

# Impact of Decreasing Blood Hemoglobin Concentration On Malondialdehyde, Reduced Glutathione Levels In Anemic Women During Pregnancy

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## الخلاصة

أجريت الدراسة الحالية لإثبات العلاقة بين تركيز هيموكلوبين الدم مع مستويات كلا من الكلوتاثاينون المختزل والمالون ثنائي الديهايد ، والتي ضمت ٥٠ امرأة من الأصحاء للمقارنة و ١٠٠ امرأة من الحوامل اللواتي يعانين من فقر الدم. قسمت عينة البحث إلى ثلاث مجموعات، تكونت الأولى من ٩ مصابات بفقر الدم خلال الفصل الأول من الحمل، والمجموعة الثانية ٥٧ حامل خلال الفصل الثاني، أما المجموعة الثالثة ضمت ٣٤ حامل في الفصل الثالث. بينت النتائج انخفاضاً كبيراً في تركيز الهيموكلوبين ( $p < 0.001$ ) للمجاميع النساء الثلاثة المصابات بفقر الدم مقارنة مع مجموعته السيطرة. على النقيض من ذلك لوحظ ارتفاع معنوي في مستويات المالون ثنائي الديهايد ( $p < 0.001$ ) في مصول النساء الحوامل، وجد كذلك نقص معنوي في تراكيز الكلوتاثاينون المختزل ( $p < 0.001$ ) خلال الفصل الثاني والثالث من الحمل، بينما أوضحت النتائج عدم وجود فروق واضحة في مستوى الكلوتاثاينون المختزل خلال الفصل الأول من الحمل، وقد لوحظ بأن المعدل الكلي لهيموكلوبين أعلى في الفصل الأول عما هي عليه في الفصل الثاني، بينما وجد بأن هناك انخفاضاً كبيراً في تركيز لهيموكلوبين في الفصل الأخير من الحمل مقارنة مع المجموعة الضابطة.

## Abstract

The present study was conducted to verify the relationship between Hemoglobin (Hb) concentration (con), Reduced glutathione (GSH), and malondialdehyde (MDA) levels in 50 healthy woman and 100 pregnant anemic women. (anemic pregnant women) were divided into three groups, group 1 consisted of (9) patients with first trimester, group 2 contained 57 patients with second trimester, and group 3 comprised 34 patients with third trimester. the result exhibits a significant decrease ( $p < 0.001$ ) in blood hemoglobin concentration in pregnant anemic groups comparison to the control groups. In contrast, a significant increase ( $p < 0.001$ ) in MDA level in sera of women with pregnant, also a significant highly decrease was found in GSH level in sera of pregnant women in second and third trimester ( $p < 0.001$ ), While there was no significant differences in GSH level for women in first trimester as compared with control groups. Average total Hb con were observed in the first trimester was higher than Hb con in the second trimester, while, largest decrease was found in the third trimester, with a drop relative to the control serum total Hb con.

**Key words:** Reduced Glutathione, Malondialdehyde, Pregnancy, Oxidative Stress

## Introduction

Anemia during pregnancy is a commonest medical disorder that can have deleterious effects on mother and as well as fetus in the form of maternal morbidity and mortality intrauterine

growth retardation, poor weight gain, premature labor, preterm delivery ,and prenatal morbidity, and mortality [1]. Among pregnant women, a about 90% of anemia cases are reported to be due to iron deficiency, because high iron requirements during pregnancy are not easily fulfilled by dietary intake alone, especially when iron bioavailability is poor [2]. Iron deficiency is the most common deficiency worldwide, with negative effects on work capacity and on motor and mental development of infants, children, and adolescents, particularly in developing countries [3- 6]. The World Health organization (WHO) defines anemia as a haemoglobin (Hb) below 13 g/dL in men over 15 years, below 12 g/dL in non-pregnant women over 15 years, and below 11 g/dL in pregnant women [7].

Pregnancy is a physiological state, which is accompanied by a high-energy demand and an increased oxygen requirement. Both of these may lead to increased oxidative stress. Oxidative stress may be defined as a condition where there is disturbance in the pro-oxidant antioxidant balance, which favors the former [8]. Living organisms possess several anti oxidative species and mechanisms protecting them against the harmful action of Reactive Oxygen Species (ROS). These include the enzymes superoxide dismutase (SOD), glutathione peroxides (GSH-Px), and reduced glutathione (GSH) , together with non enzymatic antioxidants, like selenium compounds, vitamins A, E, and C, and compounds containing thiol groups [9]. GSH plays a pivotal role in protection of cells against oxidative stress. It can act as a non-enzymatic antioxidant by direct interactions of SH group with ROS or it can be involved in the enzymatic detoxification reactions for ROS as a coenzyme [10].

