Miscarriage and Oxidative Stress of Iron Supplementation during Pregnancy

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Abstract

Background: Iron supplementations to the pregnant women in Iraq mostly considered as obligatory routine health care additive treatment without real assessing to the iron status in these people, therefore the acceptable range of iron must be estimated and the relationship between iron status and miscarriage should be explored.Objective: In miscarriage and pregnant women that supplemented with iron tablets the levels of oxidative stress should be assessed and compared to the iron status to show the type and the strength of correlation between them.Patients and methods: 96 pregnant women were participate in this study and categorized into. First group: Pregnant iron supplemented group (PIS group) includes 35 pregnant women (36.46

Index terms— iron; oxidative stress; miscarriage; free radicals.

Objective: In miscarriage and pregnant women that supplemented with iron tablets the levels of oxidative stress should be assessed and compared to the iron status to show the type and the strength of correlation between them.

Patients and methods: 96 pregnant women were participate in this study and categorized into.

First group: Pregnant iron supplemented group (PIS group) includes 35 pregnant women (36.46%) during their 1 st trimester or middle of 2 nd trimester of pregnancy **??**18-36 year) and they were received an oral iron supplementation as 250 mg of ferrous gluconate tablet, three times a day.

Second group: Pregnant iron non-supplemented group (PINS group) includes 31 pregnant women (32.29%) during their 1 st trimester or middle of 2 nd trimester of pregnancy **??**19-37 year) and they were non supplemented with iron and they considered as control group.

Third group: Miscarriage iron supplemented group (MIS group) includes 30 miscarriage pregnant women(31.3%) during their 1 st trimester or middle of 2 nd trimester **??**19-40 year) taken an oral iron supplementation as 250 mg of ferrous gluconate tablet, three times a day.

Results: The study at first assess the iron status by measuring Hb, serum ferritin and serum iron in the three groups of women and shows that there is a significant differences $p< 0.005$ between oxidative stress parameters of the miscarriage women and the pregnant women that taken oral supplements of iron tablets. The MDA and PC were higher in miscarriage in comparison to pregnant. The decrement in GSH level in miscarriage was also significant (P value< 0.025). The correlations between serum iron concentration and oxidative stress parameters MDA, PC and GSH were plotted using the regression line analysis where the r 2 values were obtained respectively r 2 = 0.63, r 2 = 0.55 and r 2 = 0.70. It is obvious that there is a high correlation between the level of iron in the circulation and the oxidative stress of the reactive oxygen species that generated by the catalytic effect of this transition metal.

1 Conclusion:

The high level of iron that obligatory supplemented to the pregnant women in Iraq should be restricted and early careful investigation should be performed to assess the status of iron before any iron supplements prescribed. The spontaneous abortion or miscarriage is highly

2 I. introduction

ron is an essential metal for hemoglobin biosynthesis of erythrocytes, oxidation-reduction reactions, and cellular proliferation, whereas excess iron accumulation causes organ dysfunction through the production of reactive oxygen species (ROS) and other mechanisms. The total amount of body iron is approximately 3-4 g in subject with 70 kg, only 1-2 mg of iron is absorbed daily from the intestinal tract and circulated in the blood (1). Although the body loses iron e.g through menstruation, this way does not considered as a systematic excretion route of iron especially during pregnancy, and therefore the cumulative effect from supplementation can be dangerous; a continuous load exceeding 1-2 mg/day can eventually result in iron overload leading to organ failure and many other complications (2,3). The benefits of improved maternal iron status by iron supplementation could be offset by increased maternal and/or infant infection risk, due to increased availability of iron to host pathogens which cause prenatal infections and miscarriage (4).

Miscarriage, also known as spontaneous abortion and pregnancy loss, is the natural death of an embryo or fetus before it is able to survive independently. Some use the cutoff of 20 weeks of gestation after which fetal death is known as a stillbirth. The most common symptoms of a miscarriage is vaginal bleeding with or without pain. Sadness, anxiety, and guilt may occur (5). Tissue or clot like material may also come out of the vagina (6).

