Journal of Pharmaceutical and Applied Chemistry
An International Journal

http://dx.doi.org/10.18576/jpac/020306

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

Nagwa M. Elsawi[1,*], *Hossam El-Din M. Omar*[2], *Ali Taha A. Hassan*[3] *and Amira M. Ahmed*[1]

[1]Department of Chemistry, Faculty of Sience, Sohage University, Sohag 82524, Egypt.
[2]Zoology Department, Faculty of Science, Assiut University, Assiut 71516, Egypt.
[3]Department of Internal Medicine, Faculty of Medicine, Sohag University, Sohag 82524, Egypt.

Received: 5 Mar. 2016, Revised: 8 Jun 2016, Accepted: 16 Jun. 2016.
Published online: 1 Sep. 2016.

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 Sohag 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

* Corresponding author E-mail: elsawinagwa@yahoo.com

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

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 subjects groups.

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].

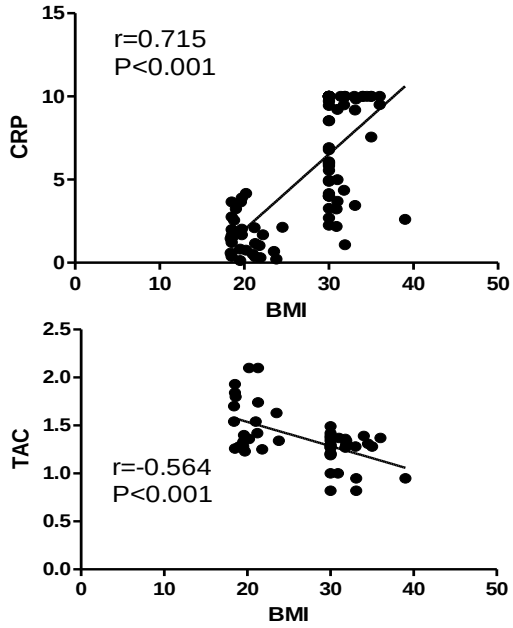

Figure (1): correlation plots between CRP, TAC and BMI.

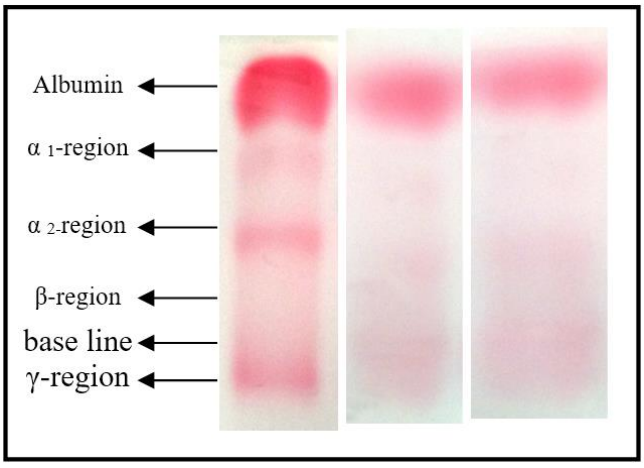

Figure (2): Serum protein fractions in sera of all subjects.

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 consistence 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 results 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 glycated 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 α1 (α 1-antitrypsin and HDL), α2 (mainly α2-macroglobulin and haptoglobin), β (β1 transferrin, with a contribution from LDL) and β2 (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 α1 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 α1, HDL and α-1anitrypsin. However, HDL lipoprotein decreased in obese non-diabetic and obese diabetic subjects compared to control, other studies have reported that α-1anitrypsin decreased in obesity and have a negatives correlation with leptin and insulin [77]. On the other hand, there was significant increase in α2 in the mean value in obese non-diabetic and

obese diabetic subjects compared to controls. Elevated levels of α2-macroglobulin in this respect, James *et al*. [78] reported that α2-macroglobulin, the major component of α2 region, are commonly seen in diabetics, especially in longstanding cases. Also Brissenden *et al*. [79] who reported that patients with α1-antitrypsin deficiency and emphysema an increased concentration of α2-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]; Sjoholm *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].

References

[1]Bray, G.A., M.B. Clearfield, D.J. Fintel, and D.S. Nelinson. 2009. Overweight and obesity: The pathogenesis of cardiometabolic risk. Clinical Cornerstone 9(4): 30–40. discussion 41–42.

[2]Garcia, C., B. Feve, P. Ferré, et al. 2010. Diabetes and inflammation: Fundamental aspects and clinical implications. Diabetes & Metabolism 36(5): 327–338.

