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Albumin- Fibrinogen Ratio- A New Marker In Rheumatoid Arthritis Patients.

Samyuktha Umashankar[1], Chitra Siva Sankari G[2]*, Amuthavalli V[3], and Jemima Ajitha[4].

[1]Final year MBBS, Madras Medical College, Chennai, Tamil Nadu, India.
[2]Senior Assistant Professor, Institute of Biochemistry, Madras Medical College, Chennai, Tamil Nadu, India.
[3]Professor, Institute of Biochemistry, Madras Medical College, Chennai, Tamil Nadu, India.
[4]Assistant Professor, Department of Biochemistry, Sree Balaji Medical College and hospital, Chennai, Tamil Nadu, India.

ABSTRACT

Rheumatoid arthritis (RA) is a chronic multi-system disease, characterised by persistent inflammatory synovitis progressing to cartilage damage and disability. Inflammatory changes play a major role in the pathogenesis of rheumatoid arthritis. Albumin and fibrinogen are vital proteins synthesised by the liver. While albumin is affected positively by inflammation, fibrinogen is a negative Acute phase reactant. In this study, albumin fibrinogen ratio was measured in RA patients and compared with healthy controls. Albumin to fibrinogen ratio acts as a promising biomarker in patients with various inflammatory conditions to detect early changes. Case control study with 101 participants recruited from RGGGH, out of which 69 RA cases based on modified criteria and 32 apparently healthy controls were included. Serum creatinine, AST, ALT, ALP, urea, albumin, fibrinogen was estimated using Beckman Coulter AU480 analyser and AFR was calculated. Statistical analysis was done using SPSS version 16.0. Analysis by independent t test revealed a significant difference in the mean value between albumin, fibrinogen and AFR between the cases and controls. Significant negative correlation was found between the AFR values of cases and controls with AFR value decreasing in cases. P value was found to be highly significant with a value < 0.001. Albumin fibrinogen ratio decreases in patients with RA even in those with seronegative arthritis. Hence measurement of AF ratio can be used as an early diagnostic marker for Rheumatoid arthritis patients helping in disability prevention.

Keywords: Albumin- fibrinogen Ratio, Rheumatoid arthritis, biomarker, EULAR criteria

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Corresponding author

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INTRODUCTION

Auto-immune disorders are those that manifest due to a dysregulated immune system which results in the body's immune system targeting selfantigens. Rheumatoid Arthritis (RA) is the most common autoimmune disorder worldwide, with a prevalence between 0.44% and 0.46% and a female predisposition between 2 to 9 times that of males [1, 2]. Prevalence in the Indian population stands at 0.75% [3].

RA is a chronic systemic disorder. While it primarily affects small joints, it can progress to involve the lungs, skin, and heart as well. Disease manifestations are not uniform across the population and are determined by various genetic and environmental triggers. Mutation in the HLA- DRB1 gene is seen in those with RF and ACPA antibodies. Females, family history, and exposure to smoke are some triggers [4, 5]. Oral contraceptive pills and breastfeeding are some protective environmental factors [5].

Loss of central tolerance in these patients results in the body's innate and adaptive immune system attacking citrullinated self-antigens. This causes inflammation and damage to the synovium leading to the recruitment of macrophages and fibroblasts causing synovial cell hypertrophy, eventually progressing to pannus formation and erosion. These changes clinically manifest as pain, swelling, stiffness, limitation of movement, and deformities [5-7].

Early detection and treatment are of paramount importance in preventing deformities and subsequent disability. Diagnosis of RA is made via modified ACR- EULAR criteria which take into consideration- symptoms, serological values like RF, ACPA, CRP, ESR, and imaging [8]. However, RA factor and ACPA testing are not available in all centers and are negative in seronegative arthritis patients. Thus, biochemical parameters like albumin and fibrinogen (both acute phase reactants) can be a viable and economic option in diagnosing RA patients and preventing a delay.

Albumin is the most abundant protein in the serum and is synthesized and excreted by the liver. It plays an important role in maintaining the oncotic pressure and in the transport of various metabolites [9]. It also acts as a negative acute phase reactant with its levels decreasing with the rise of inflammation in the body. There is a correlation between hypoalbuminemia and patients with severe RA [10, 11].

