was done between DAS28-ESR and DAS28-CRP and κ -coefficient was calculated with discordance proportion.

Results. Out of 70 patients, 50 (70%) were female and 20 (28%) were male with mean age of included patients 49.9 ± 7.5 years. Mean disease activity score, calculated using ESR was 4.1 ± 1.25 SD was higher than mean DAS28 score of 3.5 ± 1.12 SD with CRP. Twenty (28.6%) patients had High Disease Activity (HDA) (DAS28 > 5.1) when assessed by DAS28-ESR score as compared to 8 (11.4%) patients by DAS28-CRP score with 17.1% discordance and κ Coefficient of 0.402 corresponding to minimal agreement amid DAS28-ESR and DAS28-CRP for HDA ($p < 0.005$). DAS28 score using ESR as evaluating tool had 35% sensitivity and 98% specificity of detecting RA patients with High Disease Activity.

Conclusion. DAS28-ESR was preferable as compared to DAS28-CRP for monitoring disease activity and treatment decision.

Keywords. C-reactive protein (CRP), Disease activity score (DAS), Erythrocyte sedimentation rate (ESR), Joint pain, Rheumatoid arthritis, Visual analogue scale (VAS)

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INTRODUCTION

Rheumatoid arthritis (RA), a chronic inflammatory autoimmune illness primarily involving joints as idiopathic symmetrical peripheral polyarthritis which affects large as well as small joints of body, causing deformity due to stretching of tendons and ligaments [1]. The overall worldwide prevalence of RA is 1.0 - 1.5% in 3:1 women to men ratio [2]. It progresses from distal to proximal joints causing marked incapacity within 10-20 years after initial presentation in untreated patients [3]. It causes major health risks including higher rates of cardiovascular, pulmonary disease, osteoporosis, and certain types of cancer (e.g. lymphoma) specifically in untreated cases or in patients with poor response to therapy [4]. Moreover, uncontrolled inflammation and joint destruction adversely affect the quality of life causing physical function loss, inability to do routine life activities and shortened life expectancy [5]. According to 2010 American

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College of Rheumatology (ACR) / European Alliance of Associations for Rheumatology (EULAR) classification criteria, definite RA is classified in the patients with manifestation of synovitis in at least one joint, without any alternative diagnosis to explicate synovitis, and criterion score of at least 6 out of 10, from the discrete scores in four features including number and site of affected joints, Rheumatoid Factor (RF) or anti-cyclic citrullinated protein (ACPA) levels, acute phase reactants (including CRP and ESR) and symptoms duration [6].

The Disease Activity Score 28 (DAS28) is among many indices approved by the American College of Rheumatology (ACR) to monitor RA disease severity [7]. The DAS28 is assessed by adding individual score in: tender joints count; swollen joints count; global health score - calculated by visual analogue scale (VAS) and erythrocyte sedimentation rate (ESR) or alternatively Quantitative C-reactive protein (CRP-Q) value [8]. The ESR is an acute phase reactant of inflammation and its level represents disease activity and severity in the earlier weeks and helpful in monitoring the response to therapeutic modality. ESR value is subjective to certain factors like old age, gender, pregnancy, malignancy, fibrinogen levels, hyper-gammaglobulinemia, RF, anemia and polycythemia [9]. Moreover, despite these confounding factors, raised ESR in early RA is predictor and indicator of intense radiographic damage of affected joints in succeeding years despite of treatment with conservative disease-modifying anti-rheumatic drugs (DMARDs). Due to high sensitivity, ESR is believed to be used as marker for disease monitoring but low specificity makes it poor marker for diagnosis [9]. CRP is advocated as measure of RA disease activity and reflect short term changes. CRP levels are not subjective to aforesaid factors, therefore sensitive to disease activity changes. It is noteworthy that ESR and CRP values fluctuate with ongoing pathophysiological processes, hence, DAS28-CRP threshold standards are anticipated to vary from DAS28-ESR [10].

In South Asian countries including Pakistan, Qualitative and Semi-Quantitative CRP test is available and being used to monitor disease activity in primary health care setups instead of Quantitative (CRP-Q) and high sensitivity (hs-CRP) which are more sensitive in assessing joint inflammation and is closest to age adjusted ESR values [11]. Hence, it has been observed in repeated research data that, ESR is considered preferred marker to assess disease activity as compared to CRP as ESR is cost effective 900-1350 PKR (average $3 - $4.5) and readily available as compared to CRP-Q which is a bit costly 1500-2700 (average $5 - $9).

It has been noted from the data and studies that there is discordance between DAS28 scores assessed by ESR and CRP values. The rationale of our study was to observe and assess disease severity and activity in local population by comparing DAS28-ESR and DAS28-CRP scores in the light of standard values of disease activity in relation to clinical disease severity.

MATERIAL AND METHODS

This cross-sectional comparative study was conducted in Rheumatology Department of Pak-Emirates Military Hospital, Rawalpindi after seeking approval from Institutional Ethical Committee (IEC-A/28/EC/391/2022). Sample size was 70, calculated with WHO calculator keeping confidence interval 95%, margin of error 5%, and reported global prevalence of RA 0.5-1.0% [12]. Sampling was done by consecutive non-probability method.

Patients of either gender aged 30 to 65 years with diagnosed RA since at least 1 year, treated with low dose steroids (< 10mg Prednisone) or non-biologic DMARDs, no associated underlying comorbid condition and no active infection were selected for study.

RA patients with age < 30 and > 65 years, associated anemia or polycythemia and other autoimmune disease i.e. Sjogren`s syndrome, Systemic Sclerosis, Osteoarthritis, used high dose steroids (> 10mg Prednisone),

received biologic DMARDs or immune-suppressive therapy, history of infective illness active or in preceding 1-month, chronic liver or kidney disease, pregnancy, BMI > 30 kg/m^2, terminal illness or malignancy were all excluded from this study.

The study was conducted after taking informed consent from all patients included in the study. Patients with diagnosed RA were advised base line investigation including CRP and ESR on outdoor basis and DAS28 score was calculated by measuring both ESR and CRP levels. Patients who had recently received high dose steroids or previously treated with biologic DMARDs or immunosuppressive therapy were dropped from study as immunosuppressive agents can reduce or alter the ESR or CRP disproportionately without controlling overall disease activity. The activity of disease is assessed by DAS28 score and categorized as high disease activity (HDA) with DAS28 score > 5.1, moderate disease activity (MDA) score > 3.2 to < 5.1, low disease activity (LDA) score > 2.6 to < 3.2 and remission with score of < 2.6 [9].

Patient`s age, gender, disease duration, ESR and CRP values, and DAS28 scoring were noted in all patients for analysis. Categorical data were presented as numbers and percentages whereas continuous variables were as mean ± SD. Data were analyzed using Statistical Package for Social Sciences version 23 (SPSS v 23). The normality of data was tested by the Kolmogorov-Smirnov test. For analysis, the t-test was used, sensitivity and specificity for detecting high disease activity was calculated, agreement level between DAS28-ESR and CRP was calculated with κ -coefficient and discordance proportion. A p-value of ≤ 0.05 was taken as a statistically significant.

RESULTS

A total of 70 patients with established RA were included in study. Amongst them, 50 (70%) were females and 20 (28%) were males. Mean age of patients was 49.9 ± 7.5 years. Disease duration was also noted by detailed history and previous documentation which showed that 34 (48.6%) had RA since 1-3 years, 30 (42.9%) had RA for last 3-5 years and 6 (8.6%) had disease for > 5 years. ESR measurements showed that ESR were raised (> 20 mm/hour) in 61 (87.1%) patients and 9 (12.9%) had ESR in normal range whereas CRP were raised (> 10mg/L) in 62 (88.6%) patients and 8 (11.4%) had CRP in normal range. It was appreciated in final analysis that mean DAS28 score using ESR was 4.1 ± 1.25 SD which is higher than mean score 3.5 ± 1.12 SD using CRP levels. Out of total 70 patients in our study, 20 (28.6%) met the criteria for HDA (DAS28 > 5.1), 28 (40.0%) were in MDA (DAS28 > 3.2 to < 5.1), 17 (24.3%) were in LDA (DAS28 2.6 - 3.2) and 5 (7.1%) were in remission (DAS28 < 2.6) when assessed using DAS28-ESR score. On other hand, when disease activity calculated by DAS28-CRP, fewer were in HDA 8 (11.4%) and more in remission 13 (18.6%) in comparison to DAS28-ESR score (Table-I & Figure-I).

Multivariate analysis of DAS28 scores was done by assessing DAS score using ESR and CRP levels in relation with age, gender and duration of disease. It was observed that out of 70 selected patients, 35 (50%) were of < 50 years age, among which more patients were in high disease activity (HDA) in DAS28 ESR score as compared to DAS28 CRP 7 (20.0%) versus 1 (2.8%) respectively (Table-II, III).

It was also seen that DAS28-CRP score showed out of 35 (50%) patients of < 50 years age more patients 10 (28.6%) were categorized in remission (DAS28 < 2.6) as compared to 3 (8.5%) when assessed using DAS28-ESR. (Table-II, III)

DAS28 score showed that more patients 20 (28.6%) were in high disease activity (> 5.1) using DAS28-ESR as compared to DAS28-CRP 8 (11.4%) patients with 17.1% discordance and κ Coefficient of 0.402, corresponding to minimal agreement proportion between DAS28 ESR > 5.1 and DAS28 CRP > 5.1 indication high

disease activity among RA patients ($p < 0.005$) (Table-IV).

Sensitivity and specificity of DAS28 scoring using ESR and CRP was done for detection patients with high disease activity (HDA). It was observed that DAS28 score using ESR as evaluating tool had 35% sensitivity and 98% specificity of detecting RA patients with HDA

Table-I: Basic parameters of studied patients (70).

Parameter		Results n (%)
Age (mean years ± SD)		49.9 ± 7.5
Gender	Female	50 (71.4%)
	Male	20 (28.6%)
DAS28-ESR	HDA (>5.1)	20 (28.6%)
	MDA (> 3.2 to < 5.1)	28 (40.0%)
	LDA (> 2.6 to < 3.2)	17 (24.3%)
	Remission (< 2.6)	5 (7.1%)
DAS28-CRP	HDA (>5.1)	8 (11.4%)
	MDA (> 3.2 to < 5.1)	25 (35.7%)
	LDA (> 2.6 to < 3.2)	24 (34.3%)
	Remission (< 2.6)	13 (18.6%)

Table-II: Disease activity by DAS28-ESR with relation to disease duration.

		DAS28-ESR			
	Severity →	**HDA > 5.1**	**MDA: > 3.2 - < 5.1**	**LDA: > 2.6 - < 3.2**	**Remission < 2.6**
Duration of RA	**1 - 3 years** 34/70 (48.6%)	9 (26.5%)	10 (29.4%)	11 (32.4%)	4 (11.8%)
	3 - 5 years 30/70 (42.9%)	8 (26.7%)	15 (50.0%)	6 (20.0%)	1 (3.3%)
	> 5 years 6/70 (8.6%)	3 (50.0%)	3 (50.0%)	0	0

Table-III: Disease activity by DAS28 CRP with relation to disease duration.

		DAS28-CRP			
Parameter	**Severity →**	**HDA > 5.1**	**MDA: > 3.2 - < 5.1**	**LDA: > 2.6 - < 3.2**	**Remission < 2.6**
Duration of RA	**1 - 3 years** 34/70 (48.6%)	2 (5.9%)	12 (35.3%)	9 (26.5%)	11 (32.4%)
	3 - 5 years 30/70 (42.9%)	5 (16.7%)	11 (36.7%)	12 (40.0%)	2 (6.7%)
	> 5 years 6/70 (8.6%)	1 (16.7%)	2 (33.3%)	3 (50.0%)	0

Table-IV: Agreement comparison of DAS28 ESR and DAS28 CRP.

Total (n)	DAS28 ESR > 5.1 n (%)	DAS28 CRP > 5.1 n (%)	Discordance Proportion n (%)	κ - Coefficient	p-value
70	20/70 (28.6%)	8/70 (11.4%)	12/70 (17.1%)	0.402	< 0.005

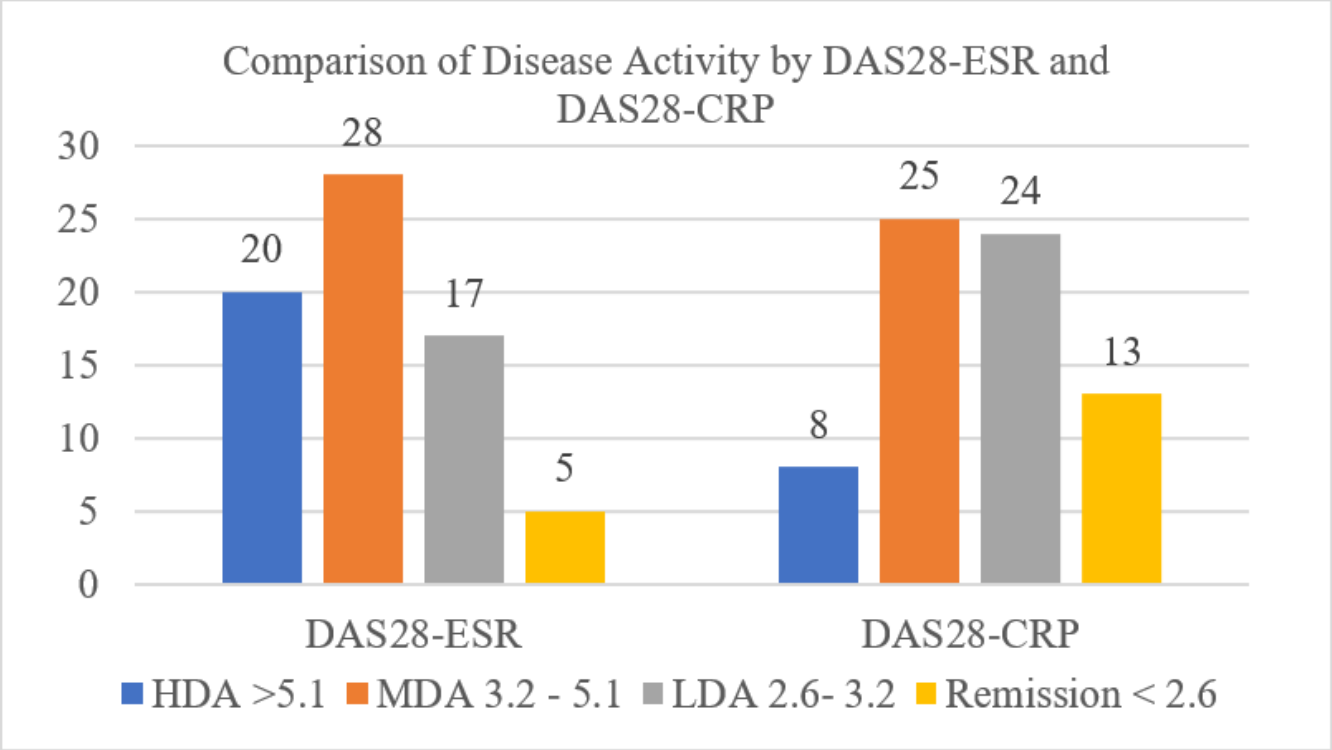

Figure-I: Comparison of disease activity by DAS28-ESR and DAS28-CRP.

DISCUSSION

RA is a chronic debilitating disease and its outcome is meticulously correlated to disease activity, which is usually assessed by markers including CRP and ESR [13]. Therefore, disease activity is consistently assessed in RA patients in decision making regarding treatment modality and assessing therapeutic response and efficacy of routinely used therapeutic approach and in clinical trials [14]. Our study results also showed that more patients fall into high disease activity when assessed by DAS28-ESR in comparison to patients assessed by DAS28-CRP with discordance proportion of 12 (17.1%). In a review study directed by Greenmyer *et al* [2020, North Dakota] concluded that 48.5% patients fall into criteria for HDA measured by DAS28-ESR compared to 14.6% patients in DAS28-CRP score with 33.9% discordance [15]. In a study conducted in Agha Khan University Hospital Karachi done by Nasir *et al* [2022, Pakistan], it was concluded that DAS28-ESR is preferred choice when assessing disease activity for initiation or maintaining therapy when combined with modified Health Assessment Questionnaire (mHAQ) [16].