[3]Pepys, M.B., and G.M. Hirschfield. 2003. C-reactive protein: A critical update. Journal of Clinical Investigation 111: 1805–1812.

[4]Yosef-Levi, I.M., E. Grad, and H.D. Danenberg. 2007. C-reactive protein and atherothrombosis—a prognostic factor or a risk factor? Harefuah 146(12): 970–974.

[5]Kahn, S.E., B. Zinman, S.M. Haffner, et al. 2006. Obesity is a major determinant of the association of C-reactive protein levels and the metabolic syndrome in type 2 diabetes. Diabetes 55(8): 2357–2364.

[6]Park, J.S., M.H. Cho, J.S. Nam, et al. 2010. Visceral adiposity and leptin are independently associated with C-reactive protein in Korean type 2 diabetic patients. Acta Diabetologica 47(2): 113–118.

[7]Kanai, A., T. Kawamura, T. Umemura, et al. 2008. Association between future events of brain infarction and soluble levels of intercellular adhesion molecule-1 and C-reactive protein in patients with type 2 diabetes mellitus. Diabetes Research and Clinical Practice 82(2): 157–164.

[8]Pu, L.J., L. Lu, X.W. Xu, et al. 2006. Value of serum glycated albumin and high-sensitivity C-reactive protein levels in the prediction of presence of coronary artery disease in patients with type 2 diabetes. Cardiovascular Diabetology 5: 27.

[9]Esposito, K.; Ciotola, M.; Giugliano, D. Oxidative stress in the Metabolic Syndrome. J. Endocrinol. Invest. **2006**, 29, 791–795.

[10]Pihl, E.; Zilmer, K.; Kullisaar, T.; Kairane, C.; Magi, A.; Zilmer, M. Atherogenic inflammatory and oxidative stress markers in relation to overweight values in male former athletes. Int. J. Obesity **2006**, 30, 141–146.

[11]Chrysohoou, C.; Panagiotakos, D.B.; Pitsavos, C.; Skoumas, I.; Papademetriou, L.; Economou, M.; Stefanadis, C. The implication of obesity on total antioxidant capacity apparently healthy men and women: The ATTICA study. Nutr. Metab. Cardiovasc. Dis. **2007**, 17, 590–597.

[12]Hartwich, J.; Goralska, J.; Siedlecka, D.; Gruca, A.; Trzos, M.; Dembinska-Kiec, A. Effec of supplementation with vitamin E and C on plasma hsCPR level and cobalt-albumin binding score as markers of plasma oxidative stress in obesity. *Genes Nutr.* **2007**, *2*, 151-154.

[13]Patel, C.; Ghanim, H.; Ravishankar, S.; Sia, C.L.; Viswanathan, P.; Mohantym, P.; Dandona, P. Prolonged reactive oxygen species generation and Nuclear Factor- kB activation after a high-fat, high-carbohydrate meal in the obese. J. Clin. Endocrinol. Metab. **2007**, 92, 4476–4479.

[14]Amirkhizi, F.; Siassi, F.; Minaie, S.; Djalali, M.; Rahimi, A.; Chamari, M. Is obesity associated with increased plasma lipid peroxidación and oxidative stress in women. ARYA Atheroscler. J. **2007**, 2, 189–192.

[15]Ozata, M.; Mergen, M.; Oktenli, C.; Aydin, A.; Sanisoglu,

S.Y.; Bolu, E.; Yilmaz, M.I.; Sayal, A.; Isimer, A.; Ozdemir, I.C. Increased oxidative stress and hypozincemia in male obesity. Clin. Biochem. **2002**, 35, 627–631.

[16]Buckley RH. Humoral immunodeficiency. Clin Immunol Immunopathol 1986; **40**:13–24.

[17]Dispenzieri A, Gertz MA, Therneau TM, Kyle RA. Retrospective cohort study of 148 patients with polyclonal gammopathy. Mayo Clin Proc 2001; **76**:476–87.

[18]Yamamoto K, Terada R, Okamoto R *et al.* A scoring system for primary biliary cirrhosis and its application for variant forms of autoimmune liver disease. J Gastroenterol 2003; **38**:52–9.

[19]Koracevic D, Koracevic G, Djordjevic V. Method of the measurement of antioxidant activity in human fluids. J. Clin. Pathol. 2001; 54: 35 - 361.

[20]John R. S., 1976.Methods on zone electrophoresis, low voltage electrophoresis on paper, serum protein, chapter 2.p 11-12.

[21]Farhangi MA, Keshavarz AS, Eshraghian M, Ostadrahimi A, Sabbor-Yaraghi

AA. White blood cell count in women: relation to inflammatory biomarkers, haematological profiles, visceral adiposity, and other cardiovascular risk factors. J Health Popul Nutr. 2013;31(1):58-64.

