

A Study on Association of Oxidative Stress in Diabetes Mellitus on High Sensitivity C - reactive protein

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Abstract: Diabetes mellitus is an oxidative stress condition. Hydrogen peroxide is produced during the advanced glycation reactions in diabetes mellitus. Ferrous Iron in presence of hydrogen peroxide results in the Fenton reaction producing hydroxyl radicals which are injurious to tissues. High sensitivity C - reactive protein (hsCRP) is an acute phase protein that is elevated in injury and inflammation. Fasting glucose, serum iron, hemoglobin and hsCRP were estimated in patients with diabetes mellitus and in age matched controls. In this study, we have shown that, in individuals with increased serum glucose and serum iron, the hsCRP levels are significantly elevated when compared with individuals with relatively lower serum glucose and iron. The increase in hsCRP in diabetes mellitus may be in response to the tissue injury caused by hydrogen peroxide and ferrous iron.

Keywords: Advanced Glycation End Products, Diabetes mellitus, High sensitivity C-reactive protein, Oxidative stress,

I. Introduction

Diabetes mellitus is an oxidative stress condition. This is due to the increased production of hydrogen peroxide in diabetes mellitus. Hydrogen peroxide is produced during the advanced glycation reactions. Amadori reaction of glucose with amino groups form ketoamine which undergoes further reaction to form the Advanced Glycation End products. Increased hydrogen peroxide produced can lead to the damaging Fenton reaction if ferrous iron is available. Ferric iron may also cause Fenton reaction if the surrounding medium contains reducing substances such as Vitamin C which will convert ferric iron to ferrous iron. [1] Advanced Glycation End products formed after Amadori product formation increases when blood glucose levels increase. Therefore, higher glucose concentration leads to the formation of higher levels of hydrogen peroxide.

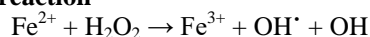
Serum urea traps hydrogen peroxide and form urea-hydrogen peroxide. Therefore, hydrogen peroxide levels may be increased in uremic patients. Both hydrogen peroxide and urea-hydrogen peroxide can be a substrate for Fenton Reaction. Urea hydrogen peroxide is more stable than hydrogen peroxide. Uremia is another cause for increase in hydrogen peroxide in serum. In this study we have selected individuals with blood urea levels within the reference interval.

High Sensitivity CRP (hsCRP) estimation is different from CRP estimation in that the former is more sensitive and quantitative CRP estimation method, where the sensitivity of the assay is 0.01mg/dL. hsCRP is an acute phase protein whose level is increased in diseases that causes inflammation, infection, injury and during repair. hsCRP is also increased in diabetes mellitus, atherosclerosis and malignancies.[2]

In this study, we are investigating to find a correlation between serum glucose levels and hsCRP and between serum iron levels and hsCRP. If blood glucose is increased, then hydrogen peroxide production is increased. If serum iron bound to transferrin is increased then amount of free serum iron may increased. This may result in Fenton reaction and cause tissue injury. Tissue injury will cause increase in hsCRP.

C-Reactive Protein(CRP) is an inflammatory marker. Inflammation is a normal response to many physical states including fever, injury, autoimmune activity and infection. CRP is synthesized by the liver in response to IL-6 released into the plasma [3] .During the acute phase response of an inflammatory reaction, CRP levels are elevated up to 1000 fold making it an excellent early indicator of infection. [4] High sensitivity CRP (hsCRP) assay is used to estimate CRP levels between 0.1-15 mg/L. Normal range 0.068-8.2mg/L [5]. High sensitivity CRP is one of the markers inflammation and injury. CRP levels were also found to be increased in certain conditions such as diabetes mellitus, atherosclerosis, malignancy and polycystic ovary. Diabetes mellitus is an oxidative stress condition where the production of hydrogen peroxide is increased. Hydrogen peroxide in the presence of ferrous iron forms hydroxyl radical (Fenton reaction). Hydroxyl radical is injurious to proteins, DNA and lipids. The source of hydrogen peroxide in diabetes mellitus is during the advanced glycation end product formation [6]. Fenton reaction is considered to be the cause of iron toxicity. It is also the reaction that is the cause of toxicity in conditions that increase the production of hydrogen peroxide, such as diabetes mellitus[6]