Fibrinogen, an important coagulation protein synthesized in the liver is a positive acute phase reactant. Levels of fibrinogen are linked to inflammation in the body. Studies have shown an increase in fibrinogen levels in patients with RA, multiple sclerosis, vascular disorders, infections, and even cancer [12].

Albumin to fibrinogen ratio, a calculated parameter is a novel nutritional and inflammatory marker(13). This study was undertaken to evaluate the role of albumin, fibrinogen, and AFR in supporting early detection of RA among patients visiting the OPD of Rajiv Gandhi Government General Hospital (RGGGH), Chennai.

Aim And Objectives

To compare the levels of AFR with associated parameters between RA patients and healthy controls

MATERIALS AND METHODS

This case-control study was conducted during the period of January 2022 to July 2023 in the Institute of Biochemistry and Institute of Rheumatology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai- 3.

Study Population

The study was conducted after getting ethical committee approval and comprised a total number of 101 participants. 69 previously diagnosed RA patients from the Institute of Rheumatology were recruited as cases. 32 apparently healthy patients visiting the OPD for non-inflammatory diseases were included as controls.

Cases: Patients diagnosed as RA according to the modified ACR- EULAR criteria.

Controls: Apparently healthy individuals

Inclusion Criteria: RA patients diagnosed according to the modified ACR-EULAR criteria

Exclusion Criteria:

- Co-morbidities like diabetes mellitus, hypertension
- Other inflammatory joint disorders and concomitant autoimmune disorders
- Any renal or liver pathology

MATERIAL AND METHODS

Sample Collection

After written informed consent was obtained. A 5 ml sterile venous blood sample was collected in red and blue vacutainers and serum was separated by centrifugation at 3000rpm for 15 mins and stored at -20^{0}c for further analysis.

Quantitative analysis of serum fibrinogen was done using the scattered light detection method. Serum albumin, creatinine, AST, ALT, and ALP were analyzed using a conventional automated (Beckman Coulter AU480) analyzer. Qualitative analysis of RA factor and ACPA was done using a latex agglutination test. The albumin to fibrinogen ratio was calculated. Serum albumin was measured using the bromocresol green, Endpoint assay. Serum creatinine was measured using modified Jaffe's method. Serum alanine aminotransferase (SGPT) and serum aspartate aminotransferase (SGOT) were estimated by Dynamic extended stability modified IFCC method; and serum alkaline phosphatase was measured using IFCC- kinetic method The data obtained was processed via SPSS software version 16.0.

Statistical Analysis

SPSS software version 16.0 was used to analyze the data. Mean value and SD deviation were obtained for albumin, fibrinogen, and AFR levels among the cases and control. Student t-test was used to compare the mean values between cases and controls. A p-value of <0.05 was taken to be significant (*). Mann- Whitney test was also used to compare the mean values between the 2 groups. To assess the sensitivity and specificity of AFR, receiver operating characteristic curve (ROC) analysis was done. Values falling under the curve are an indicator of diseased patients.

RESULTS

Table 1: Demographic details

Parameter	Cases	Controls
No. of participants	69	32
Age	47.37	48
Sex (M/F)	7/62	9/23

Table 2: Comparison of various factors in RA cases and healthy controls

Biochemical parameter	Cases	Control	P value
Albumin (g/dl)	4.36 ± 0.21	4.55 ± 0.37	0.003*
Fibrinogen (mg/dl)	473.8 ± 87.55	345.75 ± 173.39	0.001*
Albumin Fibrinogen Ratio	9.53 ± 1.93	17.05 ± 9.62	<0.001*
Serum Creatinine (mg/dl)	0.65 ± 0.13	0.75 ± 0.17	0.003*

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Serum Urea (mg/dl)	17.23 ⍰ 4.59	19.96⍰ 4.30	0.011*
AST	22.34 ⍰ 6.71	20.70 ⍰ 5.72	0.276
ALT	19.53 ⍰ 5.96	18.11 ⍰ 6.42	0.321
ALP	96.98 ⍰ 31.97	80.63 ⍰ 16.95	0.003*
RF (+/-)	21/48		
ACPA			

Table 3: Mean Concentration Of Albumin Among Cases And Controls

Parameter	Cases	Controls	p-value
Albumin (g/dL)	4.36⍰ 0.21	4.55 ⍰ 0.37	0.003*

The mean value of albumin in RA cases is 4.36⍰ 0.21 and that of controls is 4.55 ⍰ 0.37. p-value is <0.05 and it is found to be significant.

