GENDER BASED CORRELATION OF INFLAMMATORY MARKERS IN RHEUMATOID ARTHRITIS

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ABSTRACT: Rheumatoid arthritis (RA) is a chronic inflammatory disease with uncontrolled damage of synovial tissues. Nearly one percent of world population is known to be affected by RA. Clinical testing and imaging studies of joints help in the diagnosis and monitoring of disease progression. This disease is more prevalent in females but literature lacks the comparative study showing biochemical changes leading to more damaging effect of this pathophysiological condition in females in comparison to males. Study was planned to report correlation of RA with physiological and biochemical changes in females. To achieve the aim both male and female subjects attending OPD at local hospitals were selected and their blood samples were tested for inflammatory markers: free radicals estimation, levels of RA factor (RF) and C- reactive proteins (CRP). In addition disease activity score was also measured. A distinct difference in the levels of all the parameters in males and females was observed which could be correlated with inflammatory markers. It could be concluded that DAS and studied parameters can be employed to assess the progression of RA.

Keywords- Rheumatoid Factor, C- reactive proteins, serum free radicals, antioxidant activity and Erythrocyte sedimentation rate.

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INTRODUCTION

Among number of autoimmune disorders rheumatoid arthritis (RA) is the most commonly occurring disorder affecting approximately one percent of the world population [13] with females to male ratio of 4:1 [10]. RA is a chronic, systemic inflammatory disorder principally attacking the synovial joints causing inflammation of the tissue surrounding them ultimately leading to functional disability [3]. A wide array of factors including geographical location, age, gender, infection, oxidative stress and genetic predisposition are known to be involved in the list of causative agents of RA [4]. Reactive oxygen and nitrogen species have been shown to cause tissue injury and pathophysiological consequences in chronic inflammatory conditions as in RA [17].

Diagnosis of RA relies mainly on patient history and radiographic evidence of joint damage. But testing the samples in laboratory helps in differentiating RA from similar conditions like polyarthralgia and other inflammatory disorders [8] The American College of Rheumatology Subcommittee on Rheumatoid Arthritis (ACRSRA) recommends laboratory evaluations should include a complete blood cell count with differential, rheumatoid factor, and erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) [12]. RF is the most widely used laboratory marker of RA which is one of IgM, IgA and IgG antibodies acting as autoantibody [15]. CRP, an acute phase protein [6] and ESR are markers of inflammation which elevates above normal range in response to inflammatory conditions [14].

Free radicals are known to play important role in the pathogenisis of RA affecting tissues surrounding the joints. The major concerned are superoxide (Oxygen free radical) and nitric oxide NO (nitrogen free radical). They are beneficial in low quantities to fight microbial invasion in body as in respiratory burst during macrophage action, regulation of blood vessel tone, inflammation, mitochondrial functions and apoptosis [16]. But there elevated levels leads to their damaging action in the body by chemically modifying proteins, carbohydrates, DNA and lipids [1]. The concentration of their metabolites in serum, plasma, urine is used for the in vivo measurement of their presence in body.

METHODOLOGY

Subjects and sample collection

Blood samples and complete clinical profile of the subjects were collected from OPD with their consent according to the guidelines of Institutional Committee for Ethical Clearance (ICEC) for which ethical clearance by **ICEC** was already taken with ICEC No. 149/DLS/HG and 150/DLS/HG

The blood samples were subjected to various tests for physiological and biochemical analysis. Subjects were divided into five groups and the criterion followed for the division of subjects is given below:

Rheumatoid Arthritis positive female subjects	S1
Rheumatoid Arthritis negative female subjects with similar symptoms	S2
Rheumatoid Arthritis positive male subjects	S3
Normal Female subjects with no related health issues	S4
Normal Male subjects with no related health issues	S5

Table 1. Division of subjects into five different groups

Subjects suffering from any other health problems, pregnant females**,** females that undergone ovariectomy and subjects taking drugs or hormone supplements were excluded and normal subjects with no disease incidence were taken as control.

Blood sample analysis

Collected blood samples were employed for following tests.

- **Determination of C-reactive protein and RA factor by Kits based on slide reversed passive latex agglutination method [18]**

  Polystyrene latex particles coated with anti-CRP/RF monoclonal antibody provided in the kit when incubated with different dilutions of positive serum containing levels higher than normal range a clearly visible agglutination is observed macroscopically within two minutes. The highest dilution of serum sample showing agglutination corresponds to the titer of the test sample. Approximate concentration of the test agent was obtained using following formula. CRP mg/dl or RF IU/dl =S x D, where D is the highest dilution showing visible agglutination and S is the sensitivity of the test mentioned in the kit.