In our study it was seen that there was minimal proportion of agreement between DAS28 score in detecting high disease activity (> 5.1) using DAS28-ESR as compared to DAS28-CRP with κ Coefficient of 0.402, and 17.1% discordance. Also it was observed in this study that using DAS28-ESR as evaluating tool had 35% sensitivity and 98% specificity of detecting RA patients with HAD. In a comparative study done by Kuriya *et al* [2014, Canada] and published in Clinical and Experimental Rheumatology Journal it was highlighted that intended standards for DAS28-CRP were lower for remission, LDA and HDA with 2.5, 2.9 and 4.6 respectively and showing moderate level agreement with DAS28-ESR values (κ = 0.70) [17]. Similarly, in another study by Shivacheva *et al* [2020, Bulgaria] it was concluded that there was low level of agreement (κ = 0.235-0.464) and discrepancy between DAS28-ESR and DAS28-CRP when estimating activity of disease [18].

As high levels of circulating CRP in RA patients usually associated with disease activity and directly proportional to each other. Therefore, CRP levels declines with treatment representing low disease activity as explained by Pope *et al* [2021, UK] in his study that CRP is a regulator of systemic inflammation in RA that appears to play a role in pathological effects of RA. Also, reducing levels of CRP with DMARDs treatment contribute towards reduction in disease activity [19].

It was observed in our study that sensitivity and specificity of DAS28-ESR for detecting patients with high disease activity was 35 % and 95% respectively. Similarly, Hamann *et al* [2019, UK] reported that DAS28-CRP was 0.3 points lower than the DAS28-ESR for corresponding cohort when estimating activity of disease [20]. Moreover it is has been explained in a study by Hensor *et al* [2010, UK] that age-adjusted ESR (age/2 for male and age + 10/2 for female) and age-adjusted CRP (Q) (age/50 for male and age/50 + 0.5 for female) can be applied to correlate DAS28-ESR and DAS28-CRP more accurately to disease activity simultaneously [21]. Ranganath *et al* [2005, California] concluded in a study that apparent discrepancy between DAS28-ESR and DAS8-CRP scores can be minimized or removed using age adjusted values for ESR and CRP [22].

CONCLUSION

DAS28-CRP score was pointedly lower and underestimated the patients at high and moderate end of disease activity who would be undertreated whereas in actual they could have been in need of aggressive treatment. Similarly, DAS28-CRP score overestimated patients in remission who would actually need maintenance or up titration of therapy. DAS28-ESR is preferable while assessing disease activity for treat-to-target approach. Hence, ESR would be used to assess and monitor RA disease activity for treatment optimization, as it is available even at smaller setups.

LIMITATIONS

The authors of this study are well aware of its limitations, the most important being the single center study and limited sample size. Also, results could have been different, if high sensitivity CRP (hsCRP-Q) and age-adjusted ESR had been used as activity markers.

RECOMMENDATION

Further studies like RCTs including different populations of different ethnic group are required for more accurate results and comparison due to genetic polymorphism in each population. In addition, effect of anemia, co-existing infection and systemic disease on RA disease should be observed before implication of results on larger scales.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Amna Butt: Conception, study design, data acquisition, manuscript writing, analysis and interpretations, final approval, accountable for all aspects of the work

Khalid Raja, Fahad Javed Awan: Conception and study design, Analysis and interpretation, critical review, final approval, accountable for all aspects of the work

Wajahat Ahmed Khan: Analysis and interpretations, final approval, accountable for all aspects of the work

Farhan Zaid: Conception, data acquisition, critical review, accountable for all aspects of the work

Fahad Ul Hassan: Study design, Analysis and interpretation, final approval, accountable for all aspects of the work

REFERENCES

1. Weyand CM, Goronzy JJ. Immunometabolism in the development of rheumatoid arthritis. Immunol Rev. 2020; 294(1): 177–87. DOI: https://doi.org/10.1111/imr.12838
2. Giannini D, Antonucci M, Petrelli F, Bilia S, Alunno A, Puxeddu I. One year in review 2020: Pathogenesis of rheumatoid arthritis. Clin Exp Rheumatol. 2020; 38(3): 387-97. DOI:https://doi.org/10.55563/clinexprheumatol/3uj1ng
3. Littlejohn EA, Monrad SU. Early diagnosis and treatment of rheumatoid arthritis. Prim Care. 2018; 45(2): 237–55. DOI: https://doi.org/10.1016/j.pop.2018.02.010
4. Curtis JR, Flake DD, Weinblatt ME, Shadick NA, Østergaard M, Hetland ML, *et al*. Adjustment of the multi-biomarker disease activity score to account for age, sex and adiposity in patients with rheumatoid arthritis. Rheumatology. 2019; 58(5): 874–83. DOI: https://doi.org/10.1093/rheumatology/key367
5. Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: A Review. JAMA. 2018; 320(13): 1360-72. DOI: https://doi.org/10.1001/jama.2018.13103
6. Smolen JS, Landewé RBM, Bijlsma JWJ, Burmester GR, Dougados M, Kerschbaumer A, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Ann Rheum Dis. 2020; 79(6): 685–99. DOI: https://doi.org/10.1136/annrheumdis-2019-216655
7. Orr CK, Najm A, Young F, McGarry T, Biniecka M, Fearon U, *et al*. The utility and limitations of CRP, ESR and DAS28-CRP in appraising disease activity in rheumatoid arthritis. Front Med. 2018; 5: 185. DOI: https://doi.org/10.3389/fmed.2018.00185
8. Son KM, Lee SY, Seo YI, Choi JE, Kim HA. Contribution of subjective disease activity score 28 (DAS28) components to the response to treatment of rheumatoid arthritis. Clin Rheumatol. 2017; 36(6): 1221–27. DOI: https://doi.org/10.1007/s10067-017-3628-3
9. Fleischmann RM, Van Der Heijde D, Gardiner PV, Szumski A, Marshall L, Bananis E. DAS28-CRP and DAS28-ESR cut-offs for high disease activity in rheumatoid arthritis are not interchangeable. RMD Open. 2017; 3(1): e000382. DOI: https://doi.org/10.1136/rmdopen-2016-000382
10. Sengul I, Akcay-Yalbuzdag S, Ince B, Goksel-Karatepe A, Kaya T. Comparison of the DAS28-CRP and DAS28-ESR in patients with rheumatoid arthritis. Int J Rheum Dis. 2015; 18(6): 640–5. DOI: https://doi.org/10.1111/1756-185x.12695
11. Dessein PH, Joffe BI, Stanwix AE, Christian BF, Veller M. Glucocorticoids and insulin sensitivity in rheumatoid arthritis. J Rheumatol. 2004; 31(5): 867–74.
12. Black RJ, Cross M, Haile LM, Culbreth GT, Steinmetz JD, Hagins H, *et al*. Global, regional, and national burden of rheumatoid arthritis, 1990–2020, and projections to 2050: A systematic analysis of the Global Burden of Disease Study 2021. Lancet Rheumatol. 2023; 5(10): e594–610. DOI: https://doi.org/10.1016%2FS2665-9913(23)00211-4
13. Alam SM, Kidwai AA, Jafri SR, Qureshi BM, Sami A, Qureshi HH, *et al*. Epidemiology of rheumatoid arthritis in a tertiary care unit, Karachi, Pakistan. J Pak Med Assoc. 2011; 61(2): 123–6.
14. Listing J, Kekow J, Manger B, Burmester GR, Pattloch D, Zink A, *et al*. Mortality in rheumatoid arthritis: the impact of disease activity, treatment with glucocorticoids, TNFα inhibitors and rituximab. Ann Rheum Dis. 2015; 74(2): 415–21. DOI:https://doi.org/10.1136/annrheumdis-2013-204021
15. Greenmyer JR, Stacy JM, Sahmoun AE, Beal JR, Diri E. DAS28-CRP cutoffs for high disease activity and remission are lower than DAS28-ESR in rheumatoid arthritis. ACR Open Rheumatology. 2020; 2(9): 507–11. DOI: https://doi.org/10.1002/acr2.11171
16. Nasir N, Majid H, Khan A, Awan S, Riaz M. Disease activity correlates and functionality in patients with rheumatoid arthritis – real-world experience from a South Asian country. Reumatologia. 2022; 60(3): 183–91. DOI:https://doi.org/10.5114/reum.2022.117838
17. Kuriya B, Xiong J, Boire G, Haraoui B, Hitchon C, Pope J, *et al*. Earlier time to remission predicts sustained clinical remission in early rheumatoid arthritis — results from the canadian early arthritis cohort (CATCH). J Rheumatol. 2014; 41(11): 2161–6. DOI: https://doi.org/10.3899/jrheum.140137
18. Shivacheva TK. DAS28-ESR and DAS28-CRP - are they Interchangeable in measuring the activity of rheumatoid arthritis in response to treatment with biological agents? Folia Med. 2020; 62(1): 46–51. DOI: https://doi.org/10.3897/folmed.62.e47714
19. Pope JE, Choy EH. C-reactive protein and implications in rheumatoid arthritis and associated comorbidities. Semin Arthritis Rheum. 2021; 51(1): 219-29.

DOI: https://doi.org/10.1016/j.semarthrit.2020.11.005

20. Hamann PDH, Shaddick G, Hyrich K, Green A, McHugh N, Pauling JD, *et al*. Gender stratified adjustment of the DAS28-CRP improves inter-score agreement with the DAS28-ESR in rheumatoid arthritis. Rheumatol. 2019; 58(5): 831–5. DOI: https://doi.org/10.1093/rheumatology/key374
21. Hensor EMA, Emery P, Bingham SJ, Conaghan PG, YEAR Consortium. Discrepancies in categorizing rheumatoid arthritis patients by DAS-28(ESR) and DAS-28(CRP): Can they be reduced? Rheumatol. 2010; 49(8): 1521–9. DOI: https://doi.org/10.1093/rheumatology/keq117
22. Ranganath VK, Elashoff DA, Khanna D, Park G, Peter JB, Paulus HE, *et al*. Age adjustment corrects for apparent differences in erythrocyte sedimentation rate and C-reactive protein values at the onset of seropositive rheumatoid arthritis in younger and older patients. J Rheumatol. 2005; 32(6): 1040–2.

Original Article

Assessment of altitude induced effects on serum liver function, renal function and lipid profile within the population of Gilgit Baltistan's District Gamba, reporting to Combined Military Hospital Skardu

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ABSTRACT

Objective: The objective of this study was to evaluate impact of high altitude on various parameters of Liver Function Tests, Renal Function Tests, and Lipid profiles in local population reporting to CMH Skardu.

Material and Methods: This Cross-sectional observational study was conducted in the department of pathology of CMH Skardu from January 2023 to August 2023. Participants underwent a comprehensive medical examination. Serum samples were obtained for the assessment of Liver Function Tests (LFTs), Renal Function Tests (RFTs) and lipid profiles of patients in clot activator vaccutainers and analyzed on Selectra Pro XI by their respective spectrophotometric methods. One way ANOVA and Pearson correlation were used to statistical analysis between different groups according to altitude and p value <0.05 was considered significant.

Results: The study involved 150 participants, with ages ranging from 28 to 71 years with the mean age of 46.5 ±10.71 years. Altitude varied between 1700 and 2500 meters among participants with mean altitude of 2018.6±21.9 meters. A total of 105(70%) participants were male, and 45(30%) participants were female. Significant negative correlations were observed between altitude and ALT (-0.227, p = 0.005) and AST (-0.212, p = 0.009) For ALT, AST, ALP, BUN, TGs, HDL, and LDL, there are significant differences among groups (p-values < 0.05). For BIL and Creatinine, there are no significant differences among groups (p-values > 0.05)

Conclusion: This study underscores the multifaceted nature of altitude's impact on human physiology, highlighting the need for comprehensive research to optimize health and performance in high-altitude environments.

Keywords: Altitude, Gilgit, Liver Function Tests, Renal Function Tests, Lipid

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INTRODUCTION

High-altitude environment, characterized by reduced barometric pressure and lower oxygen level, have been a subject of considerable research interest, particularly regarding their impact on human physiology. Effects of high altitude on various physiological parameters, such as Liver Function Tests (LFTs), Renal Function Tests (RFTs), and Lipid profiles, collectively referred to as "LFTs-RFTs-Lipid profile," have been extensively investigated.

Liver, as a pivotal metabolic organ, is crucial for maintaining metabolic homeostasis. Studies by Lala *et al*. [1] and Cornelius *et al* [2] have explored liver function tests, including Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), revealing alterations in response to factors such as hypobaric hypoxia and hepatotoxicity. RFTs, encompassing parameters like serum Creatinine and Blood Urea Nitrogen (BUN), are indicative of kidney function. Arshad *et al*. [3] investigated renal vein thrombosis at high altitudes, shedding light on

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the impact of altitude on kidney health. Lipid profile, an essential component in assessing cardiovascular health, has also been the focus of studies related to high-altitude exposure.

Additionally, high-altitude regions, defined as areas above 1,500 meters, provide a unique natural laboratory for studying adaptive responses to hypobaric hypoxia. Studies by Naeije *et al*. [4] and He *et al*. [5] delved into the effects of high altitude on pulmonary artery pressure, exercise capacity, and vascular endothelial function.

Understanding the impact of high altitude on LFTs, RFTs, and Lipid profiles is not only academically intriguing but also holds practical implications for individuals such as mountain climbers, trekkers, and residents of high-altitude regions. Furthermore, insights into cardiovascular functions and body composition at high altitudes, as studied by Vats *et al*. [6] and Ortiz-Prado *et al*. [7], contribute significantly to the growing body of knowledge in altitude medicine. Human adaptation to high-altitude is due to characteristic adjustments at every physiological level. Differences in lipid profile and cardiovascular risk factors in altitude dwellers have been previously explored [8]. Aryal *et al*. [9] and others explored Lipid profiles, Glycosylated hemoglobin (HbA1c), and Diabetes prevalence in populations residing at high altitudes.

In this study, we have aimed to synthesize existing literature, examining the mechanisms behind changes in LFTs, RFTs, and Lipid profiles at high altitudes, and discussing their clinical implications. The study seeks to provide a holistic understanding of the effects of high-altitude exposure on these physiological parameters, contributing to the broader field of altitude medicine.

MATERIAL AND METHODS

This Cross-sectional observational study was conducted in the department of pathology of CMH Skardu from January 2023 to August 2023. Determination of sample size for this study is based on a power analysis that considers following factors: the anticipated effect size based on previous research, the desired level of statistical power, and the significance level (alpha) for the statistical tests. Given the variability in the impact of high-altitude exposure on LFTs, RFTs, and Lipid profiles reported in previous studies, a moderate effect size is anticipated.

Sample size was calculated separately for each of these parameters (LFTs, RFTs, and Lipid profiles) to ensure adequate statistical power. Previous research, such as the study by Garcia, C. M [10], will be used as a reference for effect size estimation. With 95% confidence interval and 5% margin of error sample size is estimated to be approximately 150 participants for each parameter using the WHO sample size calculator [20]. The inclusion criteria for the study are participants aged between 18 and 65 years who have resided at altitudes of 1,500 meters (4,921 feet) or higher for a minimum of six months, and who have provided written informed consent to participate. Exclusion criteria include individuals under the age of 18 or over the age of 65, patients with chronic renal or hepatobiliary diseases, and those currently taking lipid-lowering medications.

Participants underwent a comprehensive medical examination, including the measurement of vital signs including Blood pressure, pulse rate, respiratory rate and body temperature and a review of medical history. Serum samples were obtained for the assessment of LFTs including serum Bilirubin, serum alanine amino- transferase (ALT), serum aspartate amino-transferase AST, serum alkaline phosphatase (ALP), RFTs that included serum creatinine and blood urea nitrogen (BUN), and lipid profiles of patients consisting of serum Total Cholesterol, serum Triglycerides (TGs), serum low density lipoprotein (LDL) and serum High density lipoprotein (HDL) in clot activator vaccutainers. All parameters were analyzed on Selektra Pro-M Clinical Chemistry analyzer after running calibration and Quality Control for each parameter following Standard operating procedures.

Statistical analysis involved the use of t-tests, analysis of variance (ANOVA), to examine the relationships between altitude exposure and the selected physiological parameters. Adjustments for potential confounding variables, such as age and gender, was made in the analysis with at a p-value of 0.05 considered statistically significant.

This study adhered to all relevant ethical guidelines and Ethical approval was obtained from the hospital ethical review board vide letter no. CMH Skardu /ERB/23/02.