1.2.Fenton reaction



Hepcidin is a peptide that regulates iron homeostasis by inhibiting iron absorption by the small intestine and release of iron from macrophages. Its production is stimulated by iron overload and by inflammation. It has been suggested that IL-6 is the only cytokine that stimulates hepcidin transcription. Erythropoiesis mediates hepcidin expression, with increased erythropoietic activity suppressing the action of hepcidin. With regard to inflammation and infection, cytokines, in particular interleukin (IL-6) ,can induce hepcidin expression in macrophages and neutrophils in response to infection and inflammation in a manner reminiscent of the toll-like receptor 4 pathway [6]

IL-1 is made by many cells (endothelial cells, B cells, and fibroblast cells) but most abundantly by macrophages .Over production of TNF-α in adipose tissue is an important feature of obesity and contributes significantly to insulin resistance [7]

II. Materials And Methods

Fasting blood samples were taken from patients with diabetes mellitus and in age matched controls. The person should not be suffering from any diseases other than diabetes mellitus, as understood from the case history.

For estimation of Hb, blood collected in plain glass tubes which contained a pinch of EDTA as the anticoagulant. For estimation of other parameters in serum blood is taken in plain glass tubes and allowed to clot. Serum was separated by centrifugation at 3000 revolutions per minute for 4-5 minutes. hsCRP was estimated by immunoturbidimetric method in fully automated biochemical analyzer – Vitros 5,1FS.[8] Glucose is estimated by glucose-oxidase method. Serum iron is estimated by liberating bound iron at low pH from serum transferrin and reacting with Ferrozine. [9]Hemoglobin was estimated by Cyanmethemoglobin method. [9]

III. Results

In the first set of evaluation of results, individuals with fasting serum glucose between 70-85 and individuals with fasting serum glucose between 126 -200 were segregated as two groups. In these two groups, the mean and S.D of serum iron, hsCRP and hemoglobin were calculated. The values of these three parameters in these two groups were evaluated for any significant variation in their levels. It was observed that there was a significant increase in hsCRP levels in people with serum glucose levels between 126 and 200 (Table 1, Figure 1). It was also observed that there was no significant variation in serum iron levels in these two groups.

When the serum iron levels were segregated into two groups, with first group containing relatively low levels of iron and second group containing relatively higher levels of iron. Mean and standard deviation of hsCRP is calculated in these two groups. It was observed that hsCRP is significantly elevated in individuals with relatively higher levels of serum iron (Table 2 Figure 2).

3.1. Statistical analysis

The level of significance in the variation of serum hsCRP , serum iron were determined by student’s t-test All data were presented as Mean±standard deviation

	Serum glucose(n=17)	Serum iron (n=17)	hsCRP(n=17)	t value for serum iron Levels p value	t value for hs CRP levels(LS) p value
Low glucose group	79.12±5.57	158.9±41.3	0.44±0.3	0.598	4.95
High glucose group	136.88±22.04	192.5±153.9	2.25±2.1	P> 0.05	P>0.001

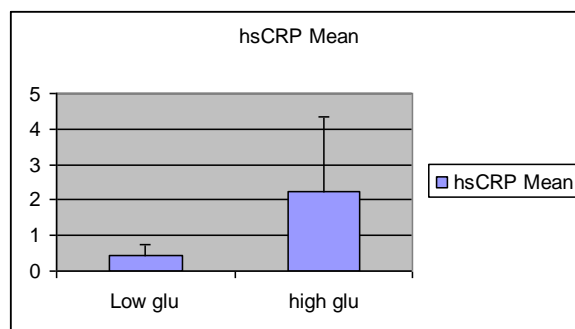


Figure 1: Serum hsCRP levels in patients with low and high glucose levels

Table 2. Serum hsCRP levels in individuals with relatively lower and higher levels of serum iron.