Ferrous iron is a principle pro-oxidant in human by being the most abundant transitional metal in human. It can initiate and potentiate the generation of free radicals through Fenton chemistry in which Fe +2 is oxidized to Fe +3 in existence of hydrogen peroxide and highly reactive hydroxyl free radical OH* is produced (7).

It is well known that the absorption of dietary iron occurs in the intestinal duodenum (8). This amount of absorbed dietary iron is enough to compensate for the estimated 1 to 2 mg of unregulated lose of iron through sweat, dermal turnover, and incidental amounts excreted in urine (9,10). The need for iron varies markedly during each trimester of pregnancy. Iron requirements decrease during the first trimester because menstruation stops (11). During the second trimester, iron requirements begin to increase and continue to do so throughout the remainder of pregnancy (12). As pregnancy progresses, iron requirements for fetal growth rise steadily in proportion to the weight of the fetus, with most of the iron accumulating during the third trimester. The average iron content of a fetus weighing >3 kg is ?270 mg (13). Pregnancy, mostly because of the mitochondrial-rich (mitochondrial mass increase with gestational age), placenta is a conduction that favors oxidative stress transition metals, especially iron, which is particularly abundant in placenta, are important in the production of free radicals . At the same time placenta is highly vascular and exposed to high maternal oxygen partial pressure (14). When exposed to excess intake of iron, it is vulnerable to oxidative damage secondary to the continuous presence of a relatively some excess of iron intake (15). This imbalance between pro-oxidants and antioxidants can lead to a number of reproductive diseases such as endometriosis, polycystic ovary syndrome (PCOS), and unexplained infertility. Pregnancy complications such as spontaneous abortion, recurrent pregnancy loss, and preeclampsia, can also develop in response to oxidative stress (16). Data from the United States National Health and Nutrition Examination Survey (NHANES) in 1999-2006 for 1171 pregnant women showed that pregnant women in the first trimester had the highest mean total body iron compared with that of pregnant women in the second or third trimesters (17,18). It has been reported by many investigators that the prevalence of fetal and maternal complications is higher in women with excess iron complications or ?-thalassemia than in the general population (19)(20)(21)(22).

In the presence of excess iron the highly reactive OH* (hydroxyl radical) species will be generated (23). In 1894, Fenton described the oxidizing potential of hydrogen peroxide when mixed with ferrous salts (24).Fe +2 + H 2 O 2 ? Fe +3 + OH * + OH -

Forty years later, in 1934, Haber and Weiss identified the hydroxyl radical as the oxidizing species in this reactions: (25).O2*-+ H 2 O 2 ? O 2 + OH -+ OH *

Overall summation of Fenton reaction in two steps gives the Haber-Weiss reaction **??**————————————Fe +2 + H 2 O 2 ? Fe +3 + OH * + OH - Fe +3 + O * 2-? Fe +2 + O 2 – O *2-+ H 2 O 2 ? O2 + OH -+ OH *

This reaction is often called the iron-catalyzed Haber-Weiss reaction, or sometimes the superoxidedriven Fenton reaction (25).

There are many different markers that can be used to demonstrate the presence of oxidative stress. In the current study reduced glutathione GSH, protein carbonyl group PC and malondialdehyde MDA all were used to monitors the oxidative stress because the free radicals are very unstable due to their high reactivity and cannot be practically determined at least by our humble laboratory (26). Protein carbonyl (PC) measurement is the most widely accepted possibility to evaluate the oxidative damage to the protein fraction as the content of carbonyl groups increases in free radical induced reactions (27,28).

The usage of (PC) groups as biomarkers of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated proteins (29). Malondialdehyde is an aldehyde (3 carbon molecules with two aldehyde groups) (30). It is considered to be the terminal compound and the most important marker for monitoring lipid peroxidation and oxidative damage induced by ROS (31). About MDA Stewart AJ et al in 2005 mentioned that this naturally occurring end product of ROS is a marker of oxidative stress and used as a biomarker to determine oxidative stress level in organisms (32).

