Effect of Interactions between Pro-Inflammatory Cytokines And Iron In Malaria-Infected Pregnant Women In ABA, Nigeria.

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Introduction

Cytokines are agonists and among the inflammatory mediators secreted principally by activated immune cells (1). They induce various transcription factors which in turn determine the fate of cells either for proliferation, differentiation, maturation or death (2). Activation of immune cells could be caused by infectious or non-infectious conditions. Most infections that activate immune cells include malaria and HIV (1,3,4,5) while non-infectious conditions include pregnancy (3,6) and iron-replete states, among others (7,8,9). Functionally, cytokines may be classified as pro and anti-inflammatory molecules1. The pro-inflammatory cytokines promote inflammation and drive immunity to cell mediated response1 while anti-inflammatory cytokines regulate the actions of pro-inflammatory cytokines and drive immunity to humoral response. The pro-inflammatory cytokines include IL1, IL6, TNFα and IFNγ. The pro-inflammatory cytokine enhances the killing of malaria parasites1 and are critical intermediates for clinical manifestations (10,11). In early pregnancy, the pro-inflammatory cytokines are known for immune-surveillance against pathogens (12); however, their upregulation at the materno-foetal interphase may compromise the viability of the foetus (13,14). Pro-inflammatory cytokine levels in pregnancy may be greatly exacerbated by infection with malaria. It is reported that cytokine secretion modulates iron levels (15) while iron chelation increases Th1-mediated immune response in P. falciparum infection (16), an effect that is associated with more efficient clearance of the parasite (17). Increased cytokine levels thus effectively reduce the iron available and consequently, the haemoglobin in circulation. On the other hand, iron is essential in the synthesis of haemoglobin and myoglobin as well as the proper functioning of some enzymes required for proper body metabolism. The body iron is obtained from dietary sources, mostly from meat and dark green vegetables. The dietary iron occurs as haem and non-haem iron; however, non-haem iron is the predominant form. Cereals, vegetables and cooking utensils are the major sources of non-haem iron while meat appears to be the major source of haem iron (18). One of the effects of maternal malaria infection is anaemia. Malaria alone without other adverse effects...
events is responsible for up to 26% of anaemia cases in pregnancy (19) in those developing countries in which malaria is endemic. In addition, malaria contributes as much as 23% of the pregnancy-related deaths in these countries (19). However, even without malaria, pregnancy is a state in which there is generally a reduction in the level of maternal haemoglobin as a result of the expanded blood volume caused by the new foetus (20,21,22). The modulation of iron levels in malaria by cytokines during pregnancy should therefore be a cause for concern as this is capable of further depressing the circulating haemoglobin level. One of the ways by which malaria reduces blood haemoglobin is by haemolysis of parasitized red blood cells (23). There is no effective excretory mechanism for iron; however, small amount of iron is lost on daily basis through various routes, even in health. In health, it is lost by desquamation, mostly by sloughing of the enterocytes through the skin and urinary tract (15). Some iron obtained from destruction of effete red cells is however recycled and brought back into the circulation for incorporation into new cells. It is also known that not all erythroblasts develop to maturation; some may die in the marrow and the iron salvaged by the macrophages (23). At the surface of the erythroids and other cells that require iron are transferrin receptors that receive iron from transferrin. Iron then enters the cells by endocytosis and is followed by series of reactions that result in the release of iron in the cytosol (18). The iron in the store could be mobilized to the circulation when it is required. The storage of iron is regulated by hepcidin; the major regulatory hormone and an inflammatory protein (24). Hepcidin binds to its putative receptor ferroportin and the binding determines the retention or the release of iron by the macrophages (9, 25). It is known that reduced iron levels protect the foetus against malaria. Again, low availability of iron in the maternal circulation restricts the amount of iron transferred to the foetus in pregnant women exposed to malaria infection, thus protecting the child against malaria ailments; the consequence may be the eventual development of iron deficiency anaemia when the baby is born. It has been stated that functional iron deficiency may result from high hepcidin concentrations that limits dietary iron absorption (24). Increased hepcidin production by hepatic cells may be induced by excess dietary iron, slowed rate of red blood cell production and inflammation (26). Inflammation is reflected in the raised blood level of C-reactive protein (C-RP). There appears to be a link between malaria, elevated levels of hepcidin and C-RP, since malaria induces inflammation that raises the levels of these biomarkers. In iron deficiency, the amount of iron-sulphur (4Fe- 4S) is relatively low, allowing for effective binding of Iron Regulatory Protein-1 (IRP1) with Iron Regulatory (responsive) Element (IRE) at 3’ downstream unsaturated region of the mRNA. This interaction increases the translation of TfR1mRNA which results in iron uptake. Conversely, the high amount of iron-sulphur in iron-replete states results in low iron uptake. Binding at the 5’ upstream unsaturated region increases the translation of ferritin mRNA resulting in increased iron store (18,23,27,28). Levels of iron in the body could be assessed by evaluating haemoglobin levels, red cell indices and assay of serum ferritin along with evidence of inflammation, infection and liver disease (29). The best combination would be estimations of haemoglobin or haematocrit, serum transferrin receptor and serum ferritin. Such a combination would reflect functional impairment, tissue avidity and iron storage (30). Evaluating C-reactive protein is commonly used to assess infection/inflammation (1,31). Under regulated conditions, sufficient amount of cytokines and iron are required to enhance immunity. Ironically, interactions between the
two molecules may modulate iron and this impairs immunity. This study is therefore aimed at determining the effects of interactions between pro-inflammatory cytokines (IFNγ, TNFα and IL6) and Iron as measured by haemoglobin (Hb), serum transferrin (sTfR) and serum ferritin (SF) in malaria parasite infected pregnant women. It is with a view to assessing the effect of the relationship between these cytokines and iron on the immune status of the pregnant woman. The findings of this study will provide understanding to one key cause of the impaired immunity of pregnant women with malaria; so that appropriate intervention can ensure successful pregnancy outcome.

