

Relationship of C-Reactive Proteins with Oxidative Stress and Obesity in Type 2 Diabetic Subjects

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Abstract: Objective: The present work was aimed to study the correlation between C-reactive proteins as marker of acute phase response, total antioxidants capacity as marker of oxidative stress and blood pictures with the electrophoresis patterns of serum protein in sera of subjects with obese and diabetic obese subjects attending Sohage University Hospital.

Subjects: The total number of study subjects was 85 subjects, 30 (35.2%) individuals with BMI 20.11 were control, 31(36.4%) subjects with BMI 31.99 were obese and 24 (28.2%) subjects with BMI 30.62 were obese diabetic type 2.

Method: In sera, C-reactive protein and immunoglobulin G was assayed by ELISA protocol, total antioxidant capacity was measured by colorimetric method, blood pictures were analyzed by haematological analyzer (CellDyne 3700) and sera proteins fractions were analyzed by electrophoresis.

Results: The present study was found that the counts of lymphocytes, monocytes and platelets were increased in non-diabetic obese and diabetic obese subjects compared to controls but still in normal range, red blood cells and hematocrite were decreased compared to control and still in the normal range, C-reactive protein was significantly elevated, however, total antioxidant capacity was decreased in non-diabetic obese and diabetic obese subjects compared to controls. In addition, immunoglobulines in electrophoretic pattern was increased compared to control.

Conclusion: The present results confirm the positive correlation between the acute phase response of inflammation and oxidative stress in non-diabetic and diabetic obese subjects.

Keywords: Obesity, type 2 Diabetes, oxidative stress, IgG, TAC, CRP, Albumin, Globulins.

1 Introduction

Obesity and type 2 diabetes mellitus (DM2) are considered to be associated with a low-grade inflammation, which may play a significant role in the development of cardiovascular complications [1,2]. C-reactive protein (CRP) is an extremely sensitive marker of systemic inflammation produced mainly by the liver under the stimulation of adipocyte-derived proinflammatory cytokines [3]. CRP has also emerged as a powerful predictor of cardiovascular diseases [4]. Elevated levels of CRP are described in DM2 subjects; however, it is not clear if they are related to the presence of obesity, diabetes, or both [5, 6]. Some studies indicate a relationship between CRP and macrovascular complications of diabetes [7,8]. It has not yet been shown whether the CRP level increases due to the metabolic effects of obesity and diabetes and plays a direct role in promoting the inflammatory component of atherosclerosis

or whether it is merely a marker of the ongoing inflammation in the vessels affected by diabetes.

ROS occur under physiological conditions and in many diseases and cause direct or indirect damage in different organs; thus, it is known that oxidative stress (OS) is involved in pathological processes such as obesity, diabetes, cardiovascular disease, and atherogenic processes. It has been reported that obesity may induce systemic OS and, in turn, OS is associated with an irregular production of adipokines, which contributes to the development of the metabolic syndrome [9]. The sensitivity of CRP and other biomarkers of oxidative damage are higher in individuals with obesity and correlate directly with BMI and the percentage of body fat, LDL oxidation, and TG levels [10]; in contrast, antioxidant defense markers are lower according to the amount of body fat and central obesity [11, 12]. A research showed that a diet high in fat and carbohydrates induces a significant increase in OS stress

and inflammation in persons with obesity [13]. When obesity persists for a long time, antioxidant sources can be depleted, decreasing the activity of enzymes such as superoxide dismutase (SOD) and catalase (CAT) [14]. The activity of SOD and glutathione peroxidase (GPx) in individuals with obesity is significantly lower compared with that in healthy persons, having implications for the development of obesity-related health problems [15].

Serum immunoglobulin levels are determined routinely in clinical practice because they provide key information on the humoral immune status. Low immunoglobulin (Ig) levels define some humoral immunodeficiencies [16]. In contrast, high immunoglobulin levels (polyclonal gammopathy) are observed in liver diseases, chronic inflammatory diseases, haematological disorders, infections and malignancies [17]. Moreover, immunoglobulin levels aid in the diagnosis of some disorders, particularly liver diseases [18].