Lipid peroxidation is an oxidative process which occurs at low levels in all cells and tissues. Under normal conditions a variety of antioxidant mechanisms serve to control this peroxidative process [11].

The present study was designed to evaluate the oxidation – anti oxidation balance in the pregnant anemic women using critical parameters (MDA as oxidative parameter and GSH as an anti oxidative parameter).

### **Subject, Materials and Methods**

The subjects enrolled in this study comprised 100 pregnant anemic women. Study samples were obtained from The medical Center of Messan , Kufa City in Najaf Government . The

patient group categorized into three groups. group 1 consisted of 9 patients with First trimester, group 2 contained 57 patients with second trimester, and group 3 comprised 34 patients with third trimester. All the study subjects were within the age range of 20-41 years. In addition 50 age matched healthy subjects were recruited to serve as a control group Hb as [11 g/dl] ranging. Patients suffered diabetes mellitus, hypertension and, hemolytic anemia renal disease and, cancer were excluded from the present work. Informed consent was obtained from each subject. Blood hemoglobin was determined using the cyanomethemoglobin method [12]. Malondialdehyd (MDA) was measured according to Draper et al. [13]. GSH estimated by Ellman [14].

**Blood Samples:** Blood samples were obtained from the study groups by vein puncture, five ml venous blood was taken from each subject at the time of recruitment and divided into two aliquots. Two ml blood was transferred to an EDTA containing evacuated tube used to determine hemoglobin (Hb). Remaining 3 ml of venous blood was also centrifuged at 3000 rpm for 15 min, serum separated and used for the estimation of MDA level and Reduced glutathione (GSH).

**Bio Statistical Analysis:** The results were expressed as mean  $\pm$  SD. Students t-test was used for assessment of the results. Significant variation was considered when the P value was less than 0.05

### **Results and Discussion**

The results in current study. The hemoglobin concentration were observed to be lower in anemic women when compared with control group. In contrast, MDA levels were found to increase significantly ( $p < 0.001$ ) during the first trimester of pregnancy when compared with those of the control group. While GSH Levels failed to illustrate the significant variation among patient at 1<sup>st</sup> trimester and control group (**Table1**).

**Table 1:** Blood Hemoglobin Con, Reduced Glutathione and Malondialdehyd Levels For Control Group and Anemic Pregnant Women In First Trimester

	<b>Control group</b>	<b>anemic women</b>	<b>P value</b>
	<b>Mean <math>\pm</math> SD</b>	<b>Mean <math>\pm</math> SD</b>	
<b>Hb (gm/dl)</b>	12.084 $\pm$ 1.242152	11.007 $\pm$ 0.66189	p<0.001
<b>GSH (<math>\mu</math>M)</b>	402.59 $\pm$ 94.13	398.02 $\pm$ 84.13	N. S
<b>MDA(<math>\mu</math>M)</b>	6.79 $\pm$ 1.11	12.93 $\pm$ 2.38	p<0.001

In the second trimester of pregnancy, the outcome results significant decreases in the GSH levels  $p < (0.001)$ , and Hb concentration  $p < 0.001$  (**Table 2**). On the other hand MDA levels recorded a significant elevations in sera of patients of 2<sup>nd</sup> trimester when compared to control group.

**Table 2:** Blood Hemoglobin Con Reduced Glutathione and, Malondialdehyde Levels For Control Group and Anemic Pregnant Women In Second Trimester

