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Assessment of Some Haemo-rheological Parameters in Descent of Rumuche, Emohua, Rivers State, Nigeria with Rheumatoid Arthritis Disease

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Authors' contributions

This work was carried out in collaboration among all authors. Author SGC designed supervised the study and performed the statistical analysis. Author MEO carried out the analysis and wrote the first draft of the manuscript. Author RBJ assisted with some of the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study assessed some haemo-rheological parameters on individuals with Rheumatoid Arthritis disease.
Study Design: A cross sectional, case-control field based design was employed with a total of eighty-six subjects consisting of thirty-one males and fifty-five females aged between 20- 80 years. Thirty-nine positive subjects for rheumatoid arthritis served as test while forty-seven negative subjects were used as control.
Methodology: Samples obtained were screened using latex agglutination method for the presence of rheumatoid factor, fibrinogen estimation was done using ELISA method, packed cell volume was determined using microhaeamatocrit method, erythrocyte sedimentation rate was determined using Westergren method and haemoglobin concentration was determined using cyanmethaemoglobin method. Data obtained were analyzed using Graph-pad prism 8.2 version.

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Results: Result showed that 45.3% of the studied population were positive for rheumatoid arthritis (females: 31.4%; males: 13.9%). Gender based percentage in relation to positivity for rheumatoid arthritis showed that females (49.0%) were more affected than males (38.7%). Between those without rheumatoid arthritis (control) and those with rheumatoid arthritis (test), there was statistically significant increase in mean values of erythrocyte sedimentation rate in test group when compared to the control group ($p < 0.0001$); there was no statistical significant difference ($p > 0.05$) in other parameters. Gender based comparison of studied parameters based on positivity and negativity of rheumatoid arthritis showed a statistically significant increase in erythrocyte sedimentation rate: 36.67 ± 14.87 mm/hr versus 10.79 ± 11.00 mm/hr($p < 0.0001$) in test group for males while packed cell volume, haemoglobin and fibrinogen showed no statistically significant difference ($p > 0.05$). In females, there was statistical significant increase in packed cell volume: 36.56 ± 3.896% versus 33.96 ± 4.501% ($p < 0.05$) and erythrocyte sedimentation rate: 50.07 ± 28.73 mm/hr versus 25.43 ± 21.77 mm/h ($p = 0.0007$) in rheumatoid positive females; haemoglobin and fibrinogen concentration showed no statistically difference ($p > 0.05$).

Conclusion: The percentage positivity for rheumatoid arthritis in the studied subjects was 45.3% with females affected more than males. No statistical significant difference was observed in fibrinogen and haemoglobin concentrations based on the presence of the disease and in-gender comparison. Rheumatoid arthritis is significantly associated with increased erythrocyte sedimentation rate in both male and female with attendant increased packed cell volume in females. This confirms the high rate of inflammation at the joint (synovium) around the microvasculature in patients with rheumatoid arthritis. The arthritis in this study is non-anaemic with respect to mean values of packed cell volume.

Keywords: Rheumatoid arthritis; fibrinogen; cyanmethaemoglobin; erythrocyte sedimentation rate; packed cell volume; haemo-rheological.

1. INTRODUCTION

Rumuche community is one of the communities in Emohua Local Government Area. It is an area located on latitude 4°52' 31" N and longitude 6°51' 39" E [1]. The major occupation of farming and hunting exposes indigenes of this community to the risk of developing multiple joint pain as a result of inflammation in parts of the body that are highly being utilized during these processes. This inflammation may eventually lead to rheumatoid arthritis. Rheumatoid arthritis is an autoimmune disease in which the body's immune system with the function of protection mistakenly attacks cells in the joints resulting in inflammation that causes the tissues lining the inside of joints (synovium) to thicken, with resultant swelling and pain in and around the joints. The synovium makes a fluid that lubricates joints and helps them move smoothly. If inflammation goes unchecked, it can damage cartilage, the elastic tissue that covers the ends of bones in a joint as well as the bones themselves. Rheumatoid arthritis most commonly affects the joints of the hands, feets, wrists, elbows, knees, and ankles. The joint effect is usually symmetrical (that is if one knee or ankle is affected it eventually affects the other with time [2]. Rheumatoid arthritis localized on joints, contributing to local destruction [3].