2 Material and methods

Blood samples were collected in the morning from the subjects after an overnight fasting. Blood pressure was measured for all subjects, also fasting and postprandial 2 hours was performed. CBC was performed for each individual by haematological analyzer (CellDyne 3700). TAC was measured by colourimetric method (koracevic *et al.*, 2001). The biochemical parameters that were measured in this study included, serum CRP and IgG were measured by enzyme immunoassay method by. Serum proteins was analysed by electrophoretic pattern according to John R. S. *et al.* (1976)

3 Statistics

Statistics was performed using the statistical graph pad

prism 5. One way analysis of variables (ANOVA) was used posted by Newman-keuls test. All results are expressed as mean \pm SE and the level of significance between groups were * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

4 Results

The total number of study subjects was 85 subjects, 30 (35.2%) individuals with BMI 20.11 were control, 31(36.4%) subjects with BMI 31.99 were obese and 24 (28.2%) subjects with BMI 30.62 were obese diabetic type 2. Table (1) showed the demographic data of the participants in which control showed waist circumference with mean 82.10 cm, but waist circumference for obese non-diabetic group was raised to 118.2 and for obese diabetic group was 119.9. Both blood glucose level and blood pressure showed a significant increase in obese non-diabetic and diabetic obese groups compared to control subjects.

Table 2 express Complete blood counts of all subjects. According to our statistic program WBC count increased non-significantly in both obese non-diabetic and obese diabetic subjects compared to control. Lymphocytes count was increased significantly in obese non-diabetic group and in obese diabetic group compared to control subjects but it still within normal range. Monocytes count significantly increased in obese non-diabetic subjects and in obese diabetic compared to control but it still within normal range. Moreover, granulocytes counts expressed non-significant changes in the three groups. RBC and HTC shown significantly decreased in obese diabetic compared to control and obese non-diabetic groups, the decrease still within the normal range. MCV expressed non-significant changes in the three groups. The level of HGB, MCH and MCHC showed non-significant changes in the different subjects groups.

Table 1: Demographic data of the study participants

Parameters	Control <i>n</i> =30	Non-diabetic obese <i>n</i> =31	Diabetic obese <i>n</i> =24
BMI (kg/m ²)	20.11 \pm 0.3217	31.99 \pm 0.4219*** ^a	30.62 \pm 0.2871 ^{*b}
Waist circumference (cm)	82.10 \pm 0.8608	118.2 \pm 1.545 *** ^a	119.9 \pm 1.505
Systolic blood pressure (mmHg)	116.0 \pm 2.724	124.6 \pm 2.665 * ^a	145.3 \pm 3.961 *** ^b
Diastolic blood pressure (mmHg)	84.93 \pm 2.820	92.17 \pm 2.454* ^a	103.5 \pm 2.245** ^b
Fasting blood glucose level	84.30 \pm 1.247	95.90 \pm 2.175 * ^a	126.7 \pm 5.938 *** ^b
Postprandial 2 hours blood glucose level	108.8 \pm 3.252	118.0 \pm 2.811	257.3 \pm 11.78*** ^b

Data are expressed as mean \pm SE, where the number of asterisk (*) indicates the levels of significant at $p < 0.05$, 0.01, 0.0001.

Letter^a referred to the significant difference between non-diabetic obese and control.

Letter^b referred to the significant difference between diabetic obese and control.

Letter^c referred to the significant difference between non-diabetic obese and diabetic obese.