**Keywords:** Malaria, Iron, Cytokines, Pregnancy, Haemoglobin

**Materials and Methods**

The study was carried out among pregnant women in Aba, Abia State, Nigeria. Aba is a cosmopolitan town and the largest commercial city of South Eastern Nigeria. The town is about 67km away from Umuahia, the State capital and located at latitude 05°10’N and longitude 07°19’E and at 205m (673ft) above sea level. The area experiences malaria transmission throughout the year. It has dirty environs characterized by heaps of refuse in dumps and stagnant water in poor drainages, which provide adequate ecological habitat for the breeding of mosquitoes. The mosquitoes are less susceptible to insecticides possibly as a result of adaptation and the majority of people in the study population sleep outside mosquito nets.

Prior to the study, ethical approval for the study was obtained from the Research and Ethics Committees of Abia State University Teaching Hospital and Living Word Mission Hospital, Aba, Nigeria. The two hospitals are of tertiary status, frequently attended by pregnant women; where samples were collected and the methods used complied with the guidelines set down in the Helsinki Declaration. Informed consent was also sought from each participant, those whose consents were received and who met the inclusion criteria were recruited into the study. Where the subjects were below 18yrs of age, parental or spouse’s consent was obtained.

The study was conducted between 2015 and 2016 as a cross sectional study. The sample size was 206 and was determined using 16% prevalence rate of malaria parasite infection in pregnancy in South Eastern Nigeria. This was determined using the formula for cross-sectional study by Daniel (52)

The sampling method was by Simple Random technique. The study participants were 286 women and were classified into four groups. The first (n=144) and second (n=62) groups were malaria parasitaemic and a-parasitaemic pregnant women respectively while the third (n=40) and fourth (n=40) groups were malaria parasite positive and a-parasitaemic non-pregnant women respectively and served as control. The participants in the control group were recruited from among the staff and students of the School of Nursing and Midwifery of the Teaching Hospital. The criterion for classification into the respective groups was based on detection of malaria parasite in the peripheral blood and without presentation of clinical malaria.

Blood samples were aseptically collected from the Subjects peripheral circulation and on screening, tested negative to HIV. They had no history of liver disease, hypertension, pre-eclampsia, tuberculosis, malignancy, gastric and duodenal ulcers. In addition, the pregnant groups had not complained of bleeding prior to 32 weeks of gestation, and had not been transfused nor had any caesarean section in previous pregnancy. Moreover, the pregnant subjects were with singleton pregnancies (based fundal height estimation and last menstrual period) from eight weeks of gestation. The gestational term of the
pregnant groups especially those that reported at second and third trimesters was confirmed by Ultrasound scan. The criteria were applied to ensure that other sources of immune stimulation aside pregnancy and malaria were eliminated and to avoid any bias in interpretation. On the contrast, subjects apparently known with chronic illnesses such as liver diseases, tuberculosis, diabetes mellitus, cardiovascular diseases and other infections and other inflammatory or pathological diseases that may alter the immune response were excluded. Also excluded were those on hormonal drugs, HIV sero-positives and those with multiple gestations. Those whose informed consents were not obtained were also excluded.