RESULTS

The study involved 150 participants, with ages ranging from 28 to 71 years with the mean age of 46.5 ±10.71 years. Altitude varied between 1700 and 2500 meters among participants with mean altitude of 2018.6±21.9 meters. A total of 105(70%) participants were male, and 45 (30%) participants were female

Means of various parameters have been shown in Table-I Below. Significant negative correlations were observed between altitude and ALT (-0.227, p = 0.005) and AST (-0.212, p = 0.009) as shown in Table-II below. For ALT, AST, ALP, BUN, TGs, HDL, and LDL, there are significant differences among groups (p-values < 0.05). For BIL and Creatinine, there are no significant differences among groups (p-values > 0.05) as shown in Table-III.

Significant differences in ALT, AST, ALP, BUN, TGs, HDL, and LDL levels among groups suggest potential influences of altitude on these biomarkers. The lack of significant differences in BIL and Creatinine may indicate that altitude may not have a substantial impact on these parameters.

Table-I: Mean and standard deviation of parameters.

Variable	Mean± Standard Deviation
Age (years)	46.49±10.71
Altitude (meters)	2018.67±210.91
ALT (U/L)	44±16.0
AST (U/L)	42±17.0
ALP (U/L)	75±17.7
Bilirubin (mg/dl)	0.91±0.60
Creatinine (mg/dl)	0.79±0.11
BUN (mg/dl)	12.88±3.59
CHOL (mmo/L)	160±78.54
TGs (mmo/L)	116±104.8
HDL (mmo/L)	34±5.18
LDL (mmo/L)	97±18.50
CHOL (mmo/L)	160±78.54
TGs (mmo/L)	116±104.8
HDL (mmo/L)	34±5.18
LDL (mmo/L)	97±18.50

Table-II: Pearson's correlation between ALT, AST and altitude.

Parameter	Altitude	P-value
ALT	-0.227	0.005
AST	-0.212	0.009

Table-III: ANOVA showing significant differences among groups-based Altitude level.

Parameter	F-value	p-value
ALT	3.876	.001
AST	3.690	.001
ALP	2.922	.007
Bilirubin	.971	.455
Creatinine	.561	.786
BUN	2.989	.006
Cholesterol	1.885	.076
Triglycerides	2.938	.007
HDL	3.211	.003
LDL	2.504	.019

DISCUSSION

Our study involved 150 participants with demographics providing a diverse and representative sample, allowing for a comprehensive exploration of the impact of high altitude on various physiological parameters. The mean values of liver function tests (LFTs) revealed intriguing insights. ALT and AST levels were negatively correlated with altitude, demonstrating significant negative correlations (-0.227, $p = 0.005$ for ALT; -0.212, $p = 0.009$ for AST) The ANOVA results indicated significant differences in ALT, AST, and ALP among groups based on altitude levels (p-values < 0.05) [10]. ALT and AST, known markers of hepatocellular damage, displayed higher levels at lower altitudes. This supports the notion that liver function is influenced by changes in altitude, potentially due to variations in oxygen availability and environmental stressors. Unlike liver function tests, creatinine and blood urea nitrogen (BUN) levels did not show significant differences among altitude groups (p-values > 0.05) This suggests that altitude may not substantially impact renal function parameters in the studied population. These results are consistent with the study by Sawka *et al*. [11], which emphasized the adaptability of blood volume to environmental stresses. The study also explored the lipid profiles of participants at different altitudes. Significant differences were observed in total cholesterol (CHOL), triglycerides (TGs), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) among altitude groups (p-values < 0.05) [12]. The elevated levels of CHOL and TGs at higher altitudes may indicate an adaptive response to the lower oxygen availability. The shifts in HDL and LDL levels further underscore the complex interplay between altitude exposure and cardiovascular health. These results are in line with studies by Ward and Milledge [13], which acknowledged Griffith Pugh's pioneering work on Everest and its implications for understanding altitude physiology.

These findings contribute to the broader field of altitude medicine, shedding light on the adaptability of the human body to hypobaric conditions. As altitude-related activities and travel become more prevalent, understanding these physiological adaptations becomes increasingly important for both medical practitioners and individuals exposed to high altitudes [14]. Further research, building upon the foundations laid by studies referenced in this discussion, will continue to unravel the complexities of high-altitude physiology. The significant correlations and differences observed in LFTs, renal function tests, and lipid profiles underscore the intricate relationship between altitude exposure and physiological adaptations [15]. These findings have practical implications for individuals residing at high altitudes, such as mountain climbers and residents of high-altitude regions.

Moreover, it is essential to consider the implications of high altitude on other physiological systems beyond those directly measured in this study. For instance, research has shown that high-altitude exposure can lead to alterations in pulmonary circulation, contributing to conditions such as high-altitude pulmonary hypertension [16]. This highlights the importance of investigating not only hepatic and renal function but also cardiopulmonary dynamics in individuals exposed to hypobaric conditions. Additionally, the effects of chronic hypoxia on oxygen transport mechanisms warrant further investigation, as they play a crucial role in the body's adaptation to high altitude [17].

Furthermore, the impact of hypoxia on oxidative stress and cellular metabolism cannot be overlooked. Studies have demonstrated an association between hypoxic environments and increased oxidative stress, which may contribute to various physiological changes observed at high altitudes [18]. Understanding the mechanisms underlying these processes is essential for developing targeted interventions to mitigate the adverse effects of altitude exposure.

In addition to physiological adaptations, it is crucial to consider the psychological and cognitive effects of high-altitude exposure. Research has shown that prolonged stays at high altitude can lead to cognitive impairments

and mood disturbances, known as acute mountain sickness (AMS) [18]. These symptoms can significantly impact individuals' quality of life and performance, particularly in demanding environments such as mountain climbing expeditions or high-altitude workplaces.

Moreover, the role of genetics in mediating individual responses to high-altitude environments merits further investigation. Studies have identified genetic variants associated with improved oxygenation and reduced susceptibility to altitude-related illnesses in certain populations, highlighting the importance of genetic factors in altitude adaptation [19].

In summary, our study contributes to the growing body of literature on altitude medicine by providing insights into the physiological adaptations of the liver, kidneys, and cardiovascular system to high-altitude exposure. However, further research is needed to elucidate the comprehensive effects of altitude on human physiology, including pulmonary circulation, oxygen transport, oxidative stress, cognitive function, and genetic predispositions. By expanding our understanding of these complex interactions, we can better support individuals living and working in high-altitude environments and optimize their health and performance.

CONCLUSION

In conclusion, this study shows the negative correlations between altitude and ALT, AST, coupled with significant differences in these biomarkers, suggest that altitude plays a role in influencing liver function. Additionally, the lack of significant differences in BIL and Creatinine indicates a potential resilience of renal function to altitude changes. The alterations in lipid profiles highlight the multifaceted nature of altitude's impact on cardiovascular health.

LIMITATIONS

However, it's crucial to acknowledge the limitations of the study. The cross-sectional design limits the establishment of causation, and confounding variables, such as lifestyle factors and pre-existing health conditions, may influence the observed outcomes. Future longitudinal studies and controlled experiments could provide deeper insights into the causal relationships between altitude and physiological parameters.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Mustajab Alam: Conception, data collection, formal analysis, investigation and resources, accountable for all aspects of the work

Hunain Habib: Drafting the manuscript and interpretation of the work, accountable for all aspects of the work

Naveed Asif: Drafting, designing the study content, review writing, accountable for all aspects of the work

Bushra Anwar: Results analysis, review of writing, accountable for all aspects of the work

Tahir Asad: Data collection, analysis, investigation, accountable for all aspects of the work

Khizar Hameed: Review writing and editing**,** accountable for all aspects of the work

REFERENCES

1. Lala V, Zubair M, Minter DA. Liver Function Tests. [Updated 2023 Jul 30]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482489/
2. Cornelius CE. Liver function tests in the differential diagnosis of hepatotoxicity. In Hepatotoxicology 2020 Jan 16 (pp. 181-213). CRC Press.
3. Arshad AR, Islam F, Qayyum M. Renal vein thrombosis at high altitude. Pak Armed Forces Med J. 2021; 71(Suppl-1): S283-85. DOI:https://doi.org/10.51253/pafmj.v71iSuppl-1.2444
4. Naeije R, Huez S, Lamotte M, Retailleau K, Neupane S, Abramowicz D, *et al*. Pulmonary artery pressure limits exercise capacity at high altitude. Eur Respir J. 2010; 36(5): 1049-55. DOI: https://doi.org/10.1183/09031936.00024410
5. He L, Jiang Y, Su H, Li J, Cheng X. Vascular endothelial function assessed by postischemic diastolic blood pressure is associated with acclimatization and acute mountain sickness. High Alt Med Biol. 2016; 17(1): 11-5.

DOI: https://doi.org/10.1089/ham.2015.0070
6. Vats P, Ray K, Majumadar D, Amitabh, Joseph DA, Bayen S, *et al*. Changes in cardiovascular functions, lipid profile, and body composition at high altitude in two different ethnic groups. High Alt Med Biol. 2013; 14(1): 45-52. DOI: https://doi.org/10.1089/ham.2012.1071
7. Ortiz-Prado E, Portilla D, Mosquera-Moscoso J, Simbaña-Rivera K, Duta D, Ochoa I, *et al*. Hematological parameters, lipid profile, and cardiovascular risk analysis among genotype-controlled indigenous Kiwcha men and women living at low and high altitudes. Front Physiol. 2021: 1769. DOI: https://doi.org/10.3389%2Ffphys.2021.749006
8. Aryal N, Weatherall M, Bhatta YK, Mann S. Lipid profiles, glycated hemoglobin, and diabetes in people living at high altitude in Nepal. Int J Environ Res Pub Health. 2017; 14(9): 1041. DOI: https://doi.org/10.3390/ijerph14091041
9. Naeije R, Chesler N. Pulmonary circulation at exercise. Compr Physiol. 2015; 5(1): 655-78. DOI: https://doi.org/10.1002%2Fcphy.c100091
10. Sawka MN, Convertino VA, Eichner ER, Schnieder SM, Young AJ. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. Med Sci Sports Exerc. 2000; 32(2): 332-48. DOI: https://doi.org/10.1097/00005768-200002000-00012
11. Appenzeller O, Martignoni E. The autonomic nervous system and hypoxia: mountain medicine. J Auton Nerv Sys. 1996; 57(1-2): 1-12. DOI: https://doi.org/10.1016/0165-1838(95)00093-3
12. Ward MP, Milledge JS. Griffith pugh, pioneer everest physiologist. High Alt Med Biol. 2002; 3(1): 77-87. DOI: https://doi.org/10.1089/152702902753639595
13. Winslow RM, Samaja M, West JB. Red cell function at extreme altitude on Mount Everest. J Appl Physiol. 1984; 56(5), 1097-1103. DOI: https://doi.org/10.1152/jappl.1984.56.1.109
14. Zhao Y, Wang X, Noviana M, Hou M. Nitric oxide in red blood cell adaptation to hypoxia. Acta Biochim Biophys Sin. 2018; 50(7): 621-34. DOI: https://doi.org/10.1093/abbs/gmy055
15. Brito J, Siques P, Pena E. Long-term chronic intermittent hypoxia: A particular form of chronic high-altitude pulmonary hypertension. Pulm Circ. 2020; 10(1_suppl): 5-12. DOI: https://doi.org/10.1177%2F2045894020934625
16. Bärtsch P, Swenson ER. Acute high-altitude illnesses. New Eng J Med. 2013;. 368(24). 2294-302. DOI: https://doi.org/10.1056/nejmcp1214870
17. Mallet RT, Burtscher J, Pialoux V, Pasha Q, Ahmad Y, Millet GP, *et al*. Molecular mechanisms of high-altitude acclimatization. Int J Mol Sci. 2023; 24(2): 1698. DOI: https://doi.org/10.3390%2Fijms24021698
18. Beall CM. Two routes to functional adaptation: Tibetan and Andean high-altitude natives. Proc Natl Acad Sci USA. 2007; 104 (suppl 1): 8655-60. DOI: https://doi.org/10.1073/pnas.0701985104
19. Sample size calculator. (2023, July 08). Retrieved from [https://www.calculator.net/sample-size-calculator.html]

Biochemical risk assessment of first trimester miscarriage by evaluating serum estradiol progesterone and beta human chronic gonadotropin levels

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ABSTRACT

Objective: The current study was carried out to predict outcome of first trimester pregnancy by evaluating serum estradiol, progesterone and beta human chorionic gonadotropin levels.

Material and Methods: This cross-sectional study was conducted at the Department of Chemical pathology and endocrinology AFIP, for a period of six months (from January 2023 to June 2023). Non-probability consecutive sampling was used for the selection of participants. All females presented with singleton pregnancy of 05-07 weeks from 21-40 years age were asked to sign informed consent and enroll in study. All patients with assisted reproductive techniques facilitated pregnancy, known thyroid, diabetes and hypertension were excluded from the study. Chemical biomarkers including Serum beta HCG, estradiol and progesterone were compared at the time of 7-9 weeks and 10-12 weeks. Statistical analysis was performed using Mann Whitney U test and relative risk analysis and p-Value ≤ 0.05 was considered significance.

Results: Median of Beta HCG at 7-9 weeks IU/L, Estradiol 7-9 weeks gestation, Beta HCG at 10-12 weeks IU/L, Estradiol at 11-12 weeks pmol/L and Progesterone at 11-12 weeks nmol/L were low in Miscarriages 6574.0 (9876.0 – 4321.0), 760.0 (913.3 – 634.0), 18356.0 (67893.0 – 15000.0), 1011.0 (1987.0 – 900.0) and 65.60 (164.0 – 32.50) respectively as compare to healthy groups and showed statistically highly significant difference as p values < 0.001. Median of Progesterone at 7-9 weeks nmol/L also low in Miscarriages group but showed non statistically significant difference as p value = 0.062.

Conclusion: It was concluded that estradiol levels can effectively predict miscarriage in the first trimester, with higher sensitivity at 7-9 weeks. Early monitoring of estradiol can aid in identifying at-risk pregnancies, enabling timely interventions to improve outcomes.

Keywords: First trimester miscarriage, Beta HCG, Estradiol, Progesterone

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INTRODUCTION

First-trimester miscarriage defined as the loss of a pregnancy before the 12th week of gestation, is a distressing event for couples and healthcare professionals alike. Unfortunate but statistically, pregnancy loss occurs in approximately 15–25% of pregnancies out of which 2.9% occurs within the first trimester of gestation. Despite living in the world, trying to explore the black-hole and set a step in meta-verse, the aforesaid loss is still the most common pregnancy complication affecting women's physical and mental health. Repeated Clinical examinations and treatments for threatened abortion also leads to economic and mental burdens on patients. In nutshell, impacts of pregnancy loss are manifold; affecting all realms of life [1]. Hossain *et al*., reported a high prevalence of miscarriages, with an estimated rate of 22.5% among women of reproductive age [2]. Understanding the underlying causes of first-trimester miscarriage is essential for appropriate management and counseling of

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affected couples. Biochemical risk assessment in the context of first-trimester miscarriage involves serum levels of estradiol, progesterone, and beta-human chorionic gonadotropin (β-hCG) that can play a crucial role in identifying potential causes and guiding interventions. These markers provide valuable information about hormonal balance, placental function, and embryonic development during early pregnancy. Factors such as maternal age, underlying medical conditions, lifestyle factors, and access to quality prenatal care may influence the incidence of miscarriages in the country [3]. Estradiol is the primary form of estrogen in the body which plays a critical role in the establishment and maintenance of pregnancy. Estradiol levels rise steadily during first trimester reflecting the activity of the developing placenta. Low estradiol levels may indicate insufficient placental function, while excessively high levels could suggest abnormal fetal development or an increased risk of gestational complications [4]. Progesterone is a hormone essential for maintaining pregnancy by promoting endometrial receptivity, inhibiting uterine contractions, and supporting early embryonic development. Low progesterone levels may result in inadequate endometrial preparation, leading to implantation failure or early pregnancy loss. Progesterone supplementation has been used as a potential intervention to support pregnancies at risk [5]. β-hCG is a hormone produced by the placenta during pregnancy. It is commonly used as a marker for pregnancy detection and monitoring. Abnormal β-hCG patterns, such as slow or fluctuating rise, can indicate an increased risk of miscarriage or potential chromosomal abnormalities in the fetus [6]. Several studies have explored the relationship between serum estradiol, progesterone, and β-hCG levels and the risk of first-trimester miscarriage. Lathi *et al*. found that lower serum estradiol and progesterone levels were significantly associated with an increased risk of miscarriage [7]. Another study by Chung *et al*. examined β-hCG levels in pregnancies with threatened miscarriage and found that higher initial β-hCG levels were associated with a reduced risk of miscarriage [8].