- **Disease activity score (DAS) measurement according to the guidelines of American Association of Rheumatism [2]**

  The Disease Activity Score (DAS) is a combined index that has been developed in Nijmegen in the eighties to measure the disease activity in patients with Rheumatoid Arthritis (RA). It was calculated from the number of swollen and tender joints along with measure of ESR or CRP. The DAS provided a number between 0 and 10, indicating activeness of the rheumatoid arthritis.

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- **Total anti oxidant activity (TAA) in blood serum [7]**
  Spectrophotometric detection of TAA of serum was based on the antioxidant capacity of serum to compete with nitro blue tetrazolium (NBT) for superoxide anion radicals of oxygen, which were resulted from aerobic interaction of the reduced form of nicotinamide adenine dinucleotide (NAD • H) and phenazine methosulfate (PMS). NBT was reduced to form formazan (blue) that was detected photometrically at 560 nm.
- **Serum nitrite levels [9]**
  It was done with griess reaction under conditions of low pH. The serum samples were incubated with freshly prepared griess reagent and incubated for 10 minutes at 37˚C. The serum nitrite levels were measured by the formation of magenta colored diazonium salt with absorption spectrum at 543 nm.
- **Erythrocyte sedimentation rate [11]**
  This test was performed to indicate the presence of infection, inflammation or malignancy. Anticoagulated whole blood was allowed to stand in a narrow vertical tube for one hour. RBCs – under the influence of gravity - settled out from the plasma. The level of clear fluid above settled erythrocytes was measured and expressed as millimetres per hour.

Statistical analyses
Statistical analysis was undertaken using the Daniel's XL Toolbox 4.0. Data in tables are expressed as the mean (SE). Two way ANOVA test was used as appropriate statistical methods for analyses. Differences were considered to be significant if p values were <0.05.

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RESULTS

Observations made from the planned study are given in Table 2.

Subjects (N)	CRP mg/dl	RA IU/dl	DAS	NITRITE mg/ml	AOA Percentage NBT reduction	ESR mm/hr
RA positive females (25)	15.95±3.543	239.6±37.11	5.03±0.21	57.01±10.07	47.21±5.54	35.96±2.89
RA positive males(6)	5.1±1.989	150.67±52.25	3.74±0.38	38.89±5.03	43.35±5.53	15±1.47
RA negative females(20)	8±1.959	Negative	Negative	43.13±0.77	10.46±3.08	20.6±1.24
Normal males(10)	Negative	Negative	Negative	2.42±2.62	2.51±0.61	11.67±1.08
Normal females(12)	Negative	Negative	Negative	4.31±0.82	5.48±2.94	14.58±0.89

Table 2 Levels of inflammatory markers in subjects of all groups

Out of 73 subjects selected for the study 42 percent were RA positive attending OPD and all the subjects were analyzed of planned biochemical and physiological parameters. Male to female ratio

of the prevalence of RA was 4:1. Using reverse slide passive latex quantitative agglutination method serum CRP and RF levels were determined in all the subjects that showed RA positive females with 15.95 mg/dl CRP and 239.6 IU/dl RF as compared to RA positive males with 8 mg/dl and 150.67 IU/dl RF levels. Normal healthy subjects were both CRP and RA negative whereas females with similar health issues as those of RA positive were CRP positive with 5.1 mg/dl serum C-reactive proteins level. DAS was 5.03 in RA positive females and 2.9 in RA positive male subjects. Results are shown graphically in Figure 1, 2 and 3.

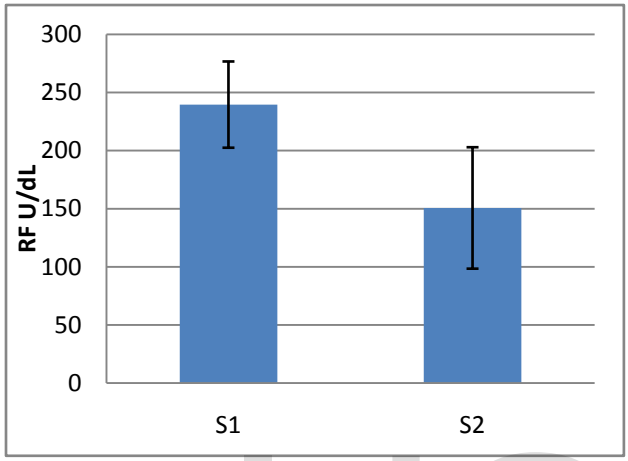

Fig 1. RA factor in diseased RA positive subjects.

