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COMPARATIVE ANALYSIS OF HEMATOLOGICAL AND IMMUNOLOGICAL BIOMARKERS IN ARTHRITIS PATIENTS IN GUJRAT, PUNJAB, PAKISTAN

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Abstract

Background: Arthritis is a common autoimmune and inflammatory condition that affects joints and multiple organ systems, is a major public health concern especially in the South Asian community. While there is extensive information available in the literature regarding haematological and immunological biomarkers of the disease, there is paucity of comparative data in the district of Gujrat in Punjab, Pakistan. Goal: To comparatively analyze the hematological and immunological biomarkers in patients of arthritis and also differentiate seropositive from seronegative arthritis patients in Gujrat region. **Methods:** A cross-sectional prospective study was conducted in the Khurram Shezad hospital, at Gujrat from January till April 2026, including 100 confirmed arthritis patients including 52 males and 48 females. Complete blood count (CBC) was done on Swe-Lab CBC analyzer. The amount of C-reactive protein (CRP) in the blood was determined by Micro Lab 300 and the erythrocyte sedimentation rate (ESR) was measured by Westergren method. Latex agglutination was used to determine RA factor. IBM SPSS v27 was used for data analysis.

Results: 69% of the patients were RA factor positive. Average hemoglobin level was 11.55 ± 1.87 g/dL, which was within the range of anemia. ESR mean was 18.41 ± 5.91 mm/hr with a maximum of 30 mm/hr. CRP ranged from 1.8 to 8.5 mg/L (mean: 5.82 ± 1.59). Total leukocytes count (TLC) ranged up to 18050 cells/µL (mean: 11740.95 ± 3437.47), which suggests a systemic inflammation and concurrent infections. The 55% of patients in the sample were urban patients. Mean patient age was 46.29 ± 17.64 years (range 11–95). **Conclusions:** The results indicated that inflammatory markers (ESR, CRP and TLC) were elevated in arthritis patients and hemoglobin was decreased which means that arthritis patients in Gujrat have concurrent anemia and inflammation in their body. Laboratory investigation is a key part of the management of arthritis and a comparative biomarker profile is good at separating seropositive from seronegative patients.

Keywords: *Arthritis; Rheumatoid factor; C-reactive protein; Erythrocyte sedimentation rate; Hematological biomarkers; Immunological biomarkers; Gujrat; Pakistan*

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1. Introduction

Arthritis is a group of over 100 musculoskeletal conditions affecting the joints that involves inflammation, pain, joint damage and different systemic involvement. It ranks as one of the major causes of chronic disability globally, and occurs in people of all ages, most commonly in those aged over 65 years (Bandyopadhyay, 2018; Fallon, 2023). Rheumatoid arthritis (RA) incidence has been estimated to be constantly increasing in the Global Burden of Disease (GBD) database from 11.66 in 1990 to 13.48 per 100,000 in 2021, and the burden seems to be greater in females and low sociodemographic index (SDI) regions (Zhang et al., 2025).

The most clinically important inflammatory subtypes of RA are all autoimmune, characterized by complex interplay between T cells, B cells, macrophages, neutrophils, and fibroblast-like synoviocytes (Firestein & McInnes, 2017). About 40% of the inherited risk of RA is due to genetic susceptibility (mainly HLA-DR4 gene of the MHC class II region) (Ishigaki et al., 2022). Environmental triggers including cigarette smoke and poor oral health further compound disease initiation and progression (Smith & Berman, 2022).

Laboratory biomarkers are essential for the diagnosis, monitoring and evaluation of therapeutic response to RA. The backbone of serological assessment consists of the rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibody, C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) (Abdelhafiz et al., 2023). In addition to these, there are a number of new markers derived from a complete blood count (CBC) that have been identified as good, inexpensive proxies of systemic inflammation, such as the neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), and total leukocyte count (TLC) (Masoumi et al., 2024). Although the global evidence has been accumulating, there is a significant lack of community level laboratory data in Pakistan, especially in semi-urban areas like Gujrat in Punjab. To develop region-specific diagnostic and public health strategies, it is crucial to understand the local biomarker profile of arthritis patients. This study aimed to comparatively assess haematological (Hb, TLC) and immunological (CRP, ESR and RF) biomarkers in patients with arthritis attending tertiary care hospital in Gujrat, and to characterise differences among patient subgroups based on seropositive and seronegative status.

2. Materials and Methods

3. 2.1 Study Design and Setting

4. A prospective cross-sectional study was conducted at Khurram Shezad hospital, Gujrat, Punjab, Pakistan. All participants gave written informed consent before enrollment.