Table 2: Complete blood counts of different subjects

Parameters	Control	Non-diabetic obese	Obese diabetic
WBC (10 ⁹ /L)	5.001±0.336	5.849±0.270	5.600±0.330
lymphocytes (10 ⁹ /L)	1.937±0.094	2.368±0.175 ^{*a}	2.743±0.124 ^{**b}
Monocytes (10 ⁹ /L)	0.39±0.020	0.489±0.021 ^{*a}	0.689±0.072 ^{***b&***c}
Eosinophils (10 ⁹ /L)	0.289±0.055	0.259±0.036	0.151±0.014
Basophils (10 ⁹ /L)	0.065±0.008	0.063±0.004	0.076±0.007
Neutrophils (10 ⁹ /L)	2.099±0.358	2.565±0.302	2.254±0.222
RBC (10 ¹² /L)	5.206±0.062	5.277±0.062	4.818±0.178 ^{*b&*c}
HTC (%)	44.50±0.608	44.70±0.482	41.38±1.660 ^{*b&*c}
MCV (fl)	85.58±0.891	85.02±0.827	86.00±1.385
HGB (g/dL)	14.57±0.177	14.95±0.154	15.41±0.861
MCH (pg)	27.50±0.719	28.41±0.343	29.15±0.447
MCHC (g/dL)	32.90±0.269	33.44±0.184	33.95±0.429
PLT (10 ⁹ /L)	215.9±7.077	254.1±9.645 ^{**a}	284.8±33.54 ^{*b}
MPV (fl)	8.765±0.238	8.617±0.197	7.913±0.418

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significant at p<0.05, 0.01, 0.0001.

Letter^a referred to the significant difference between non-diabetic obese and control.

Letter^b referred to the significant difference between diabetic obese and control.

Letter^c referred to the significant difference between non-diabetic obese and diabetic obese.

Table 3: Inflammatory biomarkers and total antioxidant capacity in sera of all subjects groups

Parameters	Control	Non-diabetic obese	Obese diabetic
CRP (μg/ml)	1.676 ±0.214	7.561 ±0.531 ^{***a}	7.068 ±0.598 ^{***b}
IgG (mg/dl)	522.5±54.88	629.92±57.10	691.67±71.29
TAC (mM/L)	1.544±0.062	1.294±0.031 ^{***a}	1.120±0.082 ^{***b}

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significant at p<0.05, 0.01, 0.0001.

Letter^a referred to the significant difference between non-diabetic obese and control.

Letter^b referred to the significant difference between diabetic obese and control.

Letter^c referred to the significant difference between non-diabetic obese and diabetic obese.

Table 4: Electrophoretic pattern of serum protein

Albumin and serum proteins	Control n=10	Non-diabetic obese n=10	Obese diabetic n=10
Albumin	31.83±1.320	29.64±0.4034	28.67±1.063
α ₁	25.44±0.7010	6.604±0.3235 ^{***a}	10.79±0.9531 ^{***b&***c}
α ₂	16.88±2.052	30.30±0.2885 ^{**a}	23.17±2.563 ^{*b&*c}
β	11.03 ±1.377	19.43 ±1.773 ^{**a}	23.43 ±1.007 ^{**b}
γ	12.42 ±0.5061	13.92 ±1.748	27.07 ±1.986 ^{***b&***c}

Data are expressed as mean ± SE, where the number of asterisk (*) indicates the levels of significant at p<0.05, 0.01, 0.0001.

Letter^a referred to the significant difference between non-diabetic obese and control.

Letter^b referred to the significant difference between diabetic obese and control.

Letter^c referred to the significant difference between non-diabetic obese and diabetic obese.

subjects groups.

The count of PLT indicated that PLT count increased significantly in obese non-diabetic and obese diabetic group compared to control and the count within the normal rang. Also MPV non-significant changes between all

5 Discussion

We evaluated the effect of human obesity on blood picture. One of the potential difficulties in studying immune status in obese population is that the effect of obesity itself on immune system can be obscured by the frequent occurrence of coexistent hyperglycaemia and dyslipidaemia. The relationship between obesity and immunity has lacked sensitive methods and sufficient numbers of subjects to identify abnormalities in immunity. Leukocyte count with its subpopulations is a clinical marker of inflammatory processes [21,22] related to cardiometabolic disorders involved in the development of cardiovascular diseases [23] especially in overweight individuals [24].