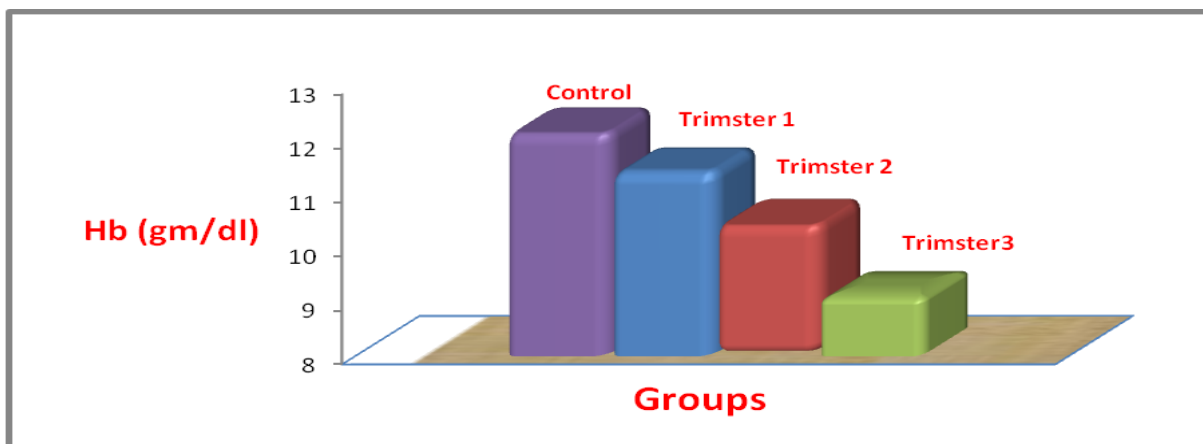
	<b>Control group</b>	<b>Anemic Women</b>	<b>P value</b>
	<b>Mean <math>\pm</math> SD</b>	<b>Mean <math>\pm</math> SD</b>	
<b>Hb (gm/dl)</b>	12.084 $\pm$ 1.242152	10.382 $\pm$ 1.227866	P<0.001
<b>GSH (<math>\mu</math>M)</b>	402.59 $\pm$ 94.13	310.59 $\pm$ 44.13	p<0.001
<b>MDA(<math>\mu</math>M)</b>	6.79 $\pm$ 1.11	15.23 $\pm$ 2.38	p<0.001

From **Table 3** it was notice that the value of Hb concentration and GSH levels are significantly  $p < 0.001$ ) decrease. On other hand, the results exhibited a significant  $p < 0.001$  rise in MDA levels in comparison to those with the control group.

**Table 3:** Blood Hemoglobin Con Reduced Glutathione And Malondialdehyde Levels For Control Group And Anemic Pregnant Women In Third Trimester

	<b>Control group</b>	<b>anemic women</b>	<b>P value</b>
	<b>Mean <math>\pm</math> SD</b>	<b>Mean <math>\pm</math> SD</b>	
<b>Hb (gm/dl)</b>	12.084 $\pm$ 1.242152	9.0818 $\pm$ 0.780406	P<0.001
<b>GSH (<math>\mu</math>M)</b>	402.59 $\pm$ 94.13	215.20 $\pm$ 69.20	p<0.001
<b>MDA(<math>\mu</math>M)</b>	6.79 $\pm$ 1.11	17.03 $\pm$ 2.38	p<0.001

When the different groups were compared together (**Figure 1**) The mean Hb concentrations in all the three trimester patients were lower than the healthy subjects. Average total Hb concentration, in the first and second trimester were less than the control Hb concentration ( $p < 0.05$ ), The clearest decrease results were observed in the third trimester, with a drop relative to the control serum total Hb concentration ( $p < 0.05$ ).



**Fig. 1:** Levels of Blood Hemoglobin Con In Three Trimester With Anemic Pregnancy Women and The Control Group

The current work results suggest the decreased of hemoglobin in all pregnant anemic groups might be due to the fact that iron is an essential constituent of hem and when its levels are decreased may lead to decreased in the hemoglobin synthesis process. Several studies had also shown that the levels of red blood cell counts Fe and ferritin found decreased in all anemic women groups [15,16]. The need for iron varies markedly during each trimester of pregnancy. Iron requirements decrease during the first trimester because menstruation stops [17]. During the second trimester, iron requirements begin to increase and continue to do so throughout the remainder of pregnancy [18].

In the present study the lipid peroxidation product i.e. MDA levels have been increased significantly in serum of the patients with anemic pregnancy women. Rise in MDA could be due to increased generation of ROS due to the excessive oxidative damage generated in these patients. It is hypothesized that free radicals were produced due to the oxidative damage to the red blood cells [19]. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids [20]. Similar reports of elevated MDA levels have been reported in patients with anemia due to iron deficiency [21, 22] the present work agreed

with these researches. Significant decrease in the levels of serum reduced glutathione (non enzymatic antioxidant defense system) in patients with anemic pregnancy women when compared to controls as recorded. This decrease in the levels of GSH may be due to the increased turnover, for preventing oxidative damage in these patients suggesting an increased defense against oxidant damage in these patients. These results were supported by the study conducted by Neelima et al [23]. In contrast to our study, Gekova K et al [24], reported the rise in the levels of GSH.

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