There is a scarcity of comprehensive and recent data specifically focused on first-trimester miscarriages in Pakistan. The aim of the study is to predict outcome of first trimester pregnancy by evaluating serum estradiol, progesterone and beta human chorionic gonadotropin levels. The study's hypothesis suggests that abnormal levels of estradiol, progesterone, and beta-human chorionic gonadotropin (β-HCG) during the first trimester can serve as early indicators of high-risk pregnancies, potentially enabling timely therapeutic interventions to improve pregnancy outcomes.

MATERIAL AND METHODS

The study was Cross Sectional conducted on 272 pregnant females at Armed Forces Institute of Pathology (AFIP) / National University of Medical Sciences (NUMS) in collaboration with Obstetrics & Gynaecology Department, Combined Military Hospital, Rawalpindi. Data was collected from January 2023 to June 2023 for the duration of 6 months after approval of Institutional Review Board (IRB).

The sample size was calculated with the help of cohort sample size calculating formula study by using the WHO calculator, with a prevalence of recurrent miscarriage being 2.9% (0.195) in the local population with a confidence interval of 95%, and 5% margin of error, sample size was come out to be 156.Sampling was done by non-probability consecutive sampling technique.

All females presented with singleton pregnancy of 05-07 weeks from 21-40 years were thoroughly informed about the study's purpose and asked to sign informed consent to enroll in study. They were assured of Confidentiality and their right to withdraw at any time. Age was categorized within 04 groups as < 25 years, 26-30 years, 31-35 years and 36-40 years. Females with pregnancy achieved by assisted reproductive technique, thyroid disease, diabetes or known hypertensive were excluded from the study.

After selection, all relevant patient's data was recorded in a profroma specially designed for this purpose. The participants were then asked to get their blood sampling for estradiol, progesterone and β-HCG levels during 5th to 7th weeks, while second sampling was done at 9th to 11th weeks of gestation again for estradiol, progesterone and β-HCG levels in. Hormone Levels were compared to assess the risk of miscarriage. Outcome of pregnancy was recorded after 13th weeks of gestation, and then the participants were divided into two groups depending on the outcome.

Data was analyzed by using Statistical Package for Social Sciences (SPSS) 22.00. Normality of data was checked by using Kolmogorov-Smirnov test. Data were not distributed normally and were represented using median (IQR). Qualitative data was represented by using percentage and frequency. Mann Whittney U-test (for non-normal quantitative data) was applied and p-value of ≤ 0.05 was considered as statistically significant".

RESULTS

Total 156 females were registered in the study during study, mean age was 35.0 (37.0 – 28.0) years. Median weight was 74.5 (83.3 – 66.0) kgs, Basal Metabolic Index for Asian population was used to determine BMI categories, results indicated maximum frequency in obesity class I (BMI of 31-34) with 106 (67.3%), followed by overweight (BMI of 25-29.9) with 24(15.4%), Obesity class II and Class III was reported in 24(15.4%), and 3 (1.9%) respectively.

Median value of gravida, parity and previous number of miscarriages were 3.0 (4.0 – 2.0), 1.0 (2.0 – 0.0) and 1.0 (2.0 – 0.0) respectively.

Patients were divided into two groups of healthy pregnancy and miscarriage; Comparison of demographics between miscarriage and healthy pregnancy groups shown in Table-I. Thyroid stimulating hormone and fasting blood glucose levels were evaluated at 10-12 weeks of gestation, indicating comparatively lower values of TSH and highest value of FBG in miscarriage groups, as 1.4 (2.9 – 1.1) and 4.3 (4.6 – 3.7) respectively. P-values were insignificant with 0.005 & 0.135 (Table-II). The Table-III compares serum hormone levels between healthy pregnancies and miscarriages at 7-9 and 10-12 weeks of gestation. Significant differences are observed in β-HCG, Estradiol, and Progesterone levels between the two groups, with lower hormone levels in miscarriages (P-Values < 0.05).

Table-03 results showed that , Median of Beta HCG at 7-9 weeks IU/L, Estradiol 7-9 weeks gestation , Beta HCG at 10-12 weeks IU/L , Estradiol at 11-12 weeks pmol/L and Progesterone at 11-12 weeks nmol/L were low in Miscarriages 6574.0 (9876.0 – 4321.0), 760.0 (913.3 – 634.0), 18356.0 (67893.0 – 15000.0), 1011.0 (1987.0 – 900.0) and 65.60 (164.0 – 32.50) respectively as compare to healthy groups and showed statistically highly significant difference as p values < 0.001. Median of Progesterone at 7-9 weeks nmol/L also low in Miscarriages group but showed non statistically significant difference as p value = 0.062.

Table-I: Comparison of demographics variables between miscarriage and healthy pregnancy groups (n=156).

Variables	Healthy Pregnancy (n=62) Median (IQR)	Miscarriages (n=94) Median (IQR)	p-Value
Age	29.0 (37.0 – 25.0)	35.0 (39.0 – 29.8)	< 0.001
BMI	34.1 (35.7 – 24.9)	31.7 (33.8 – 26.7)	0.480
Gravida	2.0 (3.0 – 1.0)	4.0 (5.0 – 3.0)	< 0.001
Parity	2.0 (1.0 – 0.0)	1.0 (2.0 – 0.0)	0.589

Table-II: Comparison thyroid stimulating hormone and fasting blood glucose levels between the groups.

Variables	Healthy Pregnancy (n=62) Median (IQR)	Miscarriages (n=94) Median (IQR)	P-Value
TSH	2.6 (3.5 – 1.2)	1.4 (2.9 – 1.1)	0.005
F. Blood Glucose	3.9 (4.4 – 3.5)	4.3 (4.6 – 3.7)	0.135

Table-III: Comparison of biochemical markers between miscarriages and healthy pregnancy groups

Variables	Healthy Pregnancy (n=62) Median (IQR)	Miscarriages (n=94) Median (IQR)	p-Value
Beta HCG at 7-9 weeks IU/L	19987.0 (20187.0 – 12897.0)	6574.0 (9876.0 – 4321.0)	< 0.001
Estradiol 7-9 weeks gestation	1076.5 (1167.0 – 971.0)	760.0 (913.3 – 634.0)	< 0.001
Progesterone at 7-9 weeks nmol/L	29.0 (53.3 – 25.0)	26.6 (39.8 – 22.0)	0.062
Beta HCG at 10-12 weeks IU/L	54384.0 (67893.0 – 23000.0)	18356.0 (67893.0 – 15000.0)	< 0.001
Estradiol at 11-12 weeks pmol/L	1997.0 (2234.0 – 1156.0)	1011.0 (1987.0 – 900.0)	< 0.001
Progesterone at 11-12 weeks nmol/L	31.70 (65.60 – 29.67)	65.60 (164.0 – 32.50)	< 0.001

DISCUSSION

Our findings confirmed prior findings that blood oestrogen, progesterone, and β-HCG levels increase by gestational phase between 7 and 12 weeks [10]. Estradiol and progesterone levels stood suggestively lesser in the miscarriage cohort. Previous research studies found that the intensities of these markers were lower, demonstrating adverse pregnancy results [11]. However, the significance of these factors in predicting miscarriage remains unknown. Our findings suggest that blood indicators within the first 9 weeks of gestation can be used to distinguish between a normal pregnancy and a first-trimester loss. Declining serum estradiol at 7-9 and 10-12 weeks, as well as progesterone levels at 7-9 weeks, were found to be predictive, but β-HCG levels had no influence [12]. β-HCG had no effect on the analytical usefulness of estradiol in combination with progesterone or estradiol at 7-9 weeks for miscarriage [13]. This is explained by the robust extrapolative effect of estradiol, which remains significant even when influenced by β-HCG [14]. At 7-9 weeks, the analytical conclusion of twin or multi-indicators was lower than at 10-12 weeks.[15] β-HCG & progesterone levels alone have little prognostic significance, but when combined with estradiol, they could boost estradiol's predictive effect at 7-9 weeks [16]. A combination of β-HCG and estradiol, in particular, might function better. As a result, decreased levels of estradiol are the greatest interpreter of miscarriage between 7 and 12 weeks. Our findings are similar to the findings of Yang Li and colleagues [17]. The aim of the study was to assess chances of miscarriage within 12 weeks, whereas Yang Li and colleagues' [17] goal was to rule out other undesirable consequences of usual pregnancy, such as biochemical ectopic pregnancy and miscarriage. In our investigation, blood progesterone increase during 7-9 weeks of conception associated to 10-12 weeks of gestation [13,18].

At 7-9 weeks, progesterone levels did not indicate miscarriage in the first trimester. However, progesterone levels at 7-9 weeks can be used to predict miscarriage, however, they are no more effective than serum estradiol levels at 7-9 weeks. Because significant progesterone production begins in the seventh gestational week, our findings suggest that progesterone might be a predictor at 7-9 weeks [19].

Similar to prior research, a high progesterone level at the time of embryo transmission projected a greater risk of continued gestation. According to the findings of Ku CW *et al*, [20] blood progesterone quantity improved with gestation till 13 weeks of typical pregnancies, but those with impulsive miscarriage exhibited a bordering rise [20]. Our findings also revealed that progesterone levels were greater at 7-9 gestational weeks than at 5-6 gestational weeks in a normal pregnancy. Similarly, around 7-9 weeks of gestation, women who miscarried had lower progesterone levels

than typical pregnant women [21]. According to the research, progesterone has an important function in promoting an early pregnancy. In our investigation, progesterone levels at 7-9 weeks that were lower than the cut-off value of 15.27ng/ml might predict miscarriage. This is consistent with the findings of the study by Lek SM *et al*. [22] They also established that the serum progesterone cut-off value (35nmol/L) has therapeutic significance and allows doctors to stratify patients into high and low-risk groups for spontaneous miscarriage [22]. Because serum HCG increases at a rate of 66% per 48 hours in normal pregnancy, a dynamic level of β-HCG may be more useful. A β-HCG rise rate of less than 66% suggests an unfavorable pregnancy outcome [23].

In comparison to those findings, β-HCG was evaluated just twice at 7-9 weeks in our investigation, which is insufficient to predict the total miscarriage fate during 12 weeks. It might be because pregnancy is an uncertain and vulnerable time. However, at 7-9 weeks, β-HCG coupled with estradiol significantly enhanced the prediction. It might be due to the HCG plateauing during this time frame. Our findings were consistent with recent research that revealed that low levels of estradiol and β-HCG, as well as low growth rates, likely signal poor pregnancy outcomes [24].

However, another study found that β-HCG and oestrogen levels were greater, not lower, during the first 6 weeks of pregnancy, indicating a new link between β-HCG, oestrogen, and threatening abortion [25].

After a woman has a vaginal hemorrhage or stomach aches, a pelvic ultrasound is frequently used to diagnose an early miscarriage. If an ultrasound at 8.5 weeks revealed embryonic survival, 95% of pregnancies would not result in miscarriage by 14 weeks. Although literature supporting ultrasound scans, such as subchorionic hemorrhage, fetal heart rate, and yolk sac diameter related to pregnancy loss has existed however the findings were argumentative [26].

As a result, prediction indicators before 8.5 weeks must be scrutinized. In our investigation, we discovered that estradiol at 7-9 weeks of gestation had a significant effect on the prediction of miscarriage in the first trimester.

The existing discoveries vary from prior studies, which found that the levels of estradiol at each gestational week from 5 to 8 weeks might calculate the threat of miscarriage. There are two explanations behind our study's 7-9 weeks and 10-12 weeks groups.

LIMITATIONS

This study has limitations due to its single-center design and small sample size, and the use of non-probability sampling techniques introduces potential bias. For more accurate results this study should be conducted on the large scale and for longer time period.

CONCLUSION

Constant assessment through ultrasound and β-HCG levels are known as the best markers to help forecast miscarriage risk and high-risk pregnancy before medical indications appear. Although the effects of estrogen and progesterone on pregnancy results are anticipated, our study is among the first to investigate the timing and results of hormone analysis for miscarriage prediction in our population. Our results discovered that estradiol levels can predict miscarriage in the first trimester, and the sensitivity of the cut-off value at 7-9 weeks of conception is greater. Additionally, our findings suggest that at 7-9 weeks of gestation, β-HCG or progesterone in combination with estradiol may offer superior predictive value. Our study contained a small number of instances, and further case and multicenter investigations are needed for validation of findings.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Saqibah Rehman: Conception, data collection, formal analysis, investigation and resources, accountable for all aspects of the work

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Zujaja Hina Haroon: Data collection, analysis, investigation, accountable for all aspects of the work

Muhammad Younas: Revisions, Accountable for all aspects of the work

REFERENCES

1. Roy P. You drowned me in tears, where did you go?" Narratives of Reproductive Loss and Grief in Middle-Class India (Doctoral dissertation, Universität Tübingen)
2. Hossain N, Khan N, Khan NH. Prevalence and risk factors of miscarriage among women in a rural community in Pakistan. J Obstet Gynaecol Res. 2012; 38(4): 765-72. DOI: https://doi.org/10.7759%2Fcureus.19057
3. Dimitriadis E, Menkhorst E, Saito S, Kutteh WH, Brosens JJ. Recurrent pregnancy loss. Nat Rev Dis Primers. 2020 Dec 10;6(1):98. DOI: https://doi.org/10.1038/s41572-020-00228-z
4. Kasvandik S, Sõritsa D, Tasa T. Low serum estradiol level in early pregnancy is associated with subsequent miscarriage in women with threatened miscarriage: A prospective observational study. Taiwan J Obstet Gynecol. 2019;58(6):820-826.
5. Di Renzo GC, Tosto V, Tsibizova V, Fonseca E. Prevention of preterm birth with progesterone. J Clin Med. 2021; 10(19): 4511. DOI: https://doi.org/10.3390%2Fjcm10194511
6. Horne AW, Bhattacharya S. Towards a more physiological, risk-based approach to the management of early pregnancy loss. Reprod Biomed Online. 2020; 40(3): 321-30.
7. Lathi RB, Massie JA, Gilani M. Serum progesterone levels predict success of artificial insemination in patients undergoing donor insemination and homologous insemination. Fertil Steril. 2011; 95(3): 1035-7.
8. Chung KH, Jeong HC, Kang JI. The impact of initial serum beta-human chorionic gonadotropin level on subsequent pregnancy outcomes after threatened miscarriage. Obstet Gynecol Sci. 2021;64(1):15-21.
9. Yasmeen R, Hussain R. Recurrent miscarriage and associated factors. Pak J Med Dentistry. 2016; 5(1): 47-50.
10. Pérez-Vargas M, Montoya JI, Santos R, Pasquale P, Gonzalez A, Alvarado S, *et al*. Association between age and estradiol/progesterone index with good quality blastocysts. Fertility Sterility. 2020; 114(3): e333.
11. Wang L, Wang L, Yang X, Jin P, Zhang R, Jiang Y, *et al*. Risk factors related to early pregnancy loss in fresh IVF/ICSI: An analysis of 954 embryo transfer cycles. Medicine. 2022 Aug 8;101(34). DOI:https://doi.org/10.1097%2FMD.0000000000030166
12. Li Y, Zhang J, Zhang K, Wang E, Shu J. Significance of dynamically monitoring serum estrogen and β-human chorionic gonadotropin in early pregnancy assessment. J Clin Lab Anal. 2021; 35(1): e23559. DOI: https://doi.org/10.1002%2Fjcla.23559
13. Neumann K, Depenbusch M, Schultze-Mosgau A, Griesinger G. Characterization of early pregnancy placental progesterone production by use of dydrogesterone in programmed frozen–thawed embryo transfer cycles. Reprod Biomed Online. 2020; 40(5): 743-51. DOI: https://doi.org/10.1016/j.rbmo.2020.01.019
14. Mu F, Wang C, Li X, Wang F. The relationship between the average decreased times of estradiol and early miscarriage: An Observational Study. Reprod Sci. 2024: 1-8. DOI: https://doi.org/10.1007/s43032-024-01570-3
15. Di Renzo GC, Giardina I, Clerici G, Brillo E, Gerli S. Progesterone in normal and pathological pregnancy. Horm Mol Biol Clin Investig. 2016; 27(1): 35-48. DOI: https://doi.org/10.1515/hmbci-2016-0038
16. Zhang Y, Li Z, Ren B, Wu W, Liu Y, Wang X, *et al*. Diagnostic value of a single β-hCG test in predicting reproductive outcomes in women undergoing cleavage embryo transfer: A retrospective analysis from a single center. Reprod Health. 2022; 19(1):145. DOI: https://doi.org/10.1186/s12978-022-01455-1
17. Puget C, Joueidi Y, Bauville E, Laviolle B, Bendavid C, Lavoué V, *et al*. Serial hCG and progesterone levels to predict early pregnancy outcomes in pregnancies of uncertain viability: A prospective study. Eur J Obstet Gynecol Reprod Biol. 2018; 220: 100-5. DOI: https://doi.org/10.1016/j.ejogrb.2017.11.020
18. Boynukalin FK, Gultomruk M, Turgut E, Demir B, Findikli N, Serdarogullari M, *et al*. Measuring the serum progesterone level on the day of transfer can be an additional tool to maximize ongoing pregnancies in single euploid frozen blastocyst transfers. Reprod Biol Endocrinol. 2019; 17(1): 1-8. DOI: https://doi.org/10.1186/s12958-019-0549-9
19. Deng W, Sun R, Du J, Wu X, Ma L, Wang M, *et al*. Prediction of miscarriage in first trimester by serum estradiol, progesterone and β-human chorionic gonadotropin within 9 weeks of gestation. BMC Pregnancy Childbirth. 2022; 22(1): 1-1. DOI: https://doi.org/10.1186/s12884-021-04158-w