Figure 1: Box Whisker Plot For Albumin Among Cases And Controls

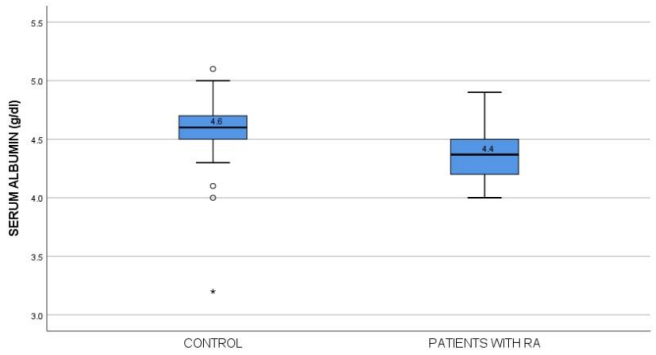

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Figure 01- shows a significant decrease in albumin concentration among cases in comparison to controls

Table 4: Mean Concentration Of Fibrinogen Between Study Groups

Parameter	Cases	Controls	p-value
Fibrinogen (mg/dL)	473.8⍰87.55	473.8⍰87.55	0.001*

The mean value of fibrinogen in RA cases is 473.8⍰87.55 and that of controls is 473.8⍰87.55. p-value is <0.05 and it is found to be significant

Figure 2: Box Whisker Plot For Fibrinogen Among Cases And Controls

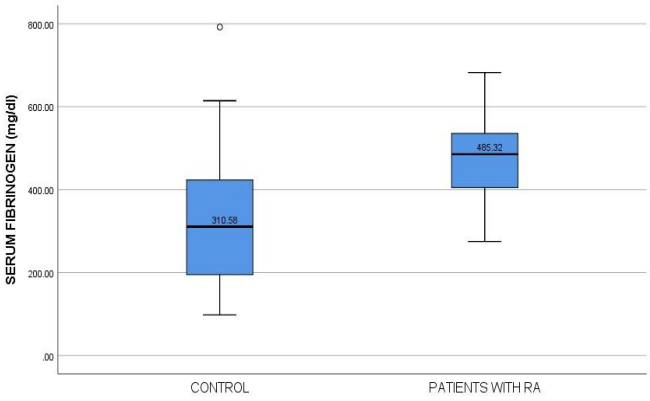

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Figure 02- shows a significant increase in fibrinogen concentration among cases in comparison to controls

Table 5: Mean Concentration Of Albumin-Fibrinogen Ratio Between Study Groups

Parameter	Cases	Controls	P- value
Albumin Fibrinogen Ratio	9.53±1.93	16.92±9.22	<0.001

The mean value of albumin- fibrinogen ratio in RA cases is 9.53±1.93 and that of controls is 16.92±9.22. p-value is <0.05 and it is found to be significant

Figure 3: Box Whisker Plot For Albumin-Fibrinogen Among Cases And Controls

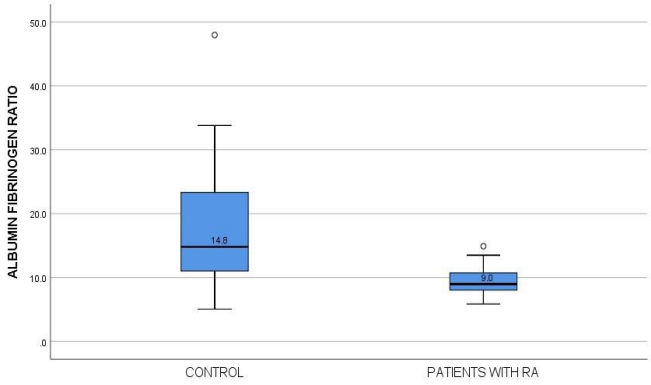

Figure 03- shows a significant decrease in albumin to fibrinogen ratio among cases in comparison to controls

Figure 4: Receiver Operations Characteristic Curve Of Albumin- Fibrinogen Ratio

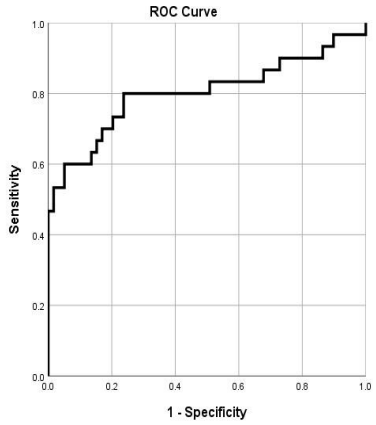

FIG 04-According to the ROC curve, a value of 7.9 of AFR, gives a specificity of 90% and sensitivity of 89%.