Pre-tested structured questionnaires were used to obtain basic anthropometric and socio-demographic information for each study participant. They were between ages 17-44 years.

About 8mls of blood was collected from the ante-cubital vein, by the phlebotomist. Out of the amount of blood collected, 3mls was dispensed into a container of 2 drops of 10% Ethylene Diamine Tetra-Acetic Acid (EDTA) container and mixed for subsequent examination for malaria parasite and haemoglobin estimation. The remaining 5mls were allowed to clot in a pyrogen-free container and centrifuged at 3000rpm for 10mins. The serum was used for serological screening for HIV and evaluation of cytokines (TNFα, IFNγ and IL-6), biochemical estimations of serum iron (SF and sTfR) and CRP.

Malaria parasitaemia was determined by Rapid Diagnostic Tests (RDT) and Thick Film Method. The HIV screening was done using the Determine and Unigold rapid test kits. Haemoglobin was evaluated using Sysmex Automated 3 parts Haematology Analyser model KX21N while serum ferritin, soluble transferrin and the cytokines were estimated by the ELISA technique. Kits for serum ferritin and soluble transferrin were sourced from Monobind INC, USA, while the cytokine kits were procured from Abcam Company, UK and the CRP kit, from Agappe Inc. Switzerland. The procedures for the tests were carried out according to the respective manufacturer’s instructions.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 21. The results were expressed as mean and standard deviation. Analysis of Variance (ANOVA) and student’s T-test were used for comparison of differences in various groups. Level of significance was set at p<0.05. The tests of association were performed using Pearson’s correlation coefficient. A two sided p-value of ≤0.05 was considered statistically significant for the tests of association.

Results

Table 1 shows comparison of the mean and SD of the pro-inflammatory cytokine levels of all the subjects. It was carried out using ANOVA and post Hoc analysis. This table shows the effect of malaria disease and pregnancy on immune cell activation. The result showed that IFNγ, TNFα and IL-6 varied among the different groups studied with respect to malaria infection and pregnancy.

Effect of Malaria Disease: for the effect of malarial disease on the subjects, comparison was made between subjects in groups 1 and 2, and between groups 3 and 4. Values obtained for groups 1 and 2 for all the cytokines, were higher in the malaria infected group and statistically significant (P<0.05) when compared with the uninfected pregnant group.

Again, between malaria infected non pregnant women (group 3) and uninfected non pregnant Women (group 4) values revealed significantly higher (P<0.05) levels for the 3 pro-inflammatory cytokines in malaria infected group than the uninfected group.
**Effect of Pregnancy**: For this assessment, comparisons of values were between groups 1 and 3 and between groups 2 and 4. On comparing values obtained for malaria infected pregnant women (group 1) and the infected, no pregnant women (group 3), IFNγ was significantly lower \((P < 0.05)\) in the former as compared with the later whereas TNFα and IL-6 were significantly higher \((P < 0.05)\) in the infected pregnant than in the infected non pregnant women. 

On comparing values obtained for malaria uninfected pregnant women (group 2) and malaria uninfected non pregnant women (group 4), IFNγ was mildly raised in the former (group 2) and statistically significant \((P<0.05)\) when compared with the later (group 4). For TNFα and IL-6, values obtained for group 2 were moderately higher and statistically significant \((P< 0.05)\) in comparison with group 4. 

Table 2: shows comparison of mean ± SD for iron levels of pregnant women using student’s t-test. The table is for assessment of the effect of malaria parasite induced immune stimulation on iron levels of the pregnant women. The values obtained shows that Hb concentration was mildly reduced in the infected pregnant women and statistically not significant \((p>0.05)\) in comparison with uninfected counterpart. The sTfR was moderately higher in the infected pregnant women and statistically significant \((p<0.05)\) when compared with the uninfected subjects. Serum ferritin for the infected pregnant women was higher and did not show statistically significant difference \((P>0.05)\) when compared with the uninfected pregnant women. Furthermore, CRP; the acute phase reactant showed that values were significantly higher \((P< 0.05)\) for the infected pregnant women than the uninfected when both groups were compared. 