20. Chu J, Hardy P, Beeson L, Coomarasamy A. What is the best method for managing early miscarriage?. BMJ. 2020; 368: l6438.
DOI: https://doi.org/10.1136/bmj.l6438
21. Zhou J, Huang Z, Pan X, Leung WT, Li C, Chen L, et al. New thoughts in exploring the pathogenesis, diagnosis, and treatment of threatened abortion. Biosci Trends. 2019; 13(3): 284-5.
DOI: https://doi.org/10.5582/bst.2019.01155
22. Xu L, Wei Q, Wu Q, Zhong Y, Li Y, Xu J, *et al*. Higher β-human chorionic gonadotropin and estrogen levels during the first 6 weeks of pregnancy are associated with threatened abortion. BioSci Trends. 2019; 13(3): 245-52.
DOI: https://doi.org/10.5582/bst.2019.01111
23. Skogler J, Moberg T, Tancredi L, Styrmisdóttir L, Hedayati E, Alarcon-Ruiz CA, et al. Association between human chorionic gonadotropin (hCG) levels and adverse pregnancy outcomes: A systematic review and meta-analysis. Pregnancy Hypertens. 2023; 34: 124-37.
DOI: https://doi.org/10.1016/j.preghy.2023.11.003
24. Devall AJ, Papadopoulou A, Podesek M, Haas DM, Price MJ, Coomarasamy A, et al. Progestogens for preventing miscarriage: A network meta-analysis. Cochrane Database Syst Rev. 2021; 4(4): CD013792.
DOI:https://doi.org/10.1002/14651858.cd013792.pub2
25. Li L, Zhang Y, Tan H, Bai Y, Fang F, Faramand A, et al. Effect of progestogen for women with threatened miscarriage: A systematic review and meta-analysis. BJOG. 2020; 127(9):1055-63.
DOI: https://doi.org/10.1111/1471-0528.16261
26. Satish S, Rufus NH. Investigation on ultrasound images for detection of fetal congenital heart defects. Biomed Phys Eng Express. 2024; 10(4): 042001.
DOI: https://doi.org/10.1088/2057-1976/ad4f91

Original Article

Bacteriological profile with phenotypic detection of MDR isolates in surgical site infections of Nishtar hospital, Multan

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ABSTRACT

Objective: To determine bacteriological profile with phenotypic detection of MDR isolates in surgical site infections.

Materials and Methods: This cross-sectional descriptive study determined the frequency of bacteria causing surgical site infections in patients admitted at Nishtar Hospital, Multan. A total of 175 wound samples were collected and processed in the laboratory. All bacterial strains were characterized, and multidrug resistant strains were identified by an antibiotic susceptibility test. Moreover, modified carbapenem inactivation method, combine disc diffusion, and double disc synergy methods were employed to identify carbapenemases, metallo beta-lactamases, and extended spectrum beta-lactamases production among gram negative bacilli, respectively. Likewise, Cefoxitin-disc diffusion method was employed to identify *S. aureus* strains as *methicillin-resistant staphylococcus aureus*.

Results: In this study, *P. aeruginosa* (40%), *E. coli* (19.4%), *Proteus* spp. (8.6%), *K. pneumoniae* (6.3%), *Enterobacter* (2.9%), and *A. baumannii* (2.2%) made up the majority of the detected Gram-Negative Bacilli, while *S. aureus* (20.6%) was the only isolated Gram-Positive Cocci. A significant proportion of Gram-Negative Bacilli showed resistance to amoxicillin/clavulanic acid, ampicillin, ceftriaxone, trimethoprim/sulfamethoxazole, ciprofloxacin, amikacin, piperacillin/tazobactam, and meropenem, while Gram Positive Cocci showed resistance to ampicillin, amoxicillin/clavulanic acid, cefoxitin, and ceftriaxone. In this study, among 139 identified Gram-Negative Bacilli, 111 (79.9%) strains were CP (+), 122 (87.8%) strains were MBL (+), and 62 (44.6%) strains were ESBL (+). Likewise, 36 isolated strains of *S. aureus* were analyzed, out of which 30 (83.3%) were *Methicillin-Resistant Staphylococcus Aureus* (+).

Conclusion: our study will help in surveillance of resistance patterns of antibiotics and provide a cornerstone for the appropriate therapeutic strategy against multidrug-resistant infection.

Keywords: Carbapenemases, ESBLs, MBLs, *methicillin-resistant staphylococcus aureus*, surgical site infections

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INTRODUCTION

The Centers for Disease Control and Prevention (CDC) defines surgical site infection as an infection of the wound that happens within 30 days of an operation or after a year (if the infection is suspected to be connected to surgery) [1]. surgical site infection is the second most prevalent hospital-acquired infection (HAIs) with a probability between 2 and 11% for all surgical procedures [2]. surgical site infection typically occurs because of microbes present in the environment of the operating room and contaminated surgical tools [3]. Surgical site infection can be avoided, but it is associated with significant mortality among patients, lengthy hospital stays, and increased expenses [4]. The opportunistic and commonly isolated Gram-positive bacteria (GPB) and gram-negative bacilli from surgical site infection are

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Acinetobacter baumannii, *Enterobacter* spp., *Enterobacteriaceae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus* spp., and *Staphylococcus aureus* [5-7]. *Staphylococcus aureus* is the most common cause of surgical site infection in the skin microbiome. Besides that, *Enterococcus* species and *Escherichia coli* from the gut microbiome cause the most surgical site infection [8].

The majority of the time, foreign and/or microbiome bacteria penetrate surgical wounds (SWs) either during the procedure (primary infection) or right after the procedure (secondary infection) [9]. The common symptoms of infected SWs are pain, discomfort, inflammation, swelling, and discharge from infection sites. The primary infections appear within five to seven days after surgery. The majority of surgical site infections are not complicated because they just infect the skin and tissue underneath. Numerous patient-specific factors (old age, food habits, concomitant illness, subpar surgical methods, and insufficient sterilization of surgical tools) might have a significant impact on the incidence of surgical site infection. In addition to these, the virulence and intrusiveness of microorganisms, the integrity of the immune system, and the condition of the surrounding tissues also play a major role [9, 10].

Intracavitary, moderate incisional, and deep incisional are different kinds of surgical site infection. Deep incisional surgical site infection usually involves more extensive debridement following surgery and frequent prophylactic antibiotic therapy than moderate surgical site infection [11]. Surgical site infection can be classified as either acute (lasting less than 30 days) or chronic (lasting longer than 30 days) wound infections. In current study patients were included with surgical site infection irrespective of duration of infection. The free-floating bacteria that cause acute wound infections tend to proceed with accelerated destruction of tissue and appearance, although they often recover. However, chronic infections are persistently undulating with many flare-ups, and they respond only partially to systematic antibiotics [11, 12].

The incorporation of antibiotics into clinical trials was a critical component in the development of the contemporary hospital system [13]. However, over time, the rise of multidrug-resistant bacteria (MDR) has become a global health concern. It further makes it difficult for medical professionals to establish the best treatment alternatives. The negative impacts of MDR bacteria spread are most severe in developing countries[14]. The resistance to antibiotics is one of the top ten health risks worldwide. According to the World Health Organization (WHO), approximately fifty percent of cases caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* exhibited resistance to many potent antibiotics [7].

The hospitals in Pakistan lack a well-organized surveillance program to track surgical site infection daily. The occurrence of surgical site infection in hospitals can be reduced if there is more published literature on the proper diagnosis of surgical site infection, the ascending problem of MDR bacteria, and the appropriate use of antibiotics. Therefore, the current study was carried out to determine the causative pathogens with their antimicrobial resistance in patients suffering from surgical site infection in different surgical wards of Nishtar Hospital, Multan. Furthermore, standard phenotypic methods were employed to determine the frequency of metallo-β-lactamases (MBLs), extended-spectrum β-lactamases (ESBLs), carbapenemase (CRs) producers, and *methicillin-resistant staphylococcus aureus* (MRSA) among isolated MDR bacteria.

MATERIAL AND METHODS

The current cross-sectional descriptive study was conducted in the microbiology laboratory of the Pathology Department, Nishtar Hospital Multan, after receiving approval from the Ethical Review Committee. A sample size of 175 was calculated by using the WHO sample size calculator after taking the confidence level (95%), the margin of error (5%), and an anticipated MDR frequency of 13 from a

previous study [15]. The patients with surgical wounds representing symptoms of swelling, pain, redness, a foul smell, and discharge from wounds were selected for the current study. However, the patients with known infections or taking antibiotic treatment were excluded from the study. A written consent was obtained from every patient to use their samples for the current study.

The sterile cotton-tipped applicators were used to collect samples from patient with surgical site infection. Firstly, adequate pressure was applied around the wounds to cause the purulent exudates to express themselves. After that, each cotton-tipped applicator was gently circulated over a 1 cm^2 area of wound for 5 seconds. Following that, each wound underwent two swabs. Finally, all collected samples were transferred to the microbiological laboratory under aseptic conditions. Gram staining was performed for the first swab, while the second swab was inoculated into blood and MacConkey agar plates. The agar plates were incubated at 37°C overnight aerobically. The bacterial isolates were further identified and characterized by microscopy, colony morphology, and standard biochemical tests. Gram negative bacilli were characterized by oxidase, TSI, SIM, urease, and citrate tests, whereas GPC were characterized by catalase, coagulase, and DNase tests.

The antibiotic susceptibility testing (AST) was performed by employing the Kirby-Bauer disc diffusion method on Muller Hinton agar (MHA) plates, and the zones of inhibition (ZOIs) were interpreted following Clinical Laboratory Standards Institute (CLSI) guidelines for 2022. In this method, 0.5 McFarland suspensions of test and control bacteria were separately prepared in 5 mL of normal saline. These suspensions were aseptically inoculated onto the MHA plates. After 20 minutes, antibiotic discs were subsequently placed onto the inoculated plates, and the plates were incubated at 37 °C for 20 hours.

For modified carbapenem inactivation (mCIM) method, 1 µL loopful of strain (*Enterobacteriaceae*) or 10 µL loopful of *P. aeruginosa* or *A. baumannii* from agar plates were emulsified in 2 mL of trypticase soy broth. A meropenem disc was then immersed in the suspension and incubated for 4 hours at 35 °C. A 0.5 McFarland suspension of *E. coli* ATCC 25922 was prepared in 5 mL of saline using the direct colony suspension method. The MHA plate was inoculated with *E. coli* ATCC 25922 using the routine disc diffusion procedure. The meropenem disc was removed from the TSB and placed on the MHA plate previously inoculated with the *E. coli* ATCC 25922 indicator strain. This plate was incubated at 35 °C in ambient air for 18−24 h. No ZOI or colonies within a 16-18 mm zone was considered a positive result, while a ZOI of ≥ 19 mm was considered a negative result.

The MBL producers were identified by the combined disc diffusion method. For this experiment, imipenem (IPM-10 µg) was soaked in a test tube containing 10 µl of 0.5 M EDTA for 10-20 seconds. The MHA plates were inoculated with bacterial suspensions (0.5 McFarland). The IPM-10+EDTA and IPM-10 discs were placed on these inoculated MHA plates at appropriate distances. The agar plates were incubated at 37 °C overnight. The next day, if the increase in ZOI with the IPM-10+EDTA disc was 7 mm or more than that of the IPM-10 disc alone, then it was considered a positive result.

The ESBL producers were detected by the double disc synergy test. For this experiment, ceftazidime (CAZ-30 µg) and clavulanic acid (CLA-10 µg) antibiotic discs were placed on MHA plates previously inoculated with bacterial suspensions (0.5 McFarland). The inoculated plates were incubated at 37 °C overnight. The next day, a ≥ 5 mm increase in the ZOI for CAZ-30 in combination with CLA-10 was considered a positive result.

All *methicillin-resistant staphylococcus aureus* strains were determined by a disc diffusion test using a cefoxitin (FOX-30 µg) disc on an MHA plate. In 4 mL of saline, an over-night grown *S. aureus* culture was suspended, and turbidity was compared to standard 0.5 MacFarland. The MHA plate was inoculated with this suspension. After that, FOX-30 was positioned on the agar plate. This plate was incubated at 33-35°C for 24 hours. A ZOI ≤ 21

mm was considered a positive result for *methicillin-resistant staphylococcus aureus* strains.

The collected data were entered into the computer and analyzed using SPSS (Statistical Package for Social Sciences) version 25. All variables (age, gender, bacterial isolates, and resistant patterns) were represented in the form of frequencies and percentages in this cross-sectional descriptive study.

RESULTS

In the current study, 175 patients were included. Among them, 53 (30.3%), 50 (28.6%), 37 (21.1%), 23 (13.1%), and 12 (6.9%) patients belonged to age groups of <15 years, 16-30 years, 31-45 years, 46-60 years, and >60 years, respectively. Similarly, 98 (56.0%) patients were male and 77 (44.0%) were female. In this study, out of 175 patients 139 (79.4%) strains were gram negative bacilli, and 36 (20.5%) were gram positive cocci. The identified gram-negative bacilli were *P. aeruginosa* (70), *E. coli* (34), *Proteus* spp. (15), *K. pneumoniae* (11), *Enterobacter* spp. (5), and *A. baumannii* (4). The only identified Gram-positive bacterium was *S. aureus* (36) (Figure-I).

In the current study, the screening of CPs, ESBLs, and MBLs production was observed in isolated gram-negative bacilli. According to the results, 111 (79.9%) strains were CP (+), while 28 (20.1%) strains were CP (-). Out of these positive strains, 3 (75%), 5 (100%), 27 (79.4%), 8 (72.7%), 12 (80%), and 56 (80%) were *A. baumannii*, *Enterobacter* spp., *E. coli*, *K. pneumoniae*, *Proteus* spp., and *P. aeruginosa*, respectively (Figure-IIa). Similarly, 122 (87.8%) strains were MBL (+), while 17 (12.2%) strains were MBL (-). Out of these positive strains, 4 (100%), 4 (80%), 31 (91.2%), 10 (90.9%), 11 (73.3%), and 62 (88.6%) were *A. baumannii*, *Enterobacter* spp., *E. coli*, *K. pneumoniae*, *Proteus* spp., and *P. aeruginosa*, respectively (Figure-IIb).

Likewise, 62 (44.6%) strains were ESBL (+), while 77 (55.4%) strains were ESBL (-). Out of these positive strains, 2 (50%), 1 (20%), 24 (70.6%), 3 (27.3%), 8 (53.3%), and 24 (34.3%) were *A. baumannii*, *Enterobacter* spp., *E. coli*, *K. pneumoniae*, *Proteus* spp., and *P. aeruginosa*, respectively (Figure-IIIa). In the current study, 30 (83.3%) isolated strains of *S. aureus* were identified as *methicillin-resistant staphylococcus aureus* (Figure-IIIb).

Table-I: Antibiogram of isolated gram-negative bacilli.