Glutathione is an essential nutrient for humans and, since it can be synthesized in the body from the amino

acids L-cysteine, L-glutamic acid, and glycine, it does not have to be present as a supplement in the diet (33). The strong evidence that glutathione depletion causes cell death comes from the studies by Li and colleagues (34). They emphasized that a decrease in GSH triggers the activation of neuronal 12-lipoxygenase pathway which leads to the production of peroxides. However the direct depletion of cytoplasmic GSH resulted in increased generation of ROS (35).

Blood hemoglobin, serum iron and ferritin parameters were used to assess the iron status in the body. After considering many indicators, a World Health Organization (WHO) and Centers for Disease Control (CDC) and technical consultation on the assessment of iron status at the population level concluded that serum iron, Hb and ferritin were the most efficient combination of indicators for monitoring changes in the iron status of a population as a consequence of iron supplementation (36).

The iron as a routine administered treatment, given to most pregnant women in Iraq, need to be studied intensively to explore its effects on oxidation status in those subjects and which range would be acceptable and safe for iron administration during pregnancy to prevent more pregnancy complications or miscarriage problems. This was a 12 months cross-sectional study, conducted in alzahra teaching hospital in Al-Najaf and some other private clinics, under supervision of specialist physicians, during 2014-2015. The study was designed in accordance with the Ethics Committee approval before the start of the practical part and blood collection. The 96 pregnant women were participate in this study and categorized into First group: Pregnant iron supplemented group (PIS group) includes 35 pregnant women (36.46%) during their 1 st trimester or middle of 2 nd trimester of pregnancy (18-36 year) and they were received an oral iron supplementation as 250 mg of ferrous gluconate tablet, three times a day.

3 Volume XVI Issue II Version I

Second group: Pregnant iron non-supplemented group (PINS group) includes 31 pregnant women (32.29%) during their 1 st trimester or middle of 2 nd trimester of pregnancy (19-37 yer) and they were non supplemented with iron and they considered as control group.

Third group: Miscarriage iron supplemented group (MIS group) includes 30 miscarriage pregnant women (31.3%) during their 1 st trimester or middle of 2 nd trimester (19-40 year)taken an oral iron supplementation as 250 mg of ferrous gluconate tablet, three times a day. The blood samples were drawn from iron supplemented group after 28 days from the time of starting the iron supplementation. The age of the pregnant women (supplemented and non supplemented group) was 18-40 years. The exclusion criteria contains diabetes mellitus, gestational hypertension, Rheumatoid, liver disease and hemolytic anemia which may interfere with our measurements.

Hemoglobin: Blood hemoglobin Concentration was determined by the Hb -Meter method. In the presence of alkaline potassium ferricyanide, hemoglobin is oxidized to methaemoglobin, which then react with potassium cyanide to form cyanmethaemoglobin complex which absorbe the wave length of 540 nm .The absorbance is directly related to total hemoglobin concentration (37).

Serum Iron: After dissociation of iron-transferrin bound in acidic medium, ascorbic acid reduced Fe 3+ iron into Fe 2+ iron, Fe 2+ iron then form a colored complex with 3-(2pyridyl)-5,-6-difuryl-1,-2,-4-triazine-disulfonate (ferene). The absorbance thus measured at 600nm (580-620) which is directly proportional to the amount of iron in the specimen. Thiourea is added in the reagent to prevent copper interference (38).

Serum ferritin: The quantitative determination of circulating ferritin concentration in human sera was done by the micro plate immunoenzymometric assay. The kit was purchased from Monobid inc, USA (39).

Serum malondialdehyde (MDA): level of serum malondialdehyde was determined spectrophotometrically by a modified procedure described by Guidet B. and Shah S.V. (40). The test is based on the reaction of MDA with thiobarbituric acid (TBA); forming MDA-TBA2 product that absorbs strongly at 532 nm.