[22]Ganguli D, Das N, Saha I, Sanapala KR, Chaudhuri D, Gosh S, et al. Association between inflammatory markes and cardiovascular risk factors in women from Kolkata, W.B, India. Arq Bras Cardiol. 2011;96(1):38-46.

[23]Tatsukawa Y, Hsu WL, Yamada M, Cologne JB, Suzuki G, Yamamoto H, et al. White blood cell count, especially neutrophil count, as a predictor of hypertension in a Japanese population. Hypertens Res. 2008;31(7):1391-7.

[24]Dixon JB, O'Brien PE. Obesity and the white blood cell count: changes with sustained weight loss. Obes Surg. 2006;16(3):251-7.

[25]Madjid M, Fatemi O. Component of the complete blood count as risk predictors for coronary heart disease: in-depth review and update. Tex Heart Inst J. 2013;40(1):17-29.

[26]Tanaka, S.-i., Isoda, F., Yamakawa, T., Ishihara, M. & Sekihara, H. (1998) T lymphopenia in genetically obese rats. Clinical Immunology,86, 219±225.

[27]Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 2006;6(10):772-83.

[28]Jaso-Friedmann L, Leary JH 3rd, Praveen K, Waldron M, Hoenig M. The effects of obesity and fatty acids on the feline immune system. Vet Immunol Immunopathol 2008; 122(1-2):146-52.

[29]Petakov M, Biljanovic-Paunovic L, Jovcic G, Stojanovic N, Todorovic V, Jelkmann W: The influence of acute sterile inflammation on erythropoiesis in rats. Exp Hematol 1998, 26(3):222–227.

[30]Miyagawa S, Kobayashi M, Konishi N, Sato T, Ueda K: Insulin and insulinlike growth factor I support the proliferation of erythroid progenitorcells in bone marrow through the sharing of receptors. Br J Haematol 2000, 109(3):555–562.

[31]Wang Y-Y, Lin S-Y, Liu P-H, Cheung BMH, Lai W-A: Association betweenhematological parameters and metabolic syndrome components in a Chinese population. J Diabetes Complications 2004, 18(6):322–327.

[32]Mardi T, Toker S, Melamed S, Shirom A, Zeltser D, Shapira I, Berliner S,Rogowski O: Increased erythropoiesis and subclinical inflammation as part of the metabolic syndrome. Diabetes Res Clin Pract 2005, 69(3):249–255.

[33]Mohammed A, Tanko Y, Okasha MA, Magaji RA, Yaro AH. Effects of aqueous leaves extract of Ocimum gratissimum on blood glucose levels of streptozotocininduced diabetic Wistar rats. Afr J Biotechnol 2007;6:2087-90.

[34]Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med 2005;352: 1011–23.

[35]Oyedemi SO, Yakubu MT, Afolayan AJ. Antidiabetic activities of aqueous leaves extract of Leonotis leonurus in streptozotocin induced diabetic rats. J Med Plant Res2011;5:119-25.

[36]Arun GS, Ramesh KG. Improvement of insulin sensitivity by perindopril in spontaneously hypertensive and streptozotocindiabetic rats. Indian J Pharmacol 2002;34:156- 64.

[37]Ayman M. M., 2013.Hematological alterations in diabetic rates – role of adipocytes and effect of citruss flavanoids.EXCLI Journal.12:647-657.

[38]Ly H. Q., Kirtane A. J., Murphy S. A., et al., "Association of platelet counts on presentation of clinical outcomes in STelevation myocardial infarction," American Journal of Cardiology, vol. 98, no. 1, pp. 1–5, 2006.

[39]McCabe D. J. H., Harrison P., Mackie I. J., et al., "Increased platelet count and leucocyte-platelet complex formation in acute symptomatic compared with asymptomatic severe carotid stenosis," Journal of Neurology, Neurosurgery and Psychiatry, vol. 76, no. 9, pp. 1249–1254, 2005.

[40]Yudkin J. S., Kumari M., Humphries S. E., and Mohamed-Ali V., "Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link?" *Atherosclerosis*, vol. 148, no. 2, pp. 209–214, 2000.

[41]Michiels J. J., Berneman Z., Schroyens W., Finazzi G., Budde U., and Van Vliet H., "The paradox of platelet activation and impaired function: platelet vonWillebrand factor interactions, and the etiology of thrombotic and hemorrhagic manifestations in essential thrombocythemia and polycythemia vera," Seminars in Thrombosis and Hemostasis, vol. 32, no. 6, pp