Bacteria	AMP		AMC		AK		CRO		CIP		MEM		SXT		TZP	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
A. baumannii	4	-	4	-	4	-	4	-	4	-	4	-	4	-	2	2
Enterobacter spp.	4	1	5	-	5	-	2	3	3	2	5	-	5	-	5	-
E. coli	13	21	24	10	11	23	12	22	19	15	31	3	34	-	29	5
K. pneumoniae	7	4	10	1	4	7	7	4	7	4	10	1	3	8	9	2
Proteus spp.	7	8	18	7	4	11	9	6	6	9	15	-	8	7	10	5
P. aeruginosa	-	-	61	9	33	37	47	23	38	32	60	10	67	3	57	13

*AMP (Ampicillin), AMC (Amoxicillin-Clavulanic Acid), AK (Amikacin), CRO (Ceftriaxone), CIP (Ciprofloxacin), MEM (Meropenem), SXT (Sulfamethoxazole-Trimethoprim), and TZP (Piperacillin-Tazobactam), R (resistant), S (sensitive)

Table-II: Antibiogram of isolated Gram-positive bacilli.

Bacteria	AMP		AMC		CRO		DA		FOX		LNZ		VA	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
S. aureus	32	4	31	5	30	6	8	28	30	6	6	30	8	28

*AMP (Ampicillin), AMC (Amoxicillin-Clavulanic Acid), CRO (Ceftriaxone), DA (Daptomycin), FOX (Cefoxitin), LNZ (Linezolid), and VA (Vancomycin), R (resistant), S (sensitive).

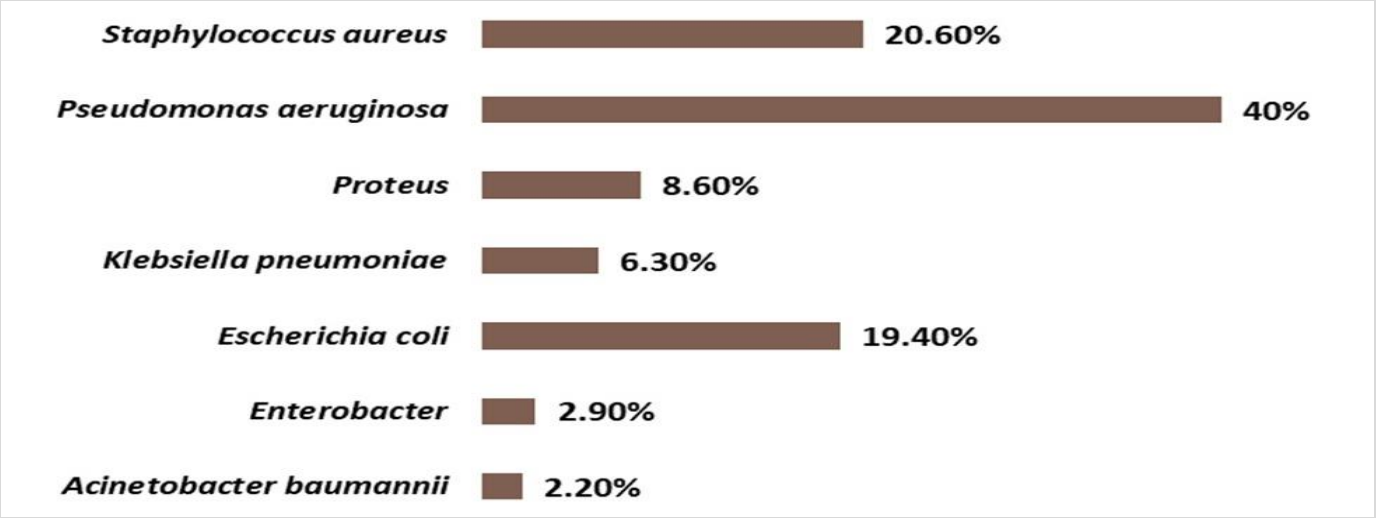

Figure-I: Bacterial strain distribution among all patients (n=175).

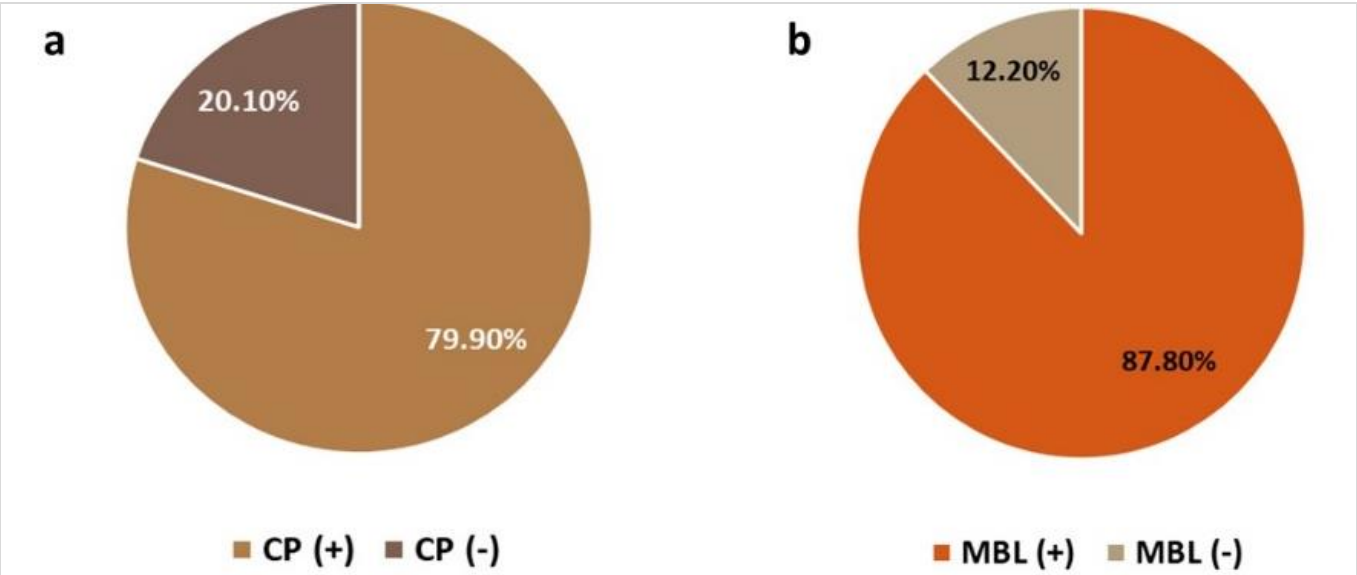

Figure-II: Pie graphs show percentages of CP producers or non-producers (a) and proportions of MBL producers or non-producers (b).

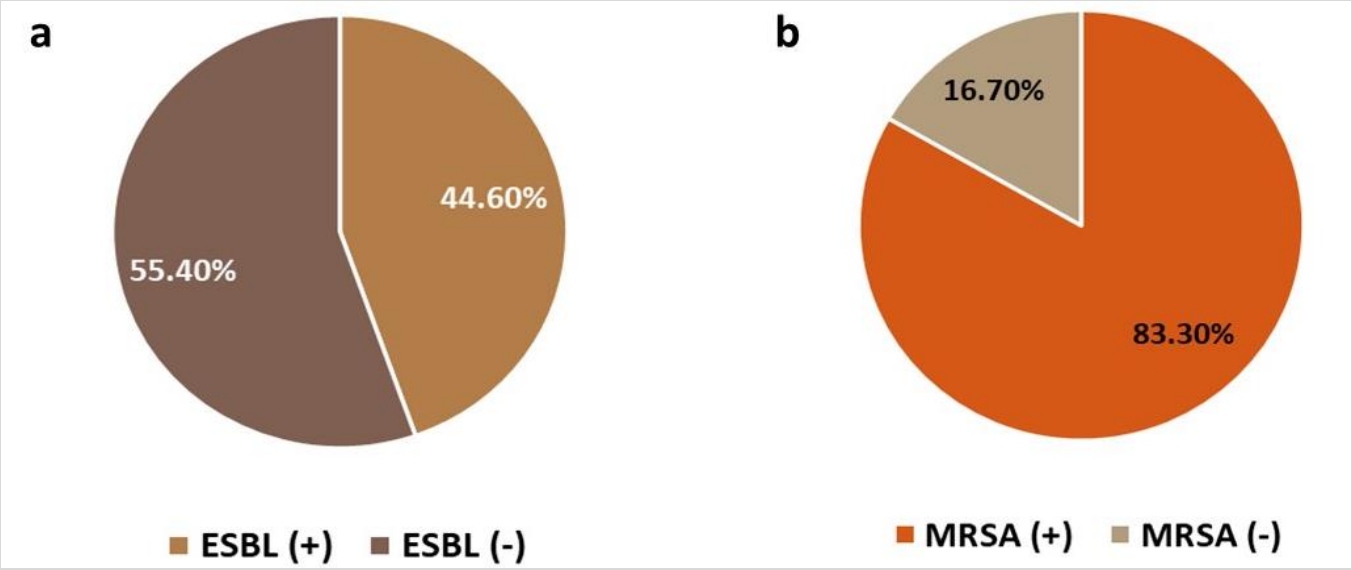

Figure-III: Pie graphs show percentages of ESBL producers or non-producers (a) proportions of *methicillin-resistant staphylococcus aureus* among isolated strains of *S. aureus* (b).

DISCUSSION

Surgical site infection is among the most often reported nosocomial infections that develop following an invasive surgical procedure [16]. Surgical site infection may result in a protracted hospital stay, a high rate of readmissions increased patient morbidity and death, reoperations, and increased medical expenses [17]. Drug resistance occurs due to the misuse and mismanagement of antibiotics is a major threat to humans. Another important dimension to the problem of surgical site infection is the recent spread of MDR bacterial pathogens [18].

In our study, a total of 175 isolated strains were isolated and further investigated, and 79.4% (139) of them were gram negative bacilli, and 20.5% (36) were GPB. The percentages of identified Gram-negative rods were *P. aeruginosa* (40%), *E. coli* (19.4%), *Proteus* spp. (8.6%), *K. pneumoniae* (6.3%), *Enterobacter* (2.9%), and *A. baumannii* (2.2%). The isolated GNB & GPC in our study were consistent with those of recent investigations performed by various researchers [19-21]. Similarly, types of causative organisms including various GNB & GPC are consistent with a previous study the main causative agents of surgical site infection are gram negative bacilli, for example, *Pseudomonas* spp., *E. coli*, *Enterobacter* spp., *A. baumannii*, *Proteus* spp., and *Klebsiella* spp. Besides that, *S. aureus* surgical site infection is common among hospitalized patients [22]

In our study, 25.2% (35), 80.6% (112), 80.6% (112), 89.9% (125), 58.3% (81), 55.4% (77), 43.9% (61), and 87.1% (121) isolated strains of Gram-negative rods showed resistance to AMP, AMC, TZP, MEM, CRO, CIP, AK, and SXT, respectively. Various studies have indicated variable resistance of Gram-negative and Gram-positive bacterial isolates to different antimicrobial agents. Other studies, similar to ours, have generally indicated that resistance patterns for Gram-negative rods are usually very high to a wide range of antibiotics. Researcher quoted resistance rates of 23% to ampicillin (AMP), 82% to amoxicillin-clavulanic acid (AMC), 79% to piperacillin-tazobactam (TZP), and 87% to meropenem (MEM), which shows quite a close pattern to our analysis. In another investigation, Jones et al. (2021) recorded resistance rates to ceftriaxone and ciprofloxacin at 60% and 56%, respectively, comparable to our findings, which revealed 58.3% and 55.4%, respectively. [23]. In addition researcher showed the following rates of resistance among Gram-negative strains: aminoglycosides (AK)-45% and sulfamethoxazole-trimethoprim (SXT)-85%, similar to our rates of 43.9% and 87.1%, respectively [23].

66.7% (24), 83.3% (30), 86.1% (31), and 69.4% (25) isolated strains of Gram-positive cocci showed resistance to AMP, AMC, FOX, and CRO, respectively, while 77.8% (28), 83.3% (30), and 77.8% (28) showed susceptibility to VA, LNZ, and DA, respectively. Some previous findings agreed with our observations in Gram-positive bacteria to a certain extent. Saka et al. described 65% of Gram-positive isolates as resistant to AMP and 80% resistant to AMC. In our study, the same flora had been resisted at rates of 66.7% and 83.3%, respectively. Moreover, they reported 70% and 74% resistance from Gram-positive strains to FOX and CRO, respectively, which agreed with our rates of resistance, 69.4% and 83.3%, respectively. On the other hand, several studies reported susceptibility rates ranging from 80% to 90% for Gram-positive strains to VA, LNZ, and DA, which agreed with our susceptibility rates of 77.8% [24].

In this study, the screening of CPs, ESBLs, and MBLs production was observed in isolated gram-negative bacilli. According to the results, the CP producers' percentages were *A. baumannii* (75%), *Enterobacter* (100%), *E. coli* (79.4%), *K. pneumoniae* (72.7%), *Proteus* (80%), and *P. aeruginosa* (80%). However, the MBL producers' percentages were *A. baumannii* (100%), *Enterobacter* (80%), *E. coli* (91.2%), *K. pneumoniae* (90.9%), *Proteus* (73.3%), and *P. aeruginosa* (88.6%). On the other hand, the ESBL producers' percentages were *A. baumannii* (50%), *Enterobacter* (20%), *E. coli* (70.6%), *K. pneumoniae* (27.3%), *Proteus* (53.3%), and *P. aeruginosa* (34.3%). In this study, 83.3% (30) isolated strains of *S. aureus*

were identified as *methicillin-resistant staphylococcus aureus*. The findings of this study are consistent with those of recent investigations that came before it [24-26].

Gram negative bacilli can acquire multi-drug or extensive drug resistance (XDR) through a variety of resistance mechanisms, such as the production of β-lactamases (ESBLS, MBLs, and AmpCs) and carbapenemases [27]. *Methicillin-resistant staphylococcus aureus* are aggressive pathogenic biovars of *S. aureus* that meet particular requirements for methicillin and cefoxitin resistance [28].

CONCLUSION

The study had a total of 175 patients. There was a very interesting distribution of age groups: <15 years constituted 30.3%, 16-30 years 28.6%, 31-45 years 21.1%, 46-60 years 13.1%, and >60 years 6.9%. The subjects were predominantly males 56.0% as opposed to females 44.0%. The microbiological examination revealed that 79.4% of the isolated strains were gram-negative bacilli, the most representative species being *Pseudomonas aeruginosa*, 40.0%, followed by *Escherichia coli*, 18.9%. Gram-positive bacteria accounted for 20.5%, with only *Staphylococcus aureus* being in the same percentage. The antibiotic susceptibility testing showed remarkable resistance among gram-negative bacteria studied. Accordingly, strains of *Pseudomonas aeruginosa* showed high resistance rates to Ceftriaxone (CRO) with a total of 61 strains and to Ciprofloxacin (CIP) with a total of 67 strains. Likewise, *Escherichia coli* revealed high resistive potential, especially to Ampicillin (AMP), with 13 resistant's versus 21 sensitive, and to Ciprofloxacin (CIP), with 31 resistant's versus 3 sensitive. Among the Gram-positive isolates, *Staphylococcus aureus* showed remarkable resistance to the commonly used antibiotics, particularly to Ampicillin: 32 resistant and 4 sensitive, Ceftriaxone: 30 resistant, 6 sensitive. Notably, 83.3% of the strains were *methicillin-resistant S. aureus*. Resistance was highly prevalent among gram-negative bacilli, including 79.9% of strains producing CPs, 87.8% producing MBLs, and 44.6% producing ESBLs. Among these, the leading proportion of CP and MBL producers was *Pseudomonas aeruginosa* and *Escherichia coli*, respectively. These data underscore the urgent need for ongoing surveillance of resistance patterns of antibiotics and provide a cornerstone for the appropriate therapeutic strategy against multidrug-resistant infection.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

AUTHORS CONTRIBUTION

Sumera Malik: Conception, manuscript writing, data collection, accountable for all aspects of the work

Blossom Neelam: Data collection, study design, interpretation of the work, accountable for all aspects of the work

Qurat Ul Ain Ayaz: Critical review, revisions, accountable for all aspects of the work

Abdul Wahab Majid: Data collection, analysis, investigation, accountable for all aspects of the work

Syed Muhammad Abbas Naqvi: Study design, proofreads, accountable for all aspects of the work