Correlation analyses were carried out on malaria infected pregnant subjects. The statistical tool used was Pearson’s Correlation co-efficient. It showcases any relationship existing between the cytokines and iron.

Among the 144 subjects, there was significant negative correlation between IFNγ and Hb \((r = -0.18, p = 0.030)\). No correlation existed between IFNγ and SF \((r = -0.77, p = 0.362)\). There was no relationship between IFNγ, and sTfR \((r = -0.011, p = 0.886)\).

There was a significantly weak negative correlation between TNFα and Hb \((r = -0.16, p = 0.05)\). No correlation existed between TNFα and SF \((r = -0.10, p = 0.241)\). Also no significant relationship existed between TNFα and sTfR \((r = 0.09, p = 0.282)\).

For IL-6, no statistically significant correlation existed between IL-6 and Hb \((r = 0.07, p = 0.402)\). Again, no correlation existed between IL-6 and SF \((r = 0.04, p = 0.607)\) and between IL-6 and sTfR \((r = -0.11, p = 0.190)\). Figures 1 and 2 revealed that significant correlation existed between IFNγ and Hb and between TNFα and Hb. Correlations that were of no statistical relevance between the cytokines and iron were not shown on scatterplots.

**Discussion**

In pregnancy, there is the activation of maternal immune system by foetal cells. This occurs at the materno-foetal interface and results in the secretion of cytokines. Cytokines diffuse into the extracellular fluid compartments and circulate in the peripheral blood, thus ensuring successful pregnancy (32). As pregnancy progresses, there is the transition of the cytokine profile with a bias away from type-1 towards type-2 but infection with malaria causes a reversal of the profile to type-1 (4).
The finding of this study in which IFN$\gamma$, TNF$\alpha$ and IL-6 were found to be significantly elevated in malaria-infected pregnant women are in agreement with the finding of investigators Nmorsi (32) and Torre (11) respectively, who reported that the pro-inflammatory cytokines were elevated in malaria infected pregnant women than their uninfected pregnant counterparts. On the other hand, the finding is at variance with the finding of Bostrom (33) who did not show any statistical difference in the level of IFN$\gamma$ and TNF$\alpha$ between the malaria infected and uninfected women. The discordance in the finding of this study and that of Bostrom (33) could be due to differences in sample size. The sample size of 144 malaria-infected pregnant women in this study is much higher than that of Bostrom in which 42 infected pregnant women were recruited. Increased pro-inflammatory cytokine level is protective against malaria. TNF$\alpha$ and IFN$\gamma$ do activate neutrophils for increased destruction of the parasites (34).

Pregnancy is a time of considerable maternal adaptation whereby iron metabolism is affected. Iron availability is commonly reflected in the haemoglobin value obtained from an individual. The data obtained in this study (Table 2) showed that the haemoglobin concentration was reduced in malaria-infected than the uninfected pregnant women but was not statistically significant on comparison. We compared our data with the data obtained by Buseri (35) who did not find any statistical difference in the haemoglobin concentration of the pregnant women with malaria and those without malaria; and it was in agreement. Conversely, the data differs from the data obtained by another investigator, Erhabor (36) who reported a significantly reduced level of haemoglobin between the infected and uninfected pregnant women. The disagreement between this study and his data could be that this study was on asymptomatic subjects whereas the latter (36) recruited subjects that also had clinical malaria. Diet and access to good ante-natal care are other factors that can influence haemoglobin studies in pregnant women among various study populations (37). On its part, malaria reduces haemoglobin level by several mechanisms including haemolysis (20). What is not clear however is, to what extent malaria causes haemolysis in asymptomatic persons.

We found that values of ferritin in this study showed that the concentration of serum ferritin was increased in the infected than the uninfected pregnant women. However, the increase was marginal and did not show any statistical relevance. Normally, constant mobilizing of iron for erythropoiesis ought to deplete the ferritin level resulting in reduced value but the presence of malaria seems to falsely elevate the value. The reason is not far-fetched since malaria potentially induces mild inflammatory response and serum ferritin, being an acute phase reactant could be elevated thereby masking the lowered level brought about by its utilization in erythropoiesis.