Serum protein carbonyl (PC): Serum protein carbonyl (P.C) groups dimerizes with 2,4-dinitrophenylhydrazine (DNPH), which leads to the formation of a stable dinitrophenyl hydrazone (DNP) product, that can be detected by enzyme-linked immunosorbent assay (ELISA), We followed the method described by Levine et al in 1990 (41).

Serum reduced glutathione (GSH): 5,5-Dithiobis(2nitrobenzoic acid) (DTNB) is a disulfide chromogen that is readily reduced by sulfahydryl group of GSH to an intensely yellow compound. The absorbance of the reduced chromogen is measured spectrophotometrically at 412 nm, which is directly proportion to the GSH concentration (42).

Statistical analysis: The results were expressed as Mean ± SD. Student's t-test was used to examine the correlation of oxidative stress parameters (MDA, PC and GSH). Significant variation was considered when Pvalue was less than 0.05. Linear regression analysis was used to explore the correlation between the values of the oxidative stress parameters and serum iron in miscarriage women.

4 III. Results

In table 1 serum iron concentration and ferritin level both were raise in PIS group women if compared to the control group (PINS group) with p value < 0.005. The level of malondialdehyde MDA and the serum protein carbonyl PC were significantly enhanced $p<0.005$ in pregnant women that taken iron supplements(PIS GROUP) in comparison to the age matched control group that didn't take any iron supplementation during pregnancy. The

reduced glutathione GSH show a significant decrement P< 0.005 in pregnant women that taken iron regularly unlike those didn't take iron supplementation during pregnancy.

It is obvious from table 2 that serum iron concentration and ferritin level both were raise in MISG women if compared to the PIS GROUP women with p value < 0.025. The level of malondialdehyde MDA and the serum protein carbonyl PC were significantly enhanced p<0.005 in miscarriage women that taken iron supplements (MISG) in comparison to the age matched pregnant women that taken a regular oral doses of iron supplementation during pregnancy (PIS GROUP). The reduced glutathione GSH show a significant decrement P< 0.025 in miscarriage women that taken iron regularly unlike the high level of GSH in pregnant group (PIS GROUP).

Table (1) : Blood hemoglobin conc., serum iron, ferritin, malondialdehyde, protein carbonyl groups and reduced glutathione in iron supplemented and non supplemented pregnant women. Table (**??**) : Blood hemoglobin conc., serum iron, ferritin, malondialdehyde, protein carbonyl group and reduced glutathione in pregnant iron supplemented and miscarriage iron supplemented women.

In figure 1 below the regression analysis was used to show the linear relationship between serum iron concentration as a dependent variable and the MDA as the independent variable, however r= 0.63 and r 2 = 0.80. The correlation was positive and significant as shown from the plotted regression line and its equation. In figure 2 the regression analysis was used to show the linear relationship between serum iron concentration as a dependent variable and protein carbonyl groups as the independent variable, however r= 0.74 and r 2 = 0.55 . The correlation was positive and progressive as shown in fig. 2 from the regression line and its equation. In figure 3 the regression analysis was used to show the linear relationship between serum iron concentration as a dependent variable and serum reduced glutathione as the independent variable, however r= -0.84 and r 2 = 0.70. The correlation was negative and descendent as shown below in the regression line and its equation.

5 Volume XVI Issue II Version I

6 IV. Discussion

In the current study the oxidative stress parameters were considerably employed to explore the role of iron supplementation in pregnant and miscarriage women and to realize the consequences of oxidative stress load complications in those individuals. Pregnancy is a physiological state accompanied by higher energy demand and an increase in oxygen requirement, with various compensate enzyme adaptation (43). The changes occur with advancing pregnancy to met the increase demands for proper body function of mother to fulfill the requirement of fetus (44,45). The increase in oxygen demand is met by increase ventilation. Such conditions are responsible for raised oxidative stress in normal pregnancy (46).