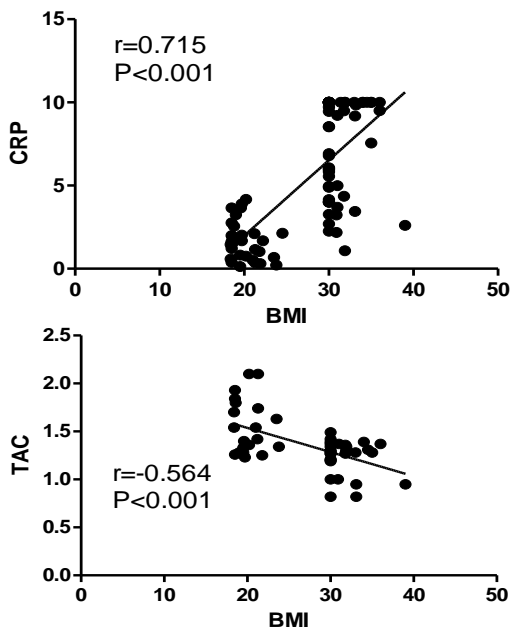


Figure (1): correlation plots between CRP, TAC and BMI.

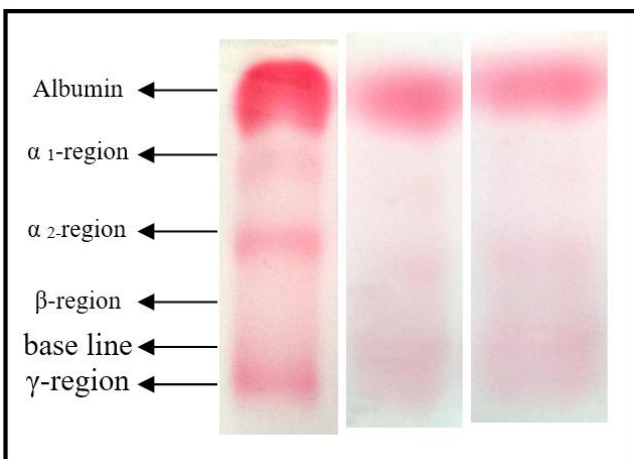


Figure (2): Serum protein fractions in sera of all subjects.

These were the primary findings that obese non-diabetic

and obese diabetic subjects represented with higher total leukocytes and subpopulation counts when compared to control but it still within normal range. Moreover, neutrophils and monocytes have been associated with obesity in adolescence and trigger the appearance of coronary artery disease [25]. In obesity T cell was impaired and this dysfunction can be recovered by adequate weight reduction [26]. Moreover, there is positive correlation BMI and total WBC, neutrophils and lymphocytes [27,28]. In the present results we found high number of lymphocytes in both obese non-diabetic and obese diabetic subjects compared to control but it still within the normal range.

Several studies highlighted the positive correlation between RBC count in diabetic and non diabetic obese subjects. This relationship might be explained by the IR mechanisms in the development of metabolic syndrome, because insulin and insulin growth factors I and II supporting erythropoiesis *in vitro in vivo* studies [29, 30]. Moreover, it was found that erythrocytes count and hematocrit (HCT) were associated with metabolic syndrome [31,32]. Other studies indicated that hyperglycemia could lead to morbidity and mortality [33], and increased non-enzymatic glycosylation of RBC membrane proteins [34, 35]. Moreover, oxidation of proteins and hyperglycaemia in DM causes an increase in the production of lipid peroxides that lead to haemolysis of RBC [36,37]. In the present study, erythrocytes count and hematocite percentage in diabetic obese subjects were significantly decreased compared to control and obese non-diabetic subjects but it still in the normal range.

It is known that higher platelet counts are associated with adverse clinical outcome in patients with myocardial infarction [38] and its activation was associated with the risk of stroke [39]. Moreover, platelet count and activation are associated with chronic inflammation such as thrombocytosis [40, 41] and inflammatory bowel diseases [42]. Nakata *et al.* [43] found that leptin receptor was expressed in platelets; hence, leptin potentiates platelet aggregation by agonists [44]. In the present investigation we observed high platelets count in obese non-diabetic and obese diabetic subjects compared to control but it still in the normal range.