Table 6: Receiver Operations Characteristic Curve Of Albumin- Fibrinogen Ratio

			Asymptotic 95% Confidence Interval	
Area	Std. Error	Asymptotic Sig. [b]	Lower Bound	Upper Bound
.802	.059	.000	.687	.917

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Under the nonparametric assumption

a. Null hypothesis: true area = 0.5

Since AUC>0.8 this diagnostic test is considered acceptable

Figure 5: Receiver Operations Characteristic Curve Comparing Values AFR, Fibrinogen, Albumin

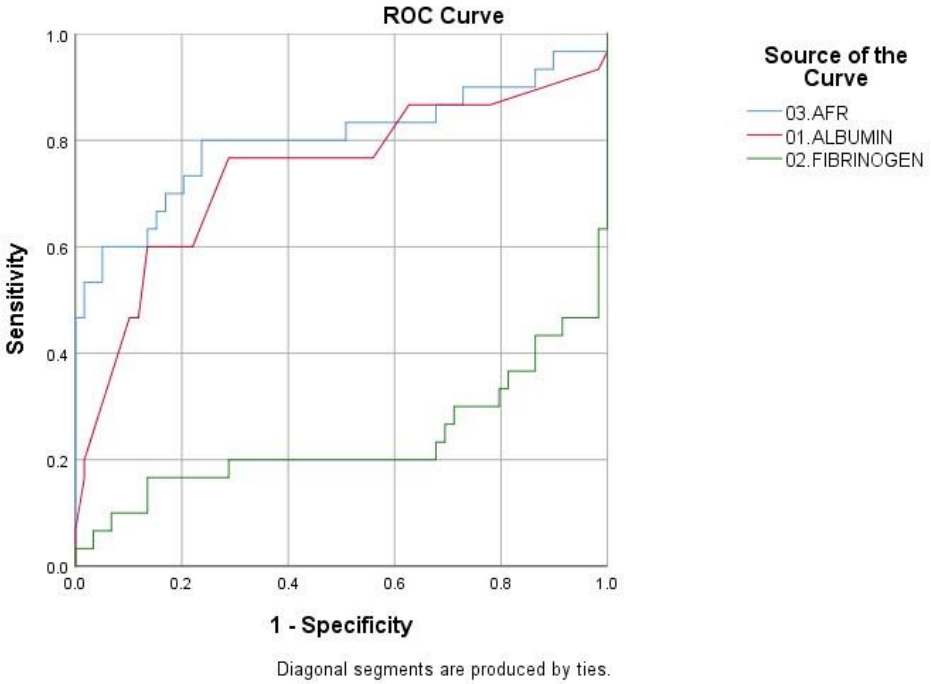

Fig 05-AFR shows the highest significance with the largest area covered

Table 7: Receiver Operations Characteristic Curve Comparing Values AFR, Fibrinogen, Albumin

Test Result Variable(s)	Area	Std. Error	Asymptotic Sig. [b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
03. AFR	.802	.059	.000	.687	.917
01.ALBUMIN	.739	.063	.000	.616	.862
02. FIBRINOGEN	.236	.065	.000	.109	.363

AFR shows the highest significance when compared to albumin and fibrinogen with the highest area under the curve.

DISCUSSION

Rheumatoid arthritis is an autoimmune disorder that commonly affects females in the reproductive age group. Clinical presentation of RA starts slowly as small joint pain (Proximal interphalangeal and Metacarpophalangeal joint). Most patients tend to ignore this or self-medicate with NSAIDs. Since the presentation of RA can mimic osteoarthritis there is a delay in proper diagnosis and start of treatment. RA is a serious and debilitating disorder. It's imperative that treatment for RA starts as early as possible preferably within 3 months since irreversible joint damage is said to occur within this time.

The delay in diagnosis and treatment is also due to a lack of testing facilities for RA factor and ACPA antibodies and a delay in confirming cases of seronegative arthritis. Recent studies show AFR as a marker of interest in several inflammatory conditions.