The results in table 1 revel that there is a general decrement in oxidative stress in the pregnant women that not taken any supplementation of iron when compared with those supplemented with iron tablets and this fact may be related to the physiological changes that associated to the process of gestation itself. However Adiga U. et al in 2009 reported that the Pregnancy is a state, which is more prone for oxidative stress. Various studies reported that there is a development of a strong defense mechanisms against free radical damage, as the pregnancy progresses (47).

The enhancement of serum iron and ferritin levels in PIS group in table 1 is attributed to the supplements of iron tablets that taken orally. Hillman and Henderson in 1969 reported that with oral iron supplementation, patients were able to achieve serum iron values between 12.5 ?M and 27.0 ?M, and red blood cell production was able to increase to four or five times normal (48).

In table 1 the levels of MDA and PC in PIS group were significantly (p< 0.005) higher than that of PINS group this result were in consistent with the outcomes of many studies for example Minic oka in 2005 (49), noticed that during the periods of intravenous iron therapy the amount of iron promotes ironmediated formation of free radical species which could result in lipid peroxidation which give raise to MDA and oxidation of plasma proteins. He concluded an elevation in serum P.C groups after intravenous iron supplementation, through a study on evaluation of oxidative stress after intravenous iron supplementation.

The same upturn was noticed in table 2, the protein carbonyl groups (PC) in sera of miscarriage women (MIS group) and serum MDA both were significantly (p< 0.005) higher than that of pregnant women in (PIS group) The attribution involve the catalyzing role of iron as in Fenton reaction. Fenton reaction lead to generation of highly reactive hydroxyl radicals which in turn would attack preferentially the proteins as a target molecules. This reaction will result in the enhancement of protein carbonyl content (50,51). Iron serves as a catalyst for protein oxidation and the formation of reactive oxygen species that in turn cause side chain modification of many amino acid and ultimately to carbonyl groups formation (52). The higher serum iron concentration were in coordinate with higher oxidative stress and raise in serum MDA concentration. This can be further demonstrated by using the linear regression analysis as in figure 1 concluded that ,there is a positive correlation between MDA level and serum iron status in young women (53).

Figure 2 shows a significant correlation between serum iron and PC groups in the circulation of the miscarriage women where r= 0.74 and r2 = 0.55. This finding was also supported by M.J.Davies et al who mentioned that the protein carbonyls are formed early during oxidative stress conditions and are not a result of one specific oxidant, thus they can be called a marker of overall protein oxidation (54).

In table 1 GSH decreased in PIS group when compared to PINS group and this may be related to the differences

in serum iron concentrations in the two groups. In the same trend the results in table 2 reveal that the serum GSH in miscarriage group was lower than that of pregnant women by ? 3.4% and the correlation between serum iron and serum GSH was shown in figure 3 which display a significant negative regression linearity where r= -0.84 and r2 = 0.70. This decrement may be in coincidence with the speculations of Rajdl D. et al who documented that the lower GSH levels may be due to the increased turnover of GSH for preventing oxidative damage in iron supplemented group (55). The generation of free radicals is dependent on the presence of various transition metal ions and the most important transition metal in vivo is believed to be the iron (56). Paik et al in 1999, demonstrated that the higher level of ferritin have lower levels of GSH (negative correlation), and this conclusion was obtained through a study on enhanced oxidative stress in hemodialysis patient receiving intravenous iron therapy (57). Mustafa et al in 2010 detected markedly higher levels of MDA and significantly lower GSH levels in the maternal blood of pregnant women (58).

Finally the oxidative stress that induced by iron supplementation in miscarriage as shown in table2 is often referred to by many other investigators. Many scientists indicated that Oxidative stress has also been implicated in early miscarriage. Jauniaux et al (59) suggested that early pregnancy loss may results from premature oxygenation of the early embryonic environment. By using an O 2 probe in women before first-trimester termination, they observed a steep rise in placental pO 2 between 8 and 12 wk of gestation.