Javairia Saeed: Revisions, proofreads, accountable for all aspects of the work

REFERENCES

1. Alkaaki A, Al-Radi OO, Khoja A, Alnawawi A, Alnawawi A, Maghrabi A, *et al*. Surgical site infection following abdominal surgery: A prospective cohort study. Can J Surg. 2019; 62(2): 111-7. DOI: https://doi.org/10.1503/cjs.004818
2. Kolasiński W. Surgical site infections–review of current knowledge, methods of prevention. Pol J Chir. 2019; 91(4): 41-7. DOI: https://doi.org/10.5604/01.3001.0012.7253
3. Liu Z, Liu H, Yin H, Rong R, Cao G, Deng Q. Prevention of surgical site infection under different ventilation systems in operating room environment. Front Environ Sci Eng. 2021; 15: 36. DOI:https://doi.org/10.1007/s11783-020-1327-9
4. Wall RT, Datta S, Dexter F, Ghyasi N, Robinson AD, Persons D, *et al*. Effectiveness and feasibility of an evidence-based intraoperative infection

control program targeting improved basic measures: a post-implementation prospective case-cohort study. J Clin Anesth. 2022; 77: 110632. DOI:https://doi.org/10.1016/j.jclinane.2021.110632
5. ALhlale MF, Humaid A, Saleh AH, Alsweedi KS, Edrees WH. Effect of most common antibiotics against bacteria isolated from surgical wounds in Aden Governorate hospitals, Yemen. Uni J Pharm Res. 2020 Mar 8; 5(1): 21-4. DOI: https://doi.org/10.22270/ujpr.v5i1.358
6. Hemmati H, Hasannejad-Bibalan M, Khoshdoz S, Khoshdoz P, Kalurazi TY, Ebrahim-Saraie HS, *et al*. Two years study of prevalence and antibiotic resistance pattern of Gram-negative bacteria isolated from surgical site infections in the North of Iran. BMC Res Notes. 2020; 13 (1): 383. DOI: https://doi.org/10.1186/s13104-020-05223-x
7. Puca V, Marulli RZ, Grande R, Vitale I, Niro A, Molinaro G, *et al*. Microbial species isolated from infected wounds and antimicrobial resistance analysis: Data emerging from a three-years retrospective study. Antibiotics. 2021; 10(10): 1162. DOI:https://doi.org/10.3390%2Fantibiotics10101162
8. Liston J, Bayles A. Surgical site infections. Surgery (Oxford). 2023 Feb 1; 41(2): 65-70.
9. Pal S, Sayana A, Joshi A, Juyal D. *Staphylococcus aureus*: A predominant cause of surgical site infections in a rural healthcare setup of Uttarakhand. J Family Med Prim Care. 2019; 8(11): 3600-6. DOI: https://doi.org/10.4103/jfmpc.jfmpc_521_19
10. Sisay M, Worku T, Edessa D. Microbial epidemiology and antimicrobial resistance patterns of wound infection in Ethiopia: a meta-analysis of laboratory-based cross-sectional studies. BMC Pharmacol Toxicol. 2019 Dec; 20: 1-9. DOI: https://doi.org/10.1186/s40360-019-0315-9
11. Hrynyshyn A, Simões M, Borges A. Biofilms in surgical site infections: Recent advances and novel prevention and eradication strategies. Antibiotics. 2022; 11(1): 69. DOI:https://doi.org/10.3390%2Fantibiotics11010069
12. Mihai MM, Preda M, Lungu I, Gestal MC, Popa MI, Holban AM, *et al*. Nanocoatings for chronic wound repair—modulation of microbial colonization and biofilm formation. Int J Mol Sci. 2018 Apr 12; 19(4): 1179. DOI: https://doi.org/10.3390/ijms19041179
13. Gaynes R. The discovery of penicillin—new insights after more than 75 years of clinical use. Emerg Infect Dis. 2017; 23(5): 849-53. DOI: https://doi.org/10.3201%2Feid2305.161556
14. Gajdács M, Spengler G. The role of drug repurposing in the development of novel antimicrobial drugs: non-antibiotic pharmacological agents as quorum sensing-inhibitors. Antibiotics. 2019 Dec 17; 8(4): 270. DOI: https://doi.org/10.3390/antibiotics8040270
15. Raouf M, Ghazal T, Kassem M, Agamya A, Amer A. Surveillance of surgical-site infections and antimicrobial resistance patterns in a tertiary hospital in Alexandria, Egypt. J Infect Dev Ctries. 2020; 14(03): 277-83. DOI: https://doi.org/10.3855/jidc.12124
16. Ahmed EF, Rasmi AH, Darwish AM, Gad GF. Prevalence and resistance profile of bacteria isolated from wound infections among a group of patients in upper Egypt: A descriptive cross-sectional study. BMC Res Notes. 2023; 16(1): 106. DOI: https://doi.org/10.1186/s13104-023-06379-y
17. Ashoobi MT, Asgary MR, Sarafi M, Fathalipour N, Pirooz A, Jafaryparvar Z, *et al*. Incidence rate and risk factors of surgical wound infection in general surgery patients: A cross-sectional study. Int Wound J. 2023; 20(7): 2640-8. DOI: https://doi.org/10.1111/iwj.14137
18. Ali A, Gebretsadik D, Desta K. Incidence of surgical site infection, bacterial isolate, and their antimicrobial susceptibility pattern among patients who underwent surgery at De surgical site infection Comprehensive Specialized Hospital, Northeast Ethiopia. SAGE Open Med. 2023; 11: 20503121231172345. DOI:https://doi.org/10.1177%2F20503121231172345
19. Dan M, Hora S, Peshattiwar P, Vijay A. Assessing the bacteriological profile and antibiotic susceptibility pattern of surgical site infections in a tertiary care hospital. Int J Acad Med Pharm. 2023; 5(3): 579-82. DOI: https://doi.org/10.47009/jamp.2023.5.3.121
20. Mukagendaneza MJ, Munyaneza E, Muhawenayo E, Nyirasebura D, Abahuje E, Nyirigira J, *et al*. Incidence, root causes, and outcomes of surgical site infections in a tertiary care hospital in Rwanda: a prospective observational cohort study. Patient Saf Surg. 2019; 13: 10. DOI: https://doi.org/10.1186/s13037-019-0190-8
21. Hogle NJ, Cohen B, Hyman S, Larson E, Fowler DL. Incidence and risk factors for and the effect of a program to reduce the incidence of surgical site infection after cardiac surgery. Surg Infect. 2014; 15(3): 299-304. DOI: https://doi.org/10.1089/sur.2013.048
22. Maab H, Ali SA, Akmal M. Antibiotic susceptibility profile of bacterial isolates from post-surgical wounds of patients in tertiary care hospitals of Peshawar, Pakistan. J Pak Med Assoc. 2018; 68: 1517-20.
23. Alebel M, Mekonnen F, Mulu W. Extended-spectrum β-lactamase and carbapenemase producing gram-negative bacilli infections among patients in intensive care units of felegehiwot referral hospital: A prospective cross-sectional study. Infect Drug Resist. 2021: 391-405. DOI: https://doi.org/10.2147%2FIDR.S292246
24. Jose LR, Gogi S. Increasing trend of antimicrobial resistance among Gram Negative organisms isolated from blood samples with special reference to ESBL and MBL producers, A study from a tertiary care center in the rural areas of Wayanad. IOSR J Dental Medical Sci. 2020; 19(5): 59-62. DOI: http://dx.doi.org/10.9790/0853-1905065962
25. Metwally W, Aamir R. Prevalence and antimicrobial resistance patterns of nosocomial pathogens causing surgical site infections in an Egyptian University hospital. Microbes Infect Dis. 2020; 1(3): 226-37. DOI:https://doi.org/10.21608/mid.2020.45255.1069

26. Lutgring JD, Limbago BM. The problem of carbapenemase-producing-carbapenesm-resistant -*Enterobacteriaceae* detection. J Clin Microbiol. 2016; 54(3): 529-34.
DOI: https://doi.org/10.1128%2FJCM.02771-15

27. Algammal AM, Hetta HF, Elkelish A, Alkhalifah DH, Hozzein WN, Batiha GE, *et al*. Methicillin-Resistant *Staphylococcus aureus* (*methicillin-resistant staphylococcus aureus*): One health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infect Drug Resist. 2020: 3255-65.
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Original Article

Estimation of donor blood component wastage in the blood bank of a tertiary care hospital

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ABSTRACT

Objective: To estimate the donor blood component wastage in the blood bank of a tertiary care hospital.

Material and Methods: This cross-sectional study was done at Blood Bank Rehman Medical Institute, Peshawar from November 2021 to August 2023. However, since FFP and CP prepared in August 2023 will not expire until August 2024, their data cannot be fully assessed for wastage within this study period. Written consent was obtained before enrolling all donors. Depending on the need from various departments, the collected units of whole blood are fractionated into various components. The donors' names, ages, genders, contact information, and kinds of donors (voluntary or replacement) were recorded using a structured proforma. The donors who were selected had to meet a few requirements, including their age (≥ 18 years old), weight (≥ 50 g/dL), having hemoglobin levels of at least 12.5 g/dL, a hematocrit level (PCV) of at least 38%, and having normal blood pressure and a pulse rate between 50 and 100 beats per minute. Various reasons for the blood wastage were noted and recorded.

Results: A total of 18397 blood donations were received. Out of which 12870 (69.95%) units were utilized as whole blood and 5527 (30.04%) were separated into different components. Among 12870 blood bags (whole blood), 661(5.13%) were wasted. 655(99.1%) wasted whole blood bags were of male donors and 6 (0.9%) blood bags were of female donors. 202 (30.56%) wasted blood bags were of blood group B^+ TTI positivity accounting for the majority of whole blood bag wastage, making up 85.17% of cases. Among the total number of cases that were TTI positive, VDRL was detected in 20.78% of the blood bags, while HBsAg was found in 50.44% of them. The highest percentage of discarded components was platelets (7.99%), followed by RCC (4.84%), CP (3.23%), and FFP (2.11%).

Conclusions: To ensure the proper utilization of blood, it is important to establish and adhere to appropriate blood transfusion guidelines. Collaborative efforts between hospital and blood bank personnel should also be made to reduce blood wastage.

Keywords: Blood discarded, Blood components. Expired blood, Blood products.

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INTRODUCTION

Transfusion of blood and blood components is essential for managing patients in modern healthcare. The provision of sufficient amounts of safe blood and its components is the primary goal of blood centers all around the world [1]. The availability of appropriate blood transfusion services prevents millions of deaths annually in routine and emergency scenarios for surgical and medical purposes. Additionally, it considerably extends and improves the quality of life for patients with a wide range of chronic and acute illnesses [2,3]. According to numbers from the World Health Organization (WHO), more than 70% of countries with limited resources only collect half the blood needed to satisfy their needs [4].

Blood shortage is an unresolved problem in our nation. Hospitals worldwide face a significant challenge in preventing the needless loss of all blood components, such as RBCs, platelets (PLT), and plasma. This issue of

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waste extends beyond blood products and is present across the healthcare system. Research on the inefficiencies of healthcare systems has analyzed the significance of procedures and protocols [5,6]. Lack of cooperation and coordination between blood donors, blood banks, hospitals, and recipients contribute to the wastage of blood. Data shows that loss of blood is attributed to inadequate inventory management, unsatisfactory manufacturing practices, and improper storage and shipment [7]. The improper management of blood products can also result in their wastage due to various reasons such as expired units, broken packaging, clotted blood, serology-positive units, and other miscellaneous factors. This is often caused by a lack of sufficient knowledge, awareness, and training. It is advised by the College of American Pathologists (CAP) to keep track of unused blood as it can lead to financial loss in the healthcare system [8]. Low blood reserve can be addressed by reducing the incidence of blood loss through the effective use of stock management and standard procedures for blood usage [9]. More strict policies should be available and utilized to make the best use of this inadequate reserve in order to address the demand for the supply of both blood and its components. Effective collaboration between healthcare professionals and blood bank personnel can minimize blood wastage resulting from expiration. In resource-limited areas, the preparation of blood components may not be optimized, underscoring the importance of maximizing available resources, human capital, and reducing waste [10].

The aim of the study was to analyze the wastage of the donor blood component in the blood bank of Rehman Medical Institute, Peshawar.

MATERIAL AND METHODS

This prospective cross-sectional study was performed at the Blood bank RMI, Peshawar after obtaining approval from the Institutional Review Board (IRB), vide reference number RMI/RMI-REC/Article Approval/143. After a thorough literature search, we calculated a sample size of 18357 via the WHO calculator, keeping the margin of error at 5%, a confidence level at 95%. Sampling was done using a non-probability consecutive sampling technique.

All collected samples were taken through screening tests after blood donation to ensure its safety and the blood's suitability for transfusion. According to the written protocol, the collected samples were tested for infectious diseases such as HIV, hepatitis B, hepatitis C, syphilis, and malaria by ELISA and CLIA. The tests were carried out on Abbott ARCHITECT i1000SR.

Nucleic Acid Testing (NAT) with Roche Cobas 6800 System was used to increase the yield of detection of viral pathogens for HIV, HCV, and HBV. This can detect a window period of infection. The blood was collected in sterile, single-use blood bags of 350 mL to 450 mL, depending on the eligibility criteria of the donor. The blood bags used in the present study were of Terumo Penpol known for high-quality leak-proof bags. An anticoagulant solution of CPDA-1 for preserving blood up to processing was attached to each blood bag.

Each blood bag, before its use, was inspected for any visible damage or contamination. After collection, the bags were again checked for any breakage, leakage, or clot formation. Any compromised bags were immediately discarded and the donor was informed.

After blood collection, the whole blood was processed into its components using

Off-centrifugation. Centrifugation was done on whole blood using a high-speed centrifuge, like the Sorvall RC3BP Plus, to separate it into red blood cells, plasma, and platelets. All the conditions for the centrifugation process were very controlled, with all specific conditions stipulated based on the diverse components that were to be attained on 4000 RPM for 10 minutes in the case of plasma separation.

Separated components were transferred into corresponding storage bags. The red blood cells were stored at a temperature of 2-6°C, plasma was frozen at -30°C, and platelets were stored at 20-24°C with continuous agitation.

Each component was examined for any visible aberration, such as discoloration or clots, and then tested further. The measurement of pH for platelets to check that it met the standard requirements. Those that did not come up to the mark were discarded.

Written consent was obtained before enrolling all donors, and their confidentiality was ensured at all levels. The donors' names, ages, genders, contact information, and kinds of donors (voluntary or replacement) were recorded using a structured proforma.

The donors who were selected had to meet a few requirements, including their age (≥ 18 years old), weight (≥ 50 g/dL), having hemoglobin levels of at least 12.5 g/dL, a hematocrit level (PCV) of at least 38%, and having normal blood pressure and a pulse rate between 50 and 100 beats per minute. All components, including the whole blood unit (WBU), RCC, PC, FFP, CP, and CPP, that were discarded or wasted because of TTI positivity, hemolysis, expired shelf life broken or leaked units, QNS (quantity not sufficient), broken segments expired QC bags, lipemia, or waste as a result of leakage were also included in the study. Donors who engaged in high-risk behavior and had a history of jaundice, non-marital sexual contacts, intravenous drug abuse, recent blood transfusion, tattooing, or recent surgery were deferred and excluded from the study. Since single donor platelets (SDP) are manufactured on demand and after the donor has been screened for transfusion-transmitted infections, their incidence of wastage was minimal and they were therefore excluded from the research.

Records of Donor, TTI, Component preparation, and Wastage were collected. Upon selecting a donor for blood donation, the phlebotomy process is conducted while adhering to strict aseptic precautions outlined in the standard operating procedure of phlebotomy. TTI testing for HIV, HBsAg, HCV, syphilis, and malaria is performed on each unit. The TTI testing for HIV, HBsAg, and HCV as well as syphilis were carried out by a senior technician and verified by a pathology consultant assigned.

The data was logged onto a Microsoft spreadsheet and later analyzed using IBM Statistics SPSS version 25 (IBM Corp., Armonk, NY). The mean (± SD) was used to report results for quantitative variables, while frequency and percentages were calculated for categorical variables. A Chi-Square test was performed to check the association between gender and blood discard due to TTIs.

RESULTS

During the study period, a total of 18397 blood donations were received from November 2021 to August 2023. Out of which 12870 (69.95%) units were utilized as whole blood and 5527 (30.04%) were separated into different components.