	Serum iron(n=14)	hsCRP(n=17)	T value for hs CRP levels(LS)
Low serum iron group	85.5±81.35	1.1±1.33	2.435
High serum iron group	249.8±157.6	3.15±2.1	p<.05

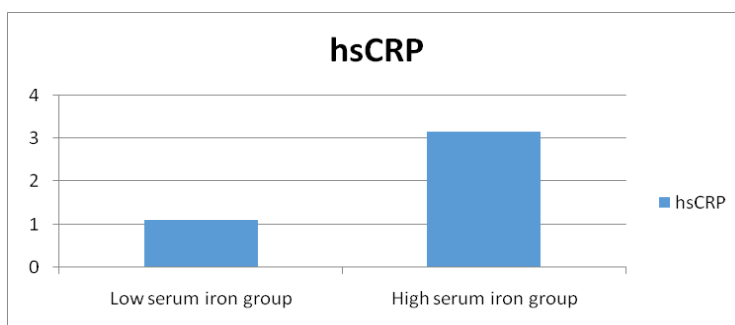


Figure2 .Serum hsCRP levels in individuals with relatively lower and higher levels of serum iron.

IV. Discussion

Hydrogen peroxide is found in the human body by repeated reduction of oxygen to superoxide and then to hydrogen peroxide. Fe²⁺ in the presence of hydrogen peroxide causes Fenton reaction resulting in the formation of hydroxyl radicals that are highly toxic. [11]Hydroxyl radicals damage lipids, proteins, and DNA. This reaction may result in a mild acute phase reaction resulting in small increase in hsCRP. In this study we are comparing the level of hsCRP in people with relatively higher and lower levels of plasma glucose. The variations in hsCRP levels were also correlated with the levels of serum iron. Serum iron is also related to the level of hemoglobin. Serum iron level is low in anaemic patients.

Iron in hemoglobin exists as Fe²⁺ and in transferrin as Fe³⁺. It is Fe²⁺ that promotes Fenton reaction. But Fe³⁺ may also participate in Fenton reaction by getting converted into the Fe²⁺ form in the presence of reducing agents such as Vitamin C.

Formation of hydrogen peroxide is increased in presence of higher glucose levels. This is because reducing sugars can react with free amino groups of proteins and form ketoaminic group, which are stable products of Amadori reaction. These products undergo further rearrangement and produce advanced glycation end products. This process is called Millard reaction. Before forming this glycation end products the early stable glycated products are formed by Amadori reaction. [12]

CRP levels are generally increased in infections, injury or inflammations. hs CRP, which is the variations of CRP at lower levels can occur when these conditions are mild or chronic. But hsCRP has been found to elevated in conditions such as diabetes mellitus, atherosclerosis and malignancy. [13] The increase in hsCRP in diabetes mellitus may be in response to the tissue injury caused by hydrogen peroxide and ferrous iron. In this study we have found that when iron and glucose are elevated, there is a significant increase the levels of hsCRP.

The two major glycation end products formed by dehydration, rearrangement and fragmentation of Amadori reaction products are pentosidine and carboxy methyl lysine. [14] Hydrogen peroxide is formed during this reaction. Therefore, oxidative damage can occur at a higher rate in the presence of higher levels of serum glucose. Hydrogen peroxide may form a relatively stable complex with urea called urea-hydrogen peroxide. Urea hydrogen peroxide also promotes Fenton reaction. In the patients studied, blood urea levels have been estimated and were found to be within physiological reference interval. This was done to avoid the increase in hydrogen peroxide levels due to the higher levels of urea-hydrogen peroxide though at present we do not know the exact pathway that proceeds from oxidative damage of tissues to increase in hsCRP. We know from these results that hsCRP is significantly increased in people with higher serum glucose and higher serum iron.

V. Conclusion

In individuals with increased serum glucose and serum iron, the hsCRP levels are significantly elevated when compared with individuals with relatively lower serum glucose and iron. The increase in hsCRP in diabetes mellitus may be in response to the tissue injury caused by hydrogen peroxide and ferrous iron.

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