Thus, it is well established that maternal metabolic disorders such as diabetes, which are associated with an increased generation of oxygen free radicals, are known to be associated with a higher incidence of miscarriages and fetal structural defects (60), indicating that the mammalian conceptus can be irreversibly damaged by oxidative stress (61).

Ruder EH in 2008 reported that in vitro fertilization is also affected by excessive ROS in embryo culture media, and the routine practice of incubating embryos at low oxygen tension can prevent embryo arrest and enhance the chances of successful fertilization (62).

7 V. Conclusion

Miscarriage in many pregnant women especially in the early first weeks of gestation affected significantly by the iron that frequently and routinely prescribed to these women in Iraq by most physicians in the form of different types of tablets and formulas. The oxidative stress that induced by the upturn of this transition metal (Fe) in the circulation may be the real causative factor in the spontaneous abortion and many other pregnancy complications. The iron supplements should be given to those who actually suffering from iron deficiency after accurate diagnosis by the suitable biochemical tools. In addition to that the administration of excess iron should be followed by supplying the necessary amount of antioxidant to neutralize the probable oxidation potential of iron during pregnancy.

8 Volume XVI Issue II Version I

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[1] Miscarriage and Oxidative Stress of Iron Supplementation during Pregnancy

Figure 1:

Parameters	PINS GROUP NO = 31 (Control group)		PIS GROUP NO = 35		P <
	Mean ± SD	Range	Mean ± SD	Range	
Hb (g/dl)	11.50 ± 2.40	7.1-13.5	11.2 ± 1.70	7.3 –13.1	N.S
S .iron (µM)	16.50 ± 3.58	10.40- 24.02	21.03 ± 3.72	11.17-26.4	0.005
S.ferritin (ng /ml)	35.53 ± 15.25	14.75-65.99	54.11 ± 17.18	28.67-79.5	0.005
MDA (µM)	9.50 ± 2.43	5.16-13.96	13.43 ± 3.22	7.51-18.30	0.005
P.C nmol / mg . protein	2. 62 ± 0.72	4.41 – 1.60	3.41 ± 0.87	1.31-5.10	0.005
GSH (µM)	173.6 ± 20.34	135 - 225	154.4 ± 13.35	124-187	0.005

1

Figure 2: Figure 1 :

Parameters	PIS GROUP NO = 35		MIS GROUP NO = 30		P <
	Mean ± SD	Range	Mean ± SD	Range	
Hb (g/dl)	11.60 ± 1.70	7.30 –13.60	10.40 ± 1.76	7.60 -13.71	0.025
S .iron (µM)	21.03 ± 3.72	11.17-26.40	23.24 ± 3.41	14.17-28.30	0.025
S .ferritin (ng /ml)	54.11 ± 17.18	28.67-79.5	57.78 ± 14.57	30.57-82.20	0.025
MDA (µM)	13.43 ± 3.22	7.51-18.30	16.59 ± 3.28	8.10 -20.73	0.005
P.C nmol / mg prot	3.41 ± 0.87	1.31-5.10	4.96 ± 0.98	1.65 – 6.25	0.005
GSH (µM)	154.4 ± 13.35	124-187	149.3 ± 11.62	125.3-160.7	0.025

2

Figure 3: Figure 2 :