Out of a total of 12870 blood bags (whole blood), 661(5.13%) were wasted for different ages, genders, and reasons. Out of 661 wasted blood bags, 655(99.1%) blood bags were of male donors and 6 (0.9%) blood bags were of female donors. Out of a total of 661 wasted blood bags maximum numbers, 202 (30.56%) wasted blood bags were of blood group B^+, and the minimum number, 7(1.06%) blood bags were of blood group AB Negative as seen in Table-I.

TTI positivity accounted for the majority of whole blood bag wastage, making up 85.17% of cases. Other causes included shelf-life expiration, leakage/breakage, QNS, QC, and hemolysis/contamination, which made up 14.83% of cases as seen in Table-II.

Among the total number of cases that were TTI positive, VDRL was detected in 20.78% of the blood bags, while HBsAg was found in 50.44% of them. HCV was detected in 27.35% of the bags, while only 1.42% of them were positive for HIV. No cases of malaria parasite were detected in the sample. Table-III showed the gender distribution and incidence of TTIs in discarded blood bags. According to the p-values, there is no significant association between gender and the incidence of transfusion transmitted infections (TTIs).

A Total of 13807 blood components were prepared during this study period out of which 609 components were discarded. The most

common blood component discarded were platelets (7.99%) followed by RCC (4.84%), CP (3.23%), and FFP (2.11%) as seen in Table-IV.

A total of 609 blood components were discarded in which the most common cause was positivity for transfusion transmitted diseases (TTI) constituted 43.84% followed by expiry of blood components constituted 21.02% as seen in Table-V.

Table-I: Wastage frequency in different blood groups.

Blood Group	Wastage Frequency n (%)
A^+	170 (25.7%)
A^-	19 (2.9%)
B^+	202 (30.6%)
B^-	9 (1.4%)
AB^+	60 (9.1%)
AB^-	7 (1.1%)
O^+	169 (25.6%)
O^-	25 (3.8%)

Table II: Analysis of reasons for wastage of blood bag (Whole Blood).

Reason of Wastage	Frequency n (%)
TTI positive	563 (85.17%)
Shelf Life Expired	37 (5.59%)
Quality Control (QC)	13 (1.96%)
Quantity Not Sufficient (QNS)	20 (3.02%)
Hemolysis/Contamination	3 (0.45%)
Leakage/Breakage	25 (3.78%)

Table-III: Analysis of discarded blood bags by gender and transfusion transmitted infections (TTIs).

TTIs		Gender		p-Value
		Male	Female	
VDRL	Positive	115 (17.4%)	2 (0.3%)	0.288
	Negative	540 (81.7%)	4 (0.6%)	
HBsAg	Positive	283 (42.8%)	1 (0.2%)	0.189
	Negative	372 (56.3%)	5 (0.8%)	
HCV	Positive	152 (23.0%)	2 (0.3%)	0.426
	Negative	503 (76.1%)	4 (0.6%)	
HIV	Positive	8 (1.2%)	0	0.929
	Negative	647 (97.9%)	6 (0.9%)	

Table-IV: Analysis of Wastage of Different Blood Components against Total Prepared Components

Types of Components	Components Prepared	Wastage of Components
RCC	5527	268 (4.84%)
FFP	5124	108 (2.11%)
PC	2753	220 (7.99%)
CP	403	13 (3.23%)
Total Components	**13807**	**609 (4.41%)**

Table-V: Analysis of reasons for discarding blood components

Blood Components	Reasons for discarding blood Components					
	TTI Positive	Expired	Leakage	QC	QNS	Hemolysis
RCC	121	53	40	32	17	5
FFP	33	24	23	19	6	3
PC	109	49	25	18	9	10
CP	4	2	2	1	3	1
Total	**267**	**128**	**90**	**70**	**35**	**19**

*Red Cell Concentrate (RCC), Fresh Frozen Plasma (FFP), Platelet Concentrate (PC), and Cryoprecipitate (CP).

DISCUSSION

Blood transfusions play a crucial role in modern healthcare. The increase in demand for blood and its components is driven by various factors, including the development of new treatment methods, the aging population, and improved identification of complex disorders that require transfusions. As a result, the need for blood transfusions is steadily rising [11]. To reduce wastage of blood and its components in blood banks, it is essential to practice effective blood management. Conducting a self-audit of whole blood and discarded blood components can help us comprehend the various reasons for their disposal.

Over the course of our investigation, a total of 12870 units of whole blood were gathered. Out of a total of 12870 blood bags (whole blood), 661 were wasted for different ages, genders, and reasons. Out of 661 wasted blood bags, 655(99.1%) were male donors and 6 (0.9%) were female donors. The blood donor statistics from this study are similar to those from studies by Bobde *et al*.8, Lakum *et al*. [2], Patil. P [14]. Out of 661 wasted blood bags, 202 (30.56%) were of blood group B+ while there were only 7 (1.06%) blood bags of blood group AB Negative.

Our study found that an average of 4.48% of blood units were discarded. In previous studies by Patil *et al*. [14], Bobde *et al*. [8], Sharma *et al*. [15]**,** Ghaflez *et al*. [16], and Deb *et al*. [17], Morish *et al*. [20], Kora *et al*. [6], Kumar *et al*. [19], Thakare *et al*. [18], Suresh *et al*. [18], the discard rates were 22.45%, 6.63%, 8.69%, 12%, 14.61%, 2.3%, 4.3%, 8.4%, 3.6%, and 7.0% respectively.

The WB discard rate in the current study was slightly lower at 5.13% compared to the discard rates recorded by Suresh *et al*.18(5.7%) and Bobde *et al*.8 (6.63%). Joshi *et al*., report the rate at which whole blood is wasted in a large blood bank to be about 4-6%, which looks very much the same as the findings in this study of 5.13%. This uniformity is indicative of some problem which is common in the working of a blood bank [12]. The most common reason for discarding whole blood was a positive TTI result, accounting for 85.17% of cases. Other reasons included expired shelf life, leakage or breakage, inability to obtain enough blood during phlebotomy due to collapsed veins, and acute donor reactions such as nausea, vomiting, perspiration, hematoma formation, or fainting during donation. Quality control and hemolysis or contamination were also contributing factors. Out of the 12,870 units of whole blood, 661 were wasted, which accounts for 5.13%. That nearly 99.1% of the blood bags were from male donor's raises questions as to whether there are problems per se with male donations or whether other factors are at work. Further investigation might be necessary to determine if there are inherent problems in the collection, storage, or processing procedures that disproportionately affect male donations. According to the study by Ravikanth *et al*., wastage may relate to demographic factors of whether the donor is male or female. Their findings showed that male donors would have high wastage rates due to a number of donations and some health-related conditions. There was a predominance of wasted male donor blood in the study, thus supporting these findings. It is therefore imperative to continue the study on certain donor-related factors [12]. The distribution of wasted blood bags by blood group indicates that the highest wastages were with blood group B+, 30.56%, while AB Negative had the least at 1.06%. This implies that the blood group B+ may be more predisposed to wastage, probably due to higher volumes of donations, mismatch between supply and demand, or some other challenge peculiar to its management. The low wastage for AB Negative may indicate that this is a rare blood group either of high demand or managed effectively within the system.

The fact that AB Negative comes back as waste in the maximum number compared to the groups of B+ only adds the relatively less frequency as another variable to studies of such kind. Xian *et al*., found that the blood groups that are more commonly found, like B+ and A+, are

wasted more because of the sheer volume of donation and demand variations. By virtue of its rarity and lesser incidence of wastage, the AB Negative group must have something to do with the lesser incidence of its donation as well as the possibility of its better demand management efficiency.

Malakar *et al*., conducted a comprehensive study on blood transfusion practices and reported that TTIs were a leading cause of blood bag discards. Their findings showed that hepatitis B and C were the most frequently detected infections, aligning with the current study's results where HBsAg and HCV were the predominant TTIs [13].

In the present study, the rate of discarding packed red cell concentrate was 5.18%. The most common reason for discarding was transfusion-transmitted infection (TTI), which accounted for 45.1% of the discarded red cell concentrate. Expiration was the second most frequent cause, comprising 9.3% of discards. In the trial conducted by Simon *et al*. [1], the rate of discarding due to expiration was significantly higher at 59%. The process of obtaining whole blood to create random donor platelets was one of the causes of expiration.

Our study found that the percentage of discarded fresh frozen plasma (FFP) was 2.03%, which is lower than the rates reported by Bobde *et al*. [8] (7.6%), Sharma *et al*.[15] (6.2%), and Simon *et al*. [1] (5.5%). Among the reasons for discarding FFP, TTI-positive cases accounted for 31.4%, followed by discarding FFP due to expiry (22.8%) and leakage (20%) which is lower than the 48% reported in the studies conducted by Simon, *et al*. [1] and Kanani, *et al* [21].

Throughout the study, PCs were the most common discarded component. PC had a lower discard rate (7.99%) than Bobde *et al*. [8] (26.2%), Kanani [21] (28.39%), Sharma, *et al*. [15] (43.6%), and Ghaflez *et al*. [16] (58.1%). TTI positive (49%) was the most frequent cause of PC discarding again, followed by leakage and expiration.

Handling and preparing blood components can contribute to a significant reduction in discarded blood units. Increasing the knowledge of blood recipients about appropriate transfusion techniques also plays a role in lowering the discard rate. Additionally, the practice of submitting fresh frozen plasma (FFP) for fractionation has further aided in reducing waste. These improvements in transfusion practices have been achieved through the implementation of appropriate policies and their consistent execution over the years.

LIMITATIONS

The study solely focused on the discard rate and its causes within the blood bank, without considering the blood that was never administered to patients due to various reasons. This could be due to patients passing away before the blood could be administered, malfunctioning cannulae systems or lack of refrigeration systems in the wards to store the blood temporarily leading to wastage of the released blood. Hence, more research is required to address this issue.

CONCLUSION

Adhering strictly to the donor selection criteria based on standard operating procedures, obtaining proper pre-donation history, giving proper counseling, identifying donors who are positive for transfusion-transmitted infections, deferring suspected professional donors who have been previously screened, using antiseptic solutions appropriately, and performing proper serological testing can minimize the wastage of blood and blood components due to TTI. Additionally, proper inventory management and knowledge of the day-to-week basis of blood requirement can reduce wastage due to expiration and outdated components.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

AUTHORS CONTRIBUTION

Iqra Zeb: Data collection, data analysis and literature search and manuscript drafting, accountable for all aspects of the work

Hina Mushtaq: Study design and concept, data interpretation, accountable for all aspects of the work

Hamid Iqbal: Revised the manuscript thoroughly for important intellectual content.

Fuad Ahmad Siddiqui: reviewed the results and approved the final version of the manuscript, accountable for all aspects of the work

Imran Khan: Literature search and critical analysis, accountable for all aspects of the work

Bilal Zeb: Questionnaire design and manuscript drafting, accountable for all aspects of the work

REFERENCES

1. Simon K, Ambroise MM, Ramdas A. Analysis of blood and blood components wastage in a tertiary care hospital in South India. J Central Res Med Sci. 2020; 6(1): 39-44. DOI: http://dx.doi.org/10.4103/jcrsm.jcrsm_9_20
2. Lakum NR, Makwana H, Agnihotri A. An analytical study of discarded units of whole blood and its components in a blood bank at a tertiary-care hospital in Zalawad region of Saurashtra. Int J Med Sci Public Health. 2016 ; 5:318-21. DOI:https://doi.org/10.5455/ijmsph.2016.06102015122
3. Joshi HJ, Patel KB, Dholu M. An analysis of wastage of blood components in blood bank at tertiary care hospital. Int J Clin Diagnostic Pathol. 2021;4(1):138-42. DOI:https://doi.org/10.33545/pathol.2021.v4.i1c.336
4. World Health Organization. Availability, safety and quality of blood products. Report by the Secretariat. Sixty-Third World Health Assembly A 63, 2009.
5. Collins RA, Wisniewski MK, Waters JH, Triulzi DJ, Yazer MH. Effectiveness of multiple initiatives to reduce blood component wastage. Am J Clin Pathol. 2015; 143(3): 329-35. DOI:https://doi.org/10.1309/AJCP42WMHSSTPHXI
6. Kora SA, Kulkarni K. An analysis of donor blood wastage in a blood bank in rural Karnataka. J Clin Diagn Res. 2011; 5(7): 1393-6.
7. Mishra S, Daga A, Gupta A. Inventory management practices in the blood bank of an institute of national importance in India. Fam Med Prim Care Rev. 2021; 10(12): 4489-92. DOI:https://doi.org/10.4103%2Fjfmpc.jfmpc_1000_21
8. Bobde V, Parate S, Kumbhalkar D. Analysis of discard of whole blood and blood components in government hospital blood bank in central India. J Evid Based Med Healthc 2015; 2(9): 1215-19. DOI: http://dx.doi.org/10.18410/jebmh/176
9. Sultan S, Jaffri SA, Irfan SM, Usman SM, Nadeem S, Waheed U, *et al*. An Insight into donor blood unit's wastage in a hospital-based blood bank from Pakistan. Int J Med Res Health Sci. 2021; 10(5): 91-5.
10. Far RM, Rad FS, Abdolazimi Z, Daneshi Kohan MM. Determination of rate and causes of wastage of blood and blood products in Iranian hospitals. DOI: https://dx.doi.org/10.4274/tjh.2012.0105
11. World Health Organization. Quality systems for blood safety: introductory module guidelines and principles for safe blood transfusion practice. Geneva: World Health Organization. 2002:65-75.
12. Ravikanth C, Babu BS, Arun R, Babu KVS. Analysis of blood and blood component wastage and its reasons among various departments in a tertiary care teaching hospital in Southern India. Nat J Lab Med. 2022; 11(2): 37-9. DOI:https://doi.org/10.7860/NJLM/2022/51736.2606
13. Malakar PC, Sharma A, Sharma S, Tiwari D, Sharma V, Dadhich N. Investigating the causes of whole blood and blood components discarded at a tertiary care hospital blood center along with discard rate detection: A prospective study. Asian J Transfus Sci. 2023; 37-9. DOI: https://doi.org/ 10.4103/ajts.ajts_162_22
14. Patil P, Bhake A, Hiwale K. Analysis of discard of whole blood and its components with suggested possible strategies to reduce it. Int J Res Med Sci. 2016; 4(2): 477-81. DOI: https://doi.org/10.18203/2320-6012.ijrms20160299
15. Sharma N, Kaushik S, Kumar R, Azad S, Acharya S, Kudesia S. Causes of wastage of blood and blood components: A retrospective analysis. Platelets. 2010; 878: 43-6. DOI: https://doi.org/10.7759%2Fcureus.59493
16. Ghaflez MB, Omeir HK, Far J, Saki N, Maatoghi JT, Naderpour M. Study of rate and causes of blood components discard among Ahwaz's hospital. Sci J Iran Blood Transfus Organ. 2014; 11(3): 197-206.
17. Deb P, Swarup D, Singh MM. Audit of blood requisition. Med J Armed Forces India. 2001; 57(1): 35-8. DOI: https://doi.org/10.1016%2FS0377-1237(01)80087-3
18. Suresh B, Babu KS, Arun R, Chandramouli P, Jothibai DS. Reasons for discarding whole blood and its components in a tertiary care teaching hospital blood bank in South India. J Clin Sci Res. 2015; 4(3): 213-9.

DOI: http://dx.doi.org/10.15380/2277-5706.JCSR.14.052

19. Kumar A, Sharma SM, Ingole NS, Gangane N. Analysis of reasons for discarding blood and blood components in a blood bank of tertiary care hospital in central India: A prospective study. Int J Med Pub Health. 2014; 4(1): 72-4. DOI: http://dx.doi.org/10.4103/2230-8598.127161
20. Thakare MM, Dixit JV, Goel NK. Reasons for discarding blood from blood bank of government medical college, Aurangabad. Asian J Transfus Sci. 2011; 5(1): 59-60. DOI: https://doi.org/10.4103%2F0973-6247.76009
21. Morish M, Ayob Y, Naim N, Salman H, Muhamad NA, Yusoff NM. Quality indicators for discarding blood in the National Blood Center, Kuala Lumpur. Asian J Transfus Sci. 2012; 6(1): 19. DOI: https://doi.org/10.4103%2F0973-6247.95045
22. Kanani A, Vachhani J, Dholakiya S, Upadhyay S. Analysis on discard of blood and its products with suggested possible strategies to reduce its occurrence in a blood bank of tertiary care hospital in Western India. Global J Transfus Med. 2017; 2(2): 130. DOI:http://dx.doi.org/10.4103/GJTM.GJTM_34_17

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