This study includes a control group of 32 and a study group of 69, with age limits in a comparable range (Mean- cases/ controls- 47.3/ 48). As tabulated in Table 1, out of the 101 study participants 62 of the 69 cases are female and 23 of the 32 controls are female. The higher female population in this study higher prevalence of RA among females.

On analyzing the various parameters in this study, they were found to be within the reference ranges. The parameters included in this study were albumin, fibrinogen, AFR, Urea, creatinine, AST, and ALT. In this study AFR was calculated and along with Fibrinogen was taken into consideration.

Albumin and RA

As shown in Table 3 and Figure 1, on comparing the mean values of albumin between cases (4.36 ± 0.21) and controls (4.55 ± 0.37) the p-value was found to be highly significant (p-value- 0.003) in this study as in the previous studies by Ganeb et al and Chekkouri et al [14, 15]. Albumin being a negative acute phase reactant was found to be low among cases raising the question of nutrition [16].

Fibrinogen and RA

In this study, fibrinogen levels were found to be highly significant, with a pvalue of 0.001, on comparing the mean values between cases (473.8±87.55) and controls (345.75 ± 173.39) as shown in Table 4 and Figure 2. The increase in fibrinogen can be attributed to its role as a positive acute phase reactant as seen in studies by Rooney et al and Sharma et al [17, 18] and it increases progressively with inflammation.

AFR and RA

In this study, the decrease in Albumin to fibrinogen ratio value was found to be highly significant (p-value- <0.001) among RA cases (9.53 ± 1.93) than controls (16.92±9.22) as shown in Table 2. This correlates with previous studies by He et al, Afifi et al and Yang et al(13,19,20). So taking the ratio AFR levels was found to more useful than taking albumin and fibrinogen separately. As shown in Fig 5 and Table 7, this was confirmed by the area under the curve (AUC) of ROC (0.9).

Liver parameters and RA

Liver involvement is not a common extra-articular presentation in RA, hence there is no significant elevation in liver parameters in RA patients. Elevated liver enzymes have been reported in RA patients due to chronic DMARDS use, especially in those on methotrexate [21]. However, liver biopsy following autopsy of RA patients shows that 65% of RA patients have diffuse lymphocytic infiltrate, periportal fibrosis, and portal hypertension [22].

In this study, AST and ALT were not found to be significant (p-value- 0.276 and 0.321respectively) and ALP was significant with a p-value of 0.003(refer to Table 2). The normal liver parameters seen among cases in this study can be because the cases were RA patients in the early stage of disease recruited from the OPD. This is in contrast to studies by Suzuki et al, Kremer et al, Fries et al and Nanke et al [23-26] where the liver parameters were found to be increased probably due to long term Methotrexate usage.

Renal parameters and RA

Renal parameters like Serum Urea and creatinine values for cases and controls was within the normal range. This is in accordance with studies by Anders et al and Cockel et al [27,28]. Renal involvement in RA patients is rare and happens in those with long-standing RA, or with long-term use of nephrotoxic drugs [29].

Patients with pre-existing renal and liver pathologies were excluded from this study because the values of albumin and fibrinogen would be influenced by the same, hence the normal values seen in renal and liver parameters cannot be taken as a limitation.

CONCLUSIONS

This study was conducted among 69 recently diagnosed rheumatoid arthritis cases and 32 age and sex-matched apparently healthy controls for the purpose. various parameters like serum albumin, fibrinogen, liver and renal parameters were taken and compared. Serum albumin values were lower and fibrinogen levels were higher indicating poor nutrition status and progress of inflammation.

Albumin- fibrinogen ratio was calculated and compared between cases and controls, and this was found to be highly significant marker for disease progression rather than comparing the individual values. This study confirms the same. This new marker of inflammation can aid in the early diagnosis and prognosis of RA in resource-poor settings and in seronegative arthritis.

Limitations Of The Study

Sample size may be increased and obtained from different secondary and tertiary care centers for more generalised interpretation.. RA patients in the late stage were not taken, and hence this study holds true only for early cases of RA. Follow up study with a large cohort is needed in the future to strengthen the study.

Scope Of The Study

- AFR can be a potential marker to differentiate between Rheumatoid arthritis and osteoarthritis.
- AFR changes can be corelated with Disease activity score- 28 score to assess changes that may arise with varying disease severity.

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