Rheumatoid arthritis can be diagnosed with the use of blood sample. Rheumatoid factor alongside anti-citrullinated protein antibody gives a clear prognosis of the disease. Erythrocyte sedimentation rate (ESR) which is one of the markers of inflammation is elevated in rheumatoid arthritis [4]. Medications such as nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids and disease-modifying anti-rheumatic drugs (DMARDs) are drug of choice for the treatment of Rheumatoid arthritis [5].

Haemo-rheological variations of blood cells and plasma component alterations leads to hyper-viscosity thereby resulting into slow blood flow. Hypo-viscosity in microcirculation is very important for interactions between the rheological factors and the surrounding tissue [6]. The haemo-rheological changes in systemic lupus erythematosus (SLE) are similar to those found in rheumatoid arthritis, which include blood and plasma viscosity, and blood cell filterability [7].

The studyevaluated some haemo-rheological parameters associated with blood flow rate such as erythrocytes sedimentation rate, fibrinogen level, packed cell volume (PCV), and haemoglobin (Hb) concentration in individuals with rheumatoid arthritis and those without the disease in Rumuche community, since there are paucity of information with regards to this

parameters, and rheumatoid arthritis in this community whose inhabitants are predominantly farmers. Comparison of these parameters were made in test subjects (those with rheumatoid arthritis) and control (those without rheumatoid arthritis, and in males and females differently.

2. MATERIALS AND METHODS

2.1 Study Design

This was a cross sectional case-control field based study, carried out in Rumuche community, Emohua Local Government Area of Rivers State. A total of eighty-six subjects consisting of thirty-one males and fifty-five females aged between 20 - 80 years were recruited. Thirty-nine positive subjects for rheumatoid arthritis served as test while forty-seven negative subjects were used as control.

2.2 Blood Sample Collection and Storage

Venous blood (5 ml) was collected aseptically from subjects with 3 ml immediately dispensed into tri-potassium ethylene diamine tetra-acetic acid (EDTA) anticoagulant bottles, containing 1.2 mg/ml concentration of anticoagulant for haematological analysis and the remaining 2 ml into a plain sterile bottle for the estimation of fibrinogen and screening for the presence of rheumatoid factor. Samples in the plain bottles were spun at 1000 g for 15 minutes to obtain serum and were stored at -20°C prior to analysis.

2.3 Methodology

2.3.1 Determination of rheumatoid factor

Method: Latex Slide Test using Skytec Medical Rheumatoid Factor Latex Kit.

Principle: Latex particles coated with IgG, react with patient's serum containing rheumatoid factor. The rheumatoid factor acts as an antibody to IgG and attaches to the Fc portion of IgG on the latex particles, to bring about visible agglutination.

Procedure: A drop of the positive and negative control and 40 µl of patient serum was placed into separate circles on a clean glass slide; a drop of rheumatoid factor latex reagent was added on each circle of sample to the test and controls. With the use of an applicator the mixture was spread over the entire area, and was tilted back and forth for 2 minutes to check for presence of agglutination.

2.3.2 Determination of haemoglobin concentration

Method: Cyanmethhaemoglobin Method. As described by Ocheiand Kolhatkar [8].

Principle: It involves diluting blood sample with potassium cyanide, potassium ferricyanide and potassium dihydrogen phosphate. The ferricyanide forms methaemoglobin which is converted to cyanmethaemoglobin by the cyanide. The amount of cyanmethaemoglobin can be measured spectrophotometrically at a wavelength of 540nm.

Procedure: Series of tubes were properly labelled for test samples, including a blank. 5 ml of cyanmethaemoglobin reagent was pipetted into each of the tubes, and 20 ul of samples were added into the various test tubes labelled, excluding the blank tube. Tubes were allowed to stand for 10 minutes and absorbance of test was read using the spectrophotometer at 540 nm wavelength with the blank solution.