As for soluble transferrin receptor, the study showed that it was elevated in malaria-infected than uninfected pregnant women. Several conditions or factors could elevate soluble transferrin receptor levels. These include pregnancy, increased erythropoiesis and haemolysis. As for pregnancy, it is known that the condition places tremendous pressure on the body’s need for iron (21). The increased requirement becomes necessary to cope with approximately 25-30% increase in red cell mass, the placental pool, the transfer of approximately 300mg of iron to the foetus and for blood loss at delivery. Besides, the plasma volume is increased by approximately 1250mls (45%) above normal by the end of gestation. So the increased red cell mass cannot match the increased plasma volume, thereby resulting in physiological anaemia (22,31). With regards to increased erythropoiesis, it has been observed that constant removal of iron to enhance
foetal growth and mobilizing of iron to compensate for the physiological anaemia induces erythropoiesis (31). Erythropoiesis involves a series of processes that results in the release of reticulocytes into the blood. The later stage of erythropoiesis involves the insertion of iron in the synthesis of haemoglobin (38). Erythropoiesis is controlled by complex interactions involving erythropoietin, cytokines, growth factor, hormones and stromal cells in the bone marrow and such elements as iron, folate and vitamin-B₁₂. Abnormalities in any of these factors can affect red blood cell mass, resulting in either anaemia or erythrocytosis (39). Thus iron and anaemia are intimately intertwined with erythropoiesis and results in elevated soluble transferrin receptor levels. As for haemolysis, it is being suggested that some degree of haemolysis may occur in asymptomatic malaria. Asymptomatic *P. falciparum* malaria is a common feature in regions with high annual malaria transmission and a positive correlation between high transmission and high asymptomatic malaria incidence has been reported in a study (40). Indeed, Eke (41) had earlier reported a high prevalence of asymptomatic malaria infection in some parts of Aba. In addition, studies have shown that malaria-infected children in Kenya exhibited the features of thrombocytopenia and lowered haemoglobin levels without symptoms (42). This being the case, haemolysis of infected red cells in pregnant women, in spite of being asymptomatic for malaria, should be more pronounced as a result of the accompanying stress, than in children. Haemolysis and other haemolytic associated conditions such as malaria, glucose 6-phosphate dehydrogenase deficiency, sickle cell disease and thalassemia lead to increased erythropoiesis (43,44,45) and increased erythropoiesis in turn results in elevated sTfR (44,46).

This study, essentially on determining relationships (Figures 1 and 2), showed statistically significant inverse relationship between IFNγ and Hb and between TNFα and Hb while Figure 3 shows a positive relationship between IL-6 and Hb. The study thus suggests that up-regulated levels of the mentioned cytokines may interfere with normal iron absorption, transport, storage and its release by the reticulo-endothelial cells in a manner characteristic of those disorders or diseases associated with anaemia of inflammation. The interference on absorption, transport, storage and utilization of iron by up-regulated pro-inflammatory cytokines is illustrated as follows; IFNγ increases the expression of divalent metal transporter protein1 in the gut enterocytes and macrophages and results in the uptake of ferrous iron. Also pro-inflammatory stimuli such as IFNγ and lipopolysaccharide (LPS) increase the uptake of Non-Transferrin Bound Iron (NTBI), favouring the down-regulation of the synthesis of ferroportin and culminating in the retention of iron within the monocytes/macrophages. Again, TNFα, IL-1, and IL-10 induce synthesis, retention and storage of ferritin within macrophages. Additionally, TNFα increases macrophage acquisition of erythrocyte iron by increasing expression of C3b receptor (31, 47, 48, 49, 50). In addition, up-regulated expression of IFNγ and TNFα are capable of suppressing growth and mitosis and may even induce apoptosis of some progenitors (1). Moreover, over-expression of pro-inflammatory cytokines may result in the formation of ceramides. Ceramides are important intermediates in the biosynthesis of sphingolipids. Accumulation of ceramides on the cell’s membrane may be injurious, resulting in the death of erythrons (51). Furthermore, during infections such as malaria, IL-6 and possibly other cytokines could be over-expressed. These in turn may induce the hepatocytes to synthesize acute phase proteins such as hepcidin (19). Hepcidin inhibits intestinal iron absorption, iron recycling by the macrophage and release of stored iron from hepatocytes,
thus decreasing the availability of body iron (25). The increased levels of IFNγ and TNFα as shown in this study could have interfered with iron, resulting in the reduction of haemoglobin concentration and this appears to conform to the lines of evidence in the above illustrations. On the contrary, this study observed a positive association between IL-6 and Hb. The reason for this presentation is not clear. However, it may mean that IL-6 does not exert a direct effect on iron; but rather alters iron homeostasis by inducing the synthesis of acute phase proteins like hepcidin.