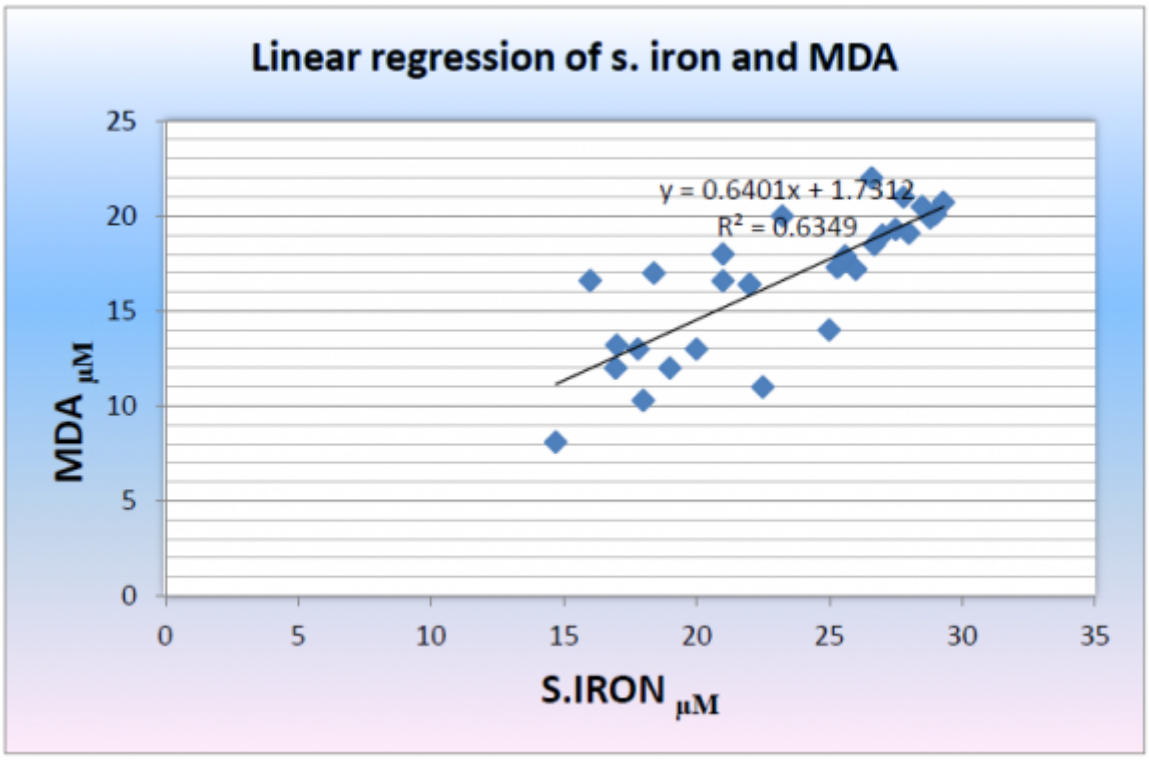

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Figure 4: Figure 3 :