2.3.3 Determination of packed cell volume

Method: Microhaematocrit Method. As described by Cheesbrough [9].

Principle: Anticoagulated blood (EDTA) in a non-heparinized capillary tube was centrifuged using a microhaematocrit centrifuge at 12000 g for 5 minutes to obtain constant packing of red cells. With the use of a microhaematocrit reader, packed cell volume is read and results expressed as percentage (%).

Procedure: A non-heparinized microhaematocrit tube was dipped into the sample container, the tube was filled to two third. The microhaematocrit tube was sealed using a sealant and the tube was placed in the microhaematocrit centrifuge. It was spun at 12000 g for 5 minutes, and after spinning results were read using the microhaematocrit reader with the base of the red cell on the "0" line and top of the plasma on the "100" line.

2.3.4 Determination of erythrocyte sedimentation rate

Method: Westergren Method. As described by Cheesbrough [9].

Principle: Citrated blood is vertically positioned using Westergren pipette which is left undisturbed. Red cells aggregate and sediment through the plasma. The ESR is the length of the column of clear plasma above the red cells and is measured in millimetre per hour (mm/hr).

Procedure: 0.4 ml of trisodium citrate was pipetted into the Westergren bucket, together with 1.6 ml of EDTA anticoagulated blood; proper mixing was done and the Westergren pipette was placed inside of the Westergren bucket, and was allowed to stand vertically undisturbed away from sunlight and vibration source. After one hour, results were read and expressed in mm/hour.

2.3.5 Determination of fibrinogen

Method: Sandwich ELISA Method. Using Human Fibrinogen Elisa kit, Elabscience Biotech Co., Ltd, China; Catelog No: E-EL-H2193, Expiry Date: 2020-07-11

Principle: It makes use of the sandwich-enzyme linked principle. The micro ELISA plate provided in the kit has been pre-coated with antibody specific to Human Fibrinogen (FG). Standards and samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human FG and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human FG, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in colour. The enzyme-substrate reaction is terminated by the addition of stop solution and the colour turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of Human FG. Calculation of the concentration of Human FG in the samples were done by comparing the OD of the samples to the standard curve.

Procedure: 100 µL of standards were added to each well labelled for standards and 100 µL of samples were also added to each well labelled for test. Samples and standards were incubated for 90 minutes at 37°C. The liquid (samples and standards) was removed. 100 µL of biotinylated detection antibody was added and Incubated for 1 hour at 37°C. Each well was aspirated and washed three times. 100 µL of horseradish peroxidase conjugate was added and Incubated for 30 minutes at 37°C. Aspiration was done on the various wells and washed five times. 90 µL of substrate reagent was added and incubated for 15 minutes at 37°C. It was then followed by pipetting 50 µL of stop solution to each well. Absorbance of tests and standards were read at 450 nm immediately. Calculation of results was performed using Beer-Lambert's law.

2.4 Statistical Anaylsis

Data were analyzed using Graph-pad prism version 8.2 to obtain values for descriptive statistics (mean and standard deviation) and values for inferential statistics (student t-test).

3. RESULTS

3.1 Demographic Profile of Participants in the Study Population

A total of 86 subjects were recruited for the study between July and August, 2019. Twenty-seven of positive case were females and 12were males (Test). While 28 of the control subjects (negative to rheumatoid factor) were females and 19 were males.

3.2 Percentage Distribution of Rheumatoid Arthritis Positivity

From the studied population, 45.3% were positive for rheumatoid arthritis (females: 31.4%; males: 13.9%). Gender based percentage in relation to positivity for rheumatoid arthritis showed that females (49.0%) were more affected than males (38.7%).