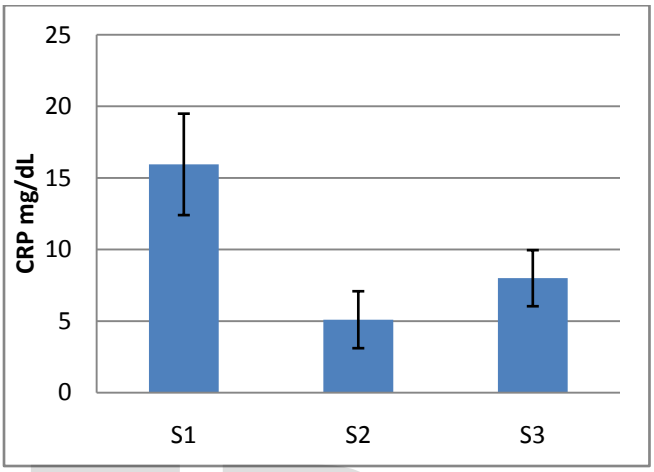

Fig 2. CRP levels in diseased RA positive and negative subjects.

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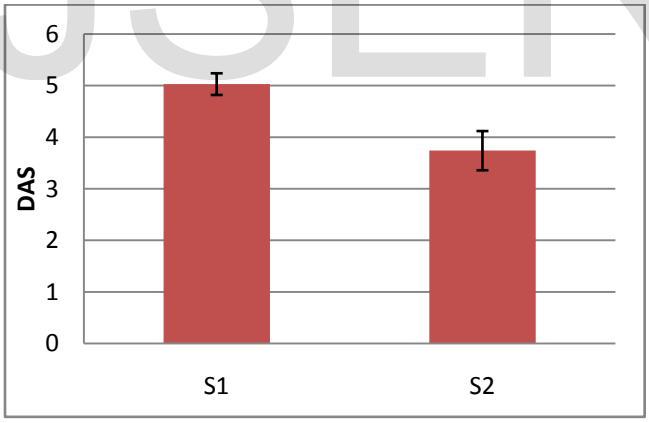

Fig 3. DAS in RA positive male and female subjects

Female subjects suffering from rheumatoid arthritis had shown 47 percent NBT and CRP positive normal female subjects were showing 43 percent NBT reduction followed by RA positive males with 10 percent. Inflammation in infections and autoimmunity leads to increased production of nitric oxide by Nitric oxide Synthase enzyme. Nitric oxide was measured indirectly from the serum free nitrite levels employing griess reaction. RA positive females had 57.02 mg/l free nitrite in serum followed by RA positive males (43.13 mg/l) and CRP positive females (38.89 mg/l). RA positive females showed highest levels of ESR (35.96 mm/hr) followed by CRP positive females (20.6 mm/hr) and RA positive males

(15 mm/hr). Normal healthy subjects were having maximum antioxidant potential, lowest nitrite levels and ESR. But according to the observations normal females had lower antioxidant potential, higher serum nitrite and blood ESR levels as compared to normal males. Results are illustrated in fig 4,5 and 6.