**Conclusion**

In conclusion, we observed that over-expression of IFNγ and TNFα interferes with normal iron absorption, transport and storage. We deduced that the effect of interactions between up-regulated levels of IFNγ and iron and between TNFα and iron decreases haemoglobin concentration and culminates in anaemia with impaired immunity among malaria parasite infected pregnant women. This occurrence appears to be the hallmark for most adverse conditions associated with malaria parasite infection during pregnancy. This study highlights the interrelationship between malaria infection, iron status, and innate immune response. The study has thus shown that malaria parasitaemia induces increased levels of certain pro-inflammatory cytokines, a situation that alters iron homeostasis with a consequence of reduced haemoglobin level which further culminates in impaired immunity for the pregnant woman. In view of these findings, we recommend that it is necessary to determine the concentrations of IFNγ and TNFα in malaria-infected pregnant women receiving prophylactic iron supplementation, especially if supplementation is not yielding the desired effect.

**Acknowledgement**

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Table 1: Comparison Of Mean±Sd Of Proinflammatory Cytokine Levels In Malaria Infected And Uninfected Pregnant Women.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>CYTOKINES(pg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>IFN-γ</td>
</tr>
<tr>
<td>G1 (n = 144)</td>
<td>22.94 ± 12.71</td>
</tr>
<tr>
<td>G2 (n = 62)</td>
<td>5.98 ± 3.11</td>
</tr>
<tr>
<td>G3 (n = 20)</td>
<td>30.07 ± 3.99</td>
</tr>
<tr>
<td>G4 (n = 20)</td>
<td>4.69 ± 2.64</td>
</tr>
<tr>
<td>F(p) Value</td>
<td>64.00</td>
</tr>
<tr>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>G1 vs G2</td>
<td>0.001*</td>
</tr>
<tr>
<td>G1 vs G3</td>
<td>0.001*</td>
</tr>
<tr>
<td>G1 vs G4</td>
<td>0.001*</td>
</tr>
<tr>
<td>G2 vs G3</td>
<td>0.001*</td>
</tr>
<tr>
<td>G2 vs G4</td>
<td>0.277</td>
</tr>
<tr>
<td>G3 vs G4</td>
<td>0.001*</td>
</tr>
</tbody>
</table>
Key++

α-level set at 0.05

*(P < 0.05) = Significant

P > 0.05 = Not Significant

G1 = Malaria Infected Pregnant Subjects

G2 = Malaria Uninfected Pregnant Subjects

G3 = Malaria Infected Non-Pregnant Subjects

G4 = Malaria Uninfected Non-Pregnant Subjects

Table 2: Comparison of Mean ± Sd of Iron Levels Of malaria Infected and Uninfected Pregnant Women.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Infected Pregnant (n = 144)</th>
<th>Uninfected Pregnant (n = 62)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/L)</td>
<td>102.42 ± 10.98^a</td>
<td>105.79 ± 14.18^a</td>
<td>0.067</td>
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<tr>
<td>STFRA (nmol/L)</td>
<td>62.78 ± 21.97^a</td>
<td>54.07 ± 23.73^a</td>
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<td>SF (ug/L)</td>
<td>43.61 ± 84.99^a</td>
<td>31.31 ± 28.18^a</td>
<td>0.267</td>
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<tr>
<td>C-RP (mg/L)</td>
<td>6.09 ± 4.88^a</td>
<td>4.49 ± 2.74^b</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Key

α-level was set at 0.05

Values not sharing the same superscript means there is a significant difference

Values sharing the same superscript imply that there is no significant difference

Figure 1: A scatter gram showing a significantly negative correlation between IFNγ and Hb (r = -0.18, p = 0.030)

Figure 2: A scattergram showing a Negative correlation between TNF-α and Hb (r = - 0.16, p = 0.05)