CRP is a sensitive physiological marker of subclinical systemic inflammation, is associated with hyperglycemia, IR, and overt T2DM [45]. There is positive association between inflammatory biomarkers and main factors of diabetic diagnosis such as IR and HbA1C. CRP is a main inflammatory factor that produced by the liver during acute infection or inflammation and its concentration in plasma can increase as much as 1000-fold during injury and infection [46]. CRP and may be independent risk factors for chronic kidney disease in patients with T2DM [47]. In the present study CRP is significantly higher in non-diabetic obese and diabetic

obese subjects compare with the healthy normoglycemic controls. This result was consistent with the result obtained by Hansen *et al.* [48]. Elevated levels of CRP in non-diabetic obese subjects predict the development of T2DM. Moreover, CRP has predictive value in chronic phase after myocardial infarction [49] and has a role in the pathogenesis of atherosclerosis [50, 51] in obese subjects. There is correlation between chronic inflammatory response, obesity and endothelial dysfunction as well as a chronic alteration of the immune function [52]. Elevation of CRP increased the risk for future myocardial infarction and stroke [53, 54] and has been reported with an increase of white blood count [55]. So, the results of the present study confirmed the well-known elevation of acute-phase proteins including CRP and leukocytosis in non-diabetic obese and diabetic obese subjects, which may be indicative for subclinical chronic inflammation associated with an increased risk for cardiovascular diseases.

Free radicals are reactive molecules produced naturally in the human body during metabolic reactions. High levels of free radicals damage cellular proteins, membrane lipids, and nucleic acids, and eventually lead to cell death. Free radicals play an important role in the pathogenesis of many chronic diseases, including atherosclerosis, myocardial failure, immune diseases, and T2DM. Free radicals include reactive oxygen species (ROS) and reactive nitrogen species [56], antioxidant compounds counter the effects of free radicals [57]. Oxidative stress is defined as the increased generation of free radicals and/or the impaired compensatory response of endogenous antioxidant defenses [58]. In the present study the total antioxidant capacity in plasma was decreased in non-diabetic obese and diabetic obese subjects compared to controls. Hence, level of plasma TAC was correlated negatively with BMI and T2DM. Hence, we can say obese subjects had a state of oxidative stress which is may be due to increased production of free radicals as complication of hyperglycemia and promotion of lipid peroxidation of LDL [59]. Hence, diabetes is considered a metabolic disorder is generally accompanied by increased levels of free radicals and decreased concentration or activity of antioxidants [56]. There is considerable evidence that oxidative stress plays a key role in IR and impaired insulin secretion [60, 61]. The increase in intra and extracellular concentration of glucose results in auto-oxidation of glucose and oxidative stress [62]. Moreover, non-enzymatic glycation of proteins activate the polyol pathway [63]. In this pathway, NADPH, a cofactor of glutathione peroxidase activity, is utilized causing increased concentration of NADH, which is essential for the activation of the enzyme NADH oxidase that causes oxidative stress [64].

There is correlation between the IgG level and BMI [65]. In the present study, we found no significant differences in the levels of serum IgG between non-diabetic obese and diabetic obese subjects compared to controls.

This result was agree with Ebtesam *et al.* [66] who mentioned that metabolic disorders did not affect serum levels of the assessed immunity indices. However, Gonzalez-Quintela *et al.* [65] found that abdominal obesity and hypertriglyceridaemia were the components of metabolic syndrome associated most strongly with serum IgA. In contrary, Over weight subjects markedly had higher measures of adiposity and serum concentrations of IgG, IgA and IgE than non-obese children; the opposite was true for IgM [67].

It is known that protein glycation is involved in the long-term complications of diabetes [68,69]. Plasma proteins are the primary targets of glycation following elevated levels of glucose in diabetes [70]. The present result of serum protein analysis showed that albumin level was decreased in non-diabetic obese and diabetic obese subjects compared to control. Albumin is one of the heavily glycosylated proteins because of its abundance, comparatively longer half-life and a higher number of free lysine and arginine residues [71]. Glycation accelerates albumin degradation via increasing catabolic rate and decreasing protein half life, thus decreasing the albumin levels in diabetes [72]. Moreover, albumin competes with other proteins for glycation [73], and low albumin level was associated with increased plasma protein glycation in diabetes [74]. This study was corroborated in a recent finding where low albumin levels were associated with increased HbA1c. Also, it has been suggested that low plasma albumin predicts the level of HbA1c in T2DM [75], thus, strongly implicating albumin in regulation of plasma protein glycation and HbA1c.