3.3 Comparison of some Haemo-rheological Parameters Based on Presence and Absence of Rheumatoid Factor

Table 1 shows the comparison of some haemo-rheological parameters based on the presence and absence of rheumatoid factor. Using student t-test, there was statistically significant increase in mean values of erythrocyte sedimentation rate in test group when compared to control group with $p < 0.0001$. There was no statistically significant difference in other parameters analyzed: haemoglobin, fibrinogen and packed cell volume, $p > 0.05$ respectively in test group when compared to the control group.

Table 1. Comparison of Some Haemo-rheological parameters based on presence and absence of rheumatoid factor

Parameters	Test (RF+) Mean ± SD	Control (RF-) Mean ± SD	p-value	Inference
Haemoglobin (g/dl)	14.47± 3.588	13.50± 2.807	0.1633	NS
PCV (%)	37.23± 4.055	36.85± 6.125	0.7412	NS
ESR (mm/hr)	46.18± 25.64	19.36± 19.49	<0.0001	HS
Fibrinogen (ng/ml)	547.2±162.2	531.2±166.7	0.6561	NS

Key: HS=Highly Significant; S=Significant; NS=Non Significant; PCV= Packed Cell Volume; ESR=Erythrocyte Sedimentation Rate; RF+=Rheumatoid Factor Positive; RF-=Rheumatoid Factor Negative; Mean ± SD= Mean ± Standard Deviation. Note: The abbreviations are applicable to all tables.

Table 2. Comparison of Some Haemo-rheological parameters in males based on presence and absence of rheumatoid factor

Parameters	RF+ Mean ± SD	RF- Mean ± SD	p-value	Inference
Haemoglobin (g/dl)	16.39± 2.951	14.92± 2.738	0.1680	NS
PCV (%)	38.75± 4.159	41.11± 5.782	0.2315	NS
ESR (mm/hr)	36.67± 14.87	10.79± 11.00	<0.0001	HS
Fibrinogen (ng/ml)	552.7±170.6	562.3±166.9	0.8779	NS

Table 3. Comparison of Some Haemo-rheological parameters in females based on presence and absence of rheumatoid factor

Parameters	RF+Mean ± SD	RF-Mean ± SD	p-value	Inference
Haemoglobin (g/dl)	13.62± 3.561	12.54± 2.462	0.1953	NS
PCV (%)	36.56± 3.896	33.96± 4.501	0.0267	S
ESR (mm/hr)	50.07± 28.73	25.43± 21.77	0.0007	HS
Fibrinogen (ng/ml)	544.7±161.6	510.2±166.1	0.4377	NS

3.4 Comparison of Some Haemo-rheological Parameters in Males Based on Presence and Absence of Rheumatoid Factor

On comparing some haemo-rheological parameters in males based on the presence and absence of rheumatoid factor (Table 2), there was statistically significant increase ($p < 0.0001$) in erythrocyte sedimentation rate in test group when compared to the control group. Other analyzed parameters showed no statistically significant difference ($p > 0.005$) in test group when compared to the control group.

3.5 Comparison of some Haemo-rheological Parameters in Females Based on Presence and Absence of Rheumatoid Factor

Table 3 shows the comparison of haemo-rheological parameters in females based on the presence and absence of rheumatoid factor. Using student t-test, there was statistically significant increase in mean values of packed cell volume and erythrocyte sedimentation rate in test group when compared to the control group, with $p < 0.05$ respectively. Other analyzed parameters showed no statistically significant difference ($p > 0.05$) in mean values of test group when compared to mean values of control group.

4. DISCUSSION

Rheumatoid arthritis is a chronic systemic disease usually manifesting as inflammation of multiple joints. Haemo-rheological abnormalities have been described in a number of autoimmune diseases. Patients with rheumatoid arthritis are known of having disturbed blood rheology (flow). This study was done to evaluate some haemo-rheological parameters on individuals experiencing multiple joint pains and at risk of developing rheumatoid arthritis. This condition may create a vicious circle between endothelial damage, thus leading to microvascular occlusion. From our finding, 45.3% of the studied population were positive for rheumatoid arthritis (females: 31.4%; males: 13.9%). Gender based percentage in relation to positivity for rheumatoid arthritis showed that females (49.0%) were more affected than males (38.7%). From the results obtained in this study, a statistically significant difference was observed on erythrocyte sedimentation rate ($p < 0.05$) in both males and females between test group and control group, while there was no statistical significant difference in haemoglobin concentration and fibrinogen, with $p > 0.05$ respectively. These