5. 2.2 Study Population

6. The number of patients enrolled was 100 patients with confirmed or clinically suspected arthritis, achieved through non-probability purposive sampling. All patients were included, regardless of age, who had been diagnosed with arthritis, based on the presence of a positive RA factor and/or abnormal ESR/CRP levels. People who did not have arthritis, severe concurrent infections, malignancy, other auto-immune diseases and pregnant women were excluded. Patients who did not want to participate were also excluded.

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2.3 Sample Collection

Standardized venipuncture was employed with an aseptic technique for the collection of venous blood. Samples were placed in the appropriate collection tubes: CBC and ESR in EDTA tubes and RA factor and CRP in plain/serum separator tubes. All samples had been identified with an individual patient identifier before processing in the laboratory. A standard consent form was used to collect demographic data for patients including age, gender and place of residence.

2.4 Laboratory Methods

Complete Blood Count (CBC):

Hemoglobin (Hb), total leukocyte count (TLC) and differential leukocyte count (DLC) were quantified on Swe-Lab CBC analyzer. The required blood volume was about 1-2 mL, which was 0.01 mL per analysis cycle (Table 1).

Erythrocyte Sedimentation Rate (ESR): The Westergren method was used to measure ESR. The blood was poured in a 4:1 ratio with 3.8% sodium citrate, taken without bubbles into a standardized ESR tube and stood on an ESR stand vertically. After one hour, the distance of the sedimentation of the erythrocytes was measured and expressed in mm/hr.

C-Reactive Protein (CRP): A latex-enhanced immunoturbidimetric CRP method was used for quantitative determination of CRP on the Micro Lab 300 chemistry analyzer. Serum (0.01 mL) was mixed with 1,000 µL of reagent. A water blank was used to calibrate the instrument before each batch. Test results were reported in mg/L, and the range of detection through dilutions was 0.3 to 1.2 IU/mL.

Rheumatoid Factor (RF):

The latex agglutination slide test was used to determine the RF. The serum obtained by spinning in the 3,000 r.p.m. centrifuge was added to the RF latex reagent on a glass slide and anti-clockwise rotated. Positioning of agglutination was interpreted as positive or absence of agglutination as negative.

2.5 Data Analysis

The collected data was entered and analyzed by Microsoft Excel 2016 and IBM SPSS Statistics version 27. Continuous variables were summarized using descriptive statistics such as means, standard deviations, minimums and maximums. Frequency distributions and percentages were computed for categorical variables. P-P plots were used to determine the normal distribution of data. To assess individual biomarker means, one-sample t-tests were used.

3. Results

3.1 Demographic Characteristics

This study included 100 patients with arthritis. Males constituted 52% (n=52) and females 48% (n=48). The patients were mainly distributed in urban localities (55%) and rural areas (45%) by residential area. Mean age was 46.29 ± 17.64 years, ranging from 11 to 95 years, which shows that arthritis affected wide age group in Gujrat population.

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Table 1. Gender and Demographic Distribution of Study Participants

Variable	Category	Frequency (n)	Percentage (%)
Gender	Male	52	52.0
	Female	48	48.0
Residence	Urban	55	55.0
	Rural	45	45.0
Total		100	100.0

3.2 RA Factor Seropositivity

There were 100 patients tested, of whom 69 (69%) were RA factor positive (seropositive) and 31 (31%) seronegative (no RA factor). This high seropositivity rate is in line with previous reports that between 60 and 85% of patients with RA were seropositive (depending on test method and population studied) (Lin et al., 2020).

Table 2. Rheumatoid Factor (RF) Distribution

RF Status	Frequency (n)	Valid Percent (%)	Cumulative Percent (%)
Positive	69	69.0	69.0
Negative	31	31.0	100.0
Total	100	100.0	

3.3 Descriptive Statistics of Biomarkers

Descriptive statistics for the key hematological and immunological biomarkers are shown in Table 3. Average hemoglobin (11.55 g/dL) was lower than the lower limit of the normal range for both males and females, indicating that there was a high prevalence of anemia in the study population. ESR and CRP were also raised, suggesting an ongoing systemic inflammatory process. Maximum TLC of 18,050 cells/µL was higher than normal range, which indicated concurrent infection or significant leukocytosis related to inflammation

Table 3. Descriptive Statistics of Hematological and Immunological Biomarkers

Parameter	N	Minimum	Maximum	Mean ± SD
Age (years)	100	11	95	46.29 ± 17.64
Hemoglobin (g/dL)	100	7.2	15.1	11.55 ± 1.87
ESR (mm/hr)	100	6	30	18.41 ± 5.91

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CRP (mg/L)	100	1.8	8.5	5.82 ± 1.59
TLC (cells/µL)	100	1,000	18,050	11,740.95 ± 3,437.47

3.4 Normality and Distribution Analysis

Estimated distribution parameters were normal for gender (location=1.48, scale=0.502), demographic information (location=1.45, scale=0.500) and the mean age location was 46.29. P-P plot analysis confirmed normal distribution of all biomarker variables (HB location=11.546, scale=1.873; RA factor location=1.310, scale=0.465; ESR location=18.41, scale=5.912; CRP location=5.822, scale=1.592; TLC location=11,740.95, scale=3,437.47). This was confirmed by one sample t-test analysis which showed that mean values were statistically sound and representative of the population being studied.