The globulins are a group of proteins consisting of $\alpha 1$ ($\alpha 1$ -antitrypsin and HDL), $\alpha 2$ (mainly $\alpha 2$ -macroglobulin and haptoglobin), β ($\beta 1$ transferrin, with a contribution from LDL) and $\beta 2$ (C3 fraction of complement)] and g fractions (IgG, IgA, IgM, IgE, and IgD), which regulate the immune system function and the transport of a variety of substances [76]. Serum immunoglobulin levels are determined routinely because they provide key information on the humoral immune status. Low immunoglobulin (Ig) levels define some humoral immunodeficiencies [16]. In contrast, high immunoglobulin levels are observed in liver diseases, chronic inflammatory diseases, haematological disorders, infections and malignancies [17].

In the present study, electrophoresis pattern showed the significant decrease of $\alpha 1$ in the mean value in both of obese non-diabetic and obese diabetic subjects compared to control. This means that there is deficiency in the major components of $\alpha 1$, HDL and $\alpha 1$ -antitrypsin. However, HDL lipoprotein decreased in obese non-diabetic and obese diabetic subjects compared to control, other studies have reported that $\alpha 1$ -antitrypsin decreased in obesity and have a negative correlation with leptin and insulin [77]. On the other hand, there was significant increase in $\alpha 2$ in the mean value in obese non-diabetic and

obese diabetic subjects compared to controls. Elevated levels of α 2-macroglobulin in this respect, James *et al.* [78] reported that α 2-macroglobulin, the major component of α 2 region, are commonly seen in diabetics, especially in longstanding cases. Also Brissenden *et al.* [79] who reported that patients with α 1-antitrypsin deficiency and emphysema an increased concentration of α 2-macroglobulin is often also present. An increased synthesis of haptoglobin occurs in patients with an acute inflammatory response and in patients with increased corticosteroid and estrogen stimulation [80], and a key factor for the increased synthesis of haptoglobin and other acute-phase reactants is IL-6 [81].

According to our data β -region also represented by high percentage in obese non-diabetic and obese diabetic subjects compared to controls. This value was expected such that LDL, component of β -region, has high level in obese non-diabetic and obese diabetic subjects compared to control group. Sun *et al.* [82]; Festa *et al.*, [83]; Pickup *et al.* [84]; Sjöholm *et al.* [85] found that complement C3 and C4 were increased in patients with an acute inflammatory reaction such as obesity and diabetes. In the present study, it was observed that γ globulin levels were higher related to higher BMI and in those who had IGT or diabetes, although this relationship was largely explained by effects of covariates such as BMI. Immunoglobulin concentrations (of IgA, IgG, and IgM classes) have previously been reported to be higher in those with diabetes [86, 18]. Higher levels of gamma globulin concentration to be related to higher BMI were reported and predict DM [87]. It was known that serum globulin levels were increased with chronic inflammation, infection, autoimmune disease and liver disease. Ardawi *et al.* [86] described the abnormalities in serum immunoglobulin concentrations in patients with diabetes. It has been hypothesized that elements of the innate immune system, such as cytokines or the acute phase reactants that they may stimulate, may contribute to the development of obesity and T2 DM [88]. In addition, γ globulin levels predict T2DM [89]. An increase in serum IgA levels is a generalized phenomenon in diabetic patients [90]. Chronic inflammation is a key feature of T2DM and obesity [91, 92] with a cluster of abnormalities characterized by IR along with specific risk factors including hyperglycaemia, visceral adiposity, dyslipidaemia and hypertension [92]. Moreover, the production of pro-inflammatory cytokines is increased in patients with metabolic syndrome [93]. These cytokines include adipocytokines such as interleukin (IL)-6 [94], which work as co-factor for immunoglobulin synthesis [95] and it was a common marker of inflammation [96].

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