findings were different from the study of Roony et al. [10], who observed that circulating fibrinogen levels was significantly higher in rheumatoid arthritis patients than in control. A recent study by Babikir et al. [11], showed that fibrinogen was statistically higher in patients with rheumatoid arthritis compared with normal healthy control group. The study carried out by Santos et al. [12], reported that there was no significant difference in fibrinogen levels and this report is in agreement with this study.

Erythrocyte sedimentation rate measures the degree of inflammation in the joints, meaning that the more red blood cell settles, the more inflammation occur in the joints. In this study, erythrocyte sedimentation rate obtained is in agreement with the study of Sharma et al. [13], who reported that erythrocyte sedimentation rate, an acute phase reactant is statistically highly significant with the test values when compared to the control values, $p < 0.0007$. Ohagwu et al. [14], reported that ESR was raised in 98/108 (90.7%) subjects. Mursal et al. [15], demonstrated that haemoglobin level of non anaemic rheumatoid arthritis patients was within a normal range with $p = 0.0001$ while in this findings, it showed that haemoglobin level was high but no statistical significance ($p > 0.05$) which is not in agreement with Mursal and colleagues. The study of Babikir et al. [11] demonstrated that there is statistically significant high level of erythrocyte sedimentation rate in patients with rheumatoid arthritis compared with the control group with p value 0.0001, which is in agreement with this study when those with rheumatoid arthritis were compared with control subjects ($p < 0.0001$). In another research work on haematological variables in rheumatoid arthritis patients, carried out by Okoroiwu et al. [16], it was observed that ESR was highly elevated in both male and female with the occurrence of rheumatoid arthritis which is in agreement with this study.

Packed cell volume measures the proportion of red cells in whole blood, occurrence of inflammatory state decreases the level of packed cell volume resulting into anaemia. This may be due to inflammatory response by cytokines, leading to suppression of erythropoietin which could be seen in anaemic individual. Anaemic syndrome is as a result of the manifestation caused by chronic inflammation which results into the reduction of oxygen supply. Findings from this study shows that packed cell volume of patients with arterial disease was statistically significant, having a mean value of 36.56 ± 3.895% when compared with those of the control 33.96 ± 4.501% ($p < 0.05$), and this partially agrees with another study by Ricci et al. [17]. According to the study carried out by Cimato et al. [18], result showed that packed cell volume expressed a normal value in rheumatoid arthritis in both control and test group ($p < 0.05$), and this report is in agreement with this study having a mean value of 36.56 ± 3.895% when compared with those of the control 33.96 ± 4.501% at $p < 0.05$. Study carried out by Santos et al. [12], also reported that packed cell volume of 45% were significantly lower in patients than in the control group.

5. CONCLUSION

The percentage positivity for rheumatoid arthritis in descent of Rumuche recruited for the study was 45.3% with females affected more than males. No statistical significant difference was observed in fibrinogen and haemoglobin based on the presence of the disease and in-gender comparison. Levels of erythrocyte sedimentation ratewas significantly elevated in individuals (males and females) who tested positive for rheumatoid factor with attendant increased packed cell volume in females. The increase in erythrocyte sedimentation rate predicts high rate of inflamed rheumatoid arthritis synovium, particularly around the microvasculature which is a risk factor for coronary artery disease. There is correlation between anaemia, non-anaemia and rheumatoid arthritis; the packed cell volume increase in females who tested positive for rheumatoid factor predicts that these females are non-anaemic for rheumatoid arthritis in this study.

6. LIMITATION

The study did not take into cognizance the grade of the disease and the duration of the disease.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

A written consent was obtained from each participant. Approval to conduct the research

was granted by the Department of Medical Laboratory Science, Rivers State University, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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