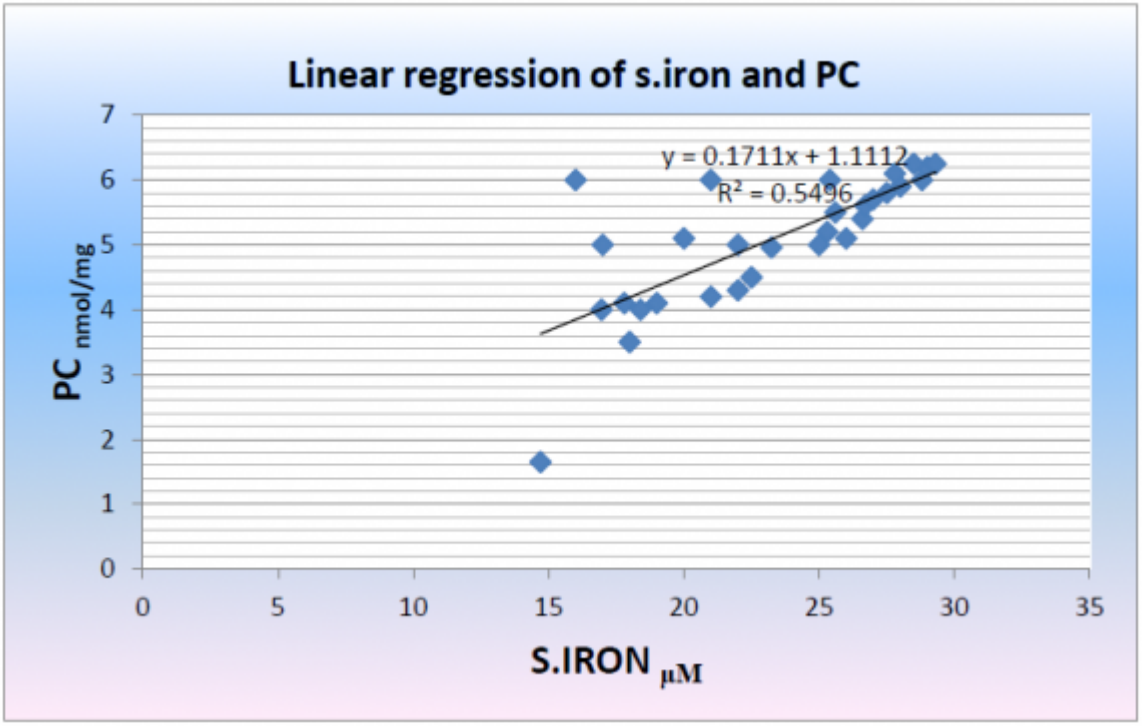

Figure 5:

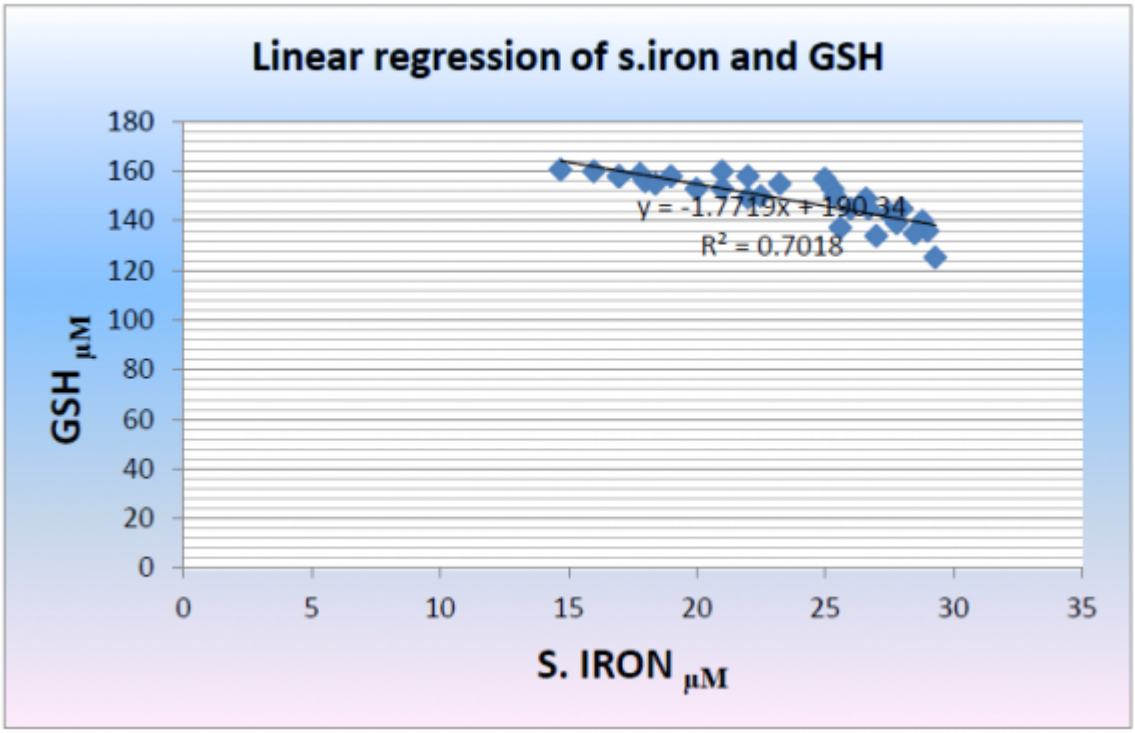

Figure 6:

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