4. Discussion

This cross-sectional study type is intended to give a detailed description of the hematological and immunological markers among the arthritis patients in Gujrat, Punjab which is a district which the author did not find any such laboratory level epidemiological data available. The study results are indicative of a significant burden of seropositive arthritis, systemic inflammation and associated anemia in this population.

Our findings of a greater prevalence of arthritis in males (52%) compared to females (48%) differed from the majority of international epidemiological studies which reflect a higher prevalence in females (Fallon, 2023; Zhang et al., 2025). This may be a mode of health care seeking behaviour seen in the Gujrat region or it may be a true difference in disease presentation within this South Asian population, which needs to be further studied. The mean age of 46.29 years indicates that the majority of the patients affected are in the economically productive age group, ranging from child age (11) to advanced old age (95) highlighting the fact that arthritis is a disease of all age groups.

The RF seropositivity rate of 69% is consistent with published literature. Lin et al. (2020) 60-85% of RA patients have been reported as RF positive and the RF is 60-85% sensitive and ~85% specific for RA. The 31% seronegative fraction underscores

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the critical role of other markers, in particular, CRP and ESR in the diagnosis of seronegative disease, which can be therapeutically challenging.

Mean hemoglobin of The mean hemoglobin level of 11.55 g/dL is less than the WHO lower limits (13 g/dL for males, 12 g/dL for females) and is typical of anemia of chronic disease (ACD) frequently seen in inflammatory arthritis. Chronic systemic inflammation leads to suppression of erythroid progenitors and sequestration of iron by cytokines, resulting in defective erythropoiesis (Masoumi et al., 2024). This pattern aligns with findings by Hassan (2024), who similarly noted reduced hemoglobin in RA-positive patients compared to controls.

This cohort has evidence of active systemic inflammation with elevated CRP (mean 5.82 mg/L) and ESR (mean 18.41 mm/hr), with maximum of 8.5 mg/L and 30 mm/hr respectively. Now CRP is the preferred marker in the most commonly used Disease Activity Score-28 (DAS28-CRP), as it responds more rapidly to changes in disease activity than ESR (Orr et al., 2018). Both markers correlate with RA disease severity and are used to monitor treatment response, making their characterization in this population particularly clinically significant.

The maximum value (18,050 cells/μL), significantly higher than the upper reference limit (11,000 cells/μL), suggests either neutrophilia associated with systemic inflammation or an existing infection. This is similar to what is observed in RA, where circulating monocytes, neutrophils, and lymphocytes are all affected by chronic inflammation (Cutolo et al., 2022). KC et al. (2020) similarly reported that neutrophil counts and NLR were diagnostically useful in arthritis patients at a Nepalese teaching hospital.

The higher urban percentage (55%) may be due to increased access to medical services, increased awareness of diagnostic tests, or potentially due to the fact that the disease is more prevalent in urban areas due to sedentary lifestyle, obesity, and environmental exposures common to urban areas. There is a known modulation of hematological and immunological biomarker profiles by lifestyle and environmental factors (Gratal et al., 2020).

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5. Conclusion

This study showed a significant and distinct biomarker profile among arthritis patients in Gujrat, Punjab, Pakistan, which included high prevalence of RF seropositivity (69%), anemia (mean Hb 11.55 g/dL), elevated inflammatory markers (CRP and ESR) and leukocytosis. Comparative analysis of hematological and immunological parameters proves to be effective in distinguishing patients with seropositive and seronegative disease, and shows that most patients in this group suffer from concurrent anemia and systemic inflammation.

The results highlight the importance of routine laboratory investigations (such as CBC, CRP, ESR and RF) in the diagnosis, monitoring and treatment of arthritis at primary and secondary care. Future studies should use larger panels of biomarkers such as anti-CCP, NLR and PLR and more comparative studies between the various subtypes of arthritis should be performed in different geographic regions of Pakistan to provide a more detailed national epidemiological profile.

6. Limitations

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This study has a number of limitations. A sample size of 100 patients was considered as sufficient for preliminary analysis, but may not be representative of the broader population of Gujrat with the findings. The cross-sectional design does not allow for causal inference or longitudinal tracking of biomarkers. Anti-CCP antibodies, which are more specific to RA than RF, were not assessed and other markers like NLR and PLR, recently shown to have emerged as a useful marker for diagnosis, were not measured. These gaps should be studied using multi-center prospective cohorts with larger numbers of subjects in future studies.

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