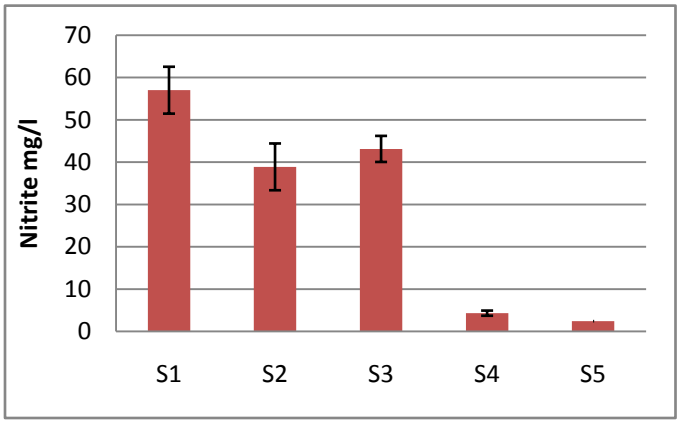

Fig 4. Serum nitrite levels in subjects of different group

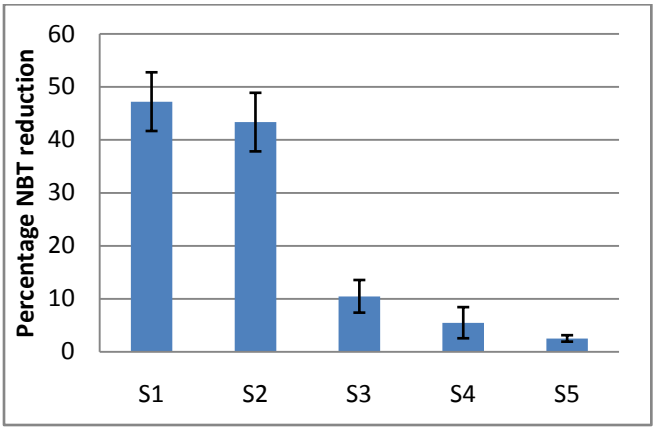

Fig 5. Percentage NBT reduction showing free oxygen radicals

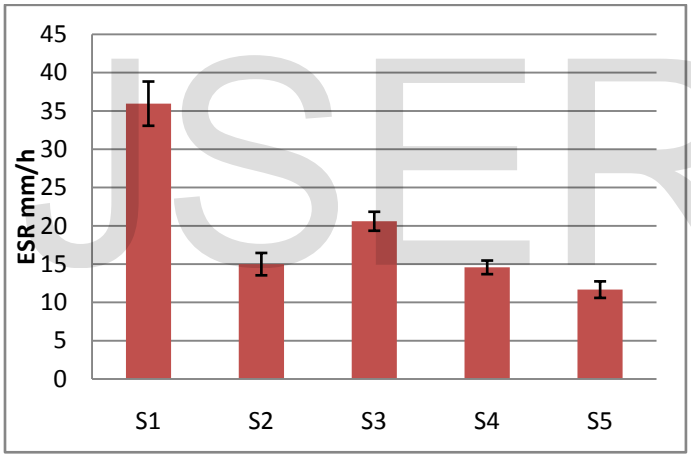

Fig 6. ESR levels in different groups of subjects

DISCUSSION

The immune system is altered by physiological and biochemical changes in human body. One example of such alterations is autoimmunity. Autoimmunity leads the body into the state of functional damage. Prior to organ/ tissue damage number of alterations takes place in the body that are directly linked to the immune response. Rheumatoid arthritis, an inflammatory disorder is one of the generalized conditions of autoimmunity. The pathophysiology involved in RA is alteration in the levels of some inflammatory markers that results in the cascade of the effects leading to partial or complete functional lose of joint.

The present work was done to study gender based correlation of inflammatory markers in diseased subjects in comparison to the healthy subjects. Our study showed clinically relevant results with 89 percent RA positive subjects as females showing higher levels of inflammatory markers in the body as

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compared to the males. Similar outcomes were observed by Nago *et al*., 2014 which suggested female predominance in RA. Diseased females have lowest antioxidant potential in their serum as they have shown maximum NBT reduction among all the groups followed by RA negative females with similar symptoms. Maximum serum nitrite level that is indirect measurement of the activity of nitric oxide synthase was observed in RA positive females followed by RA positive males. Our results corroborates with Ersoy, 2002 showing that RA positive subjects had maximum concentration of free radicals in their serum. Rindfleisch, 2005 reported that ESR a marker of inflammation was above 30mm/hr in RA positive subjects females subjects. RF in RA positive females was 1.6 times higher than RA positive males. CRP levels were maximum in RA positive females followed by RA positive males and RA negative females with similar symptoms. DAS was calculated in RA positive patients from the number of tender joints, swollen joints and blood markers of inflammation i.e. either ESR or CRP. DAS28 less than 3.2 indicate low disease activity whereas more than 5.1 show high disease activity. Out of all the RA positive female subjects 56% patients had moderate disease activity of 4.206 and 44% patients have high disease activity of 6.08. RA positive male subjects had shown low disease activity of 2.9.

CONCLUSION

Results of the study concluded a correlation between the biochemical and physiological state of RA with gender. Both RA positive and normal females had shown higher levels of all the studied inflammatory markers as compared to males of corresponding groups. Hormonal variations in females can be attributed as the major cause of raised levels of inflammations. Sex hormones regulate the function of immune system and hormonal variations leads to elevated inflammatory conditions in the females. As males do not showed any such variations the presence of inflammation is low in them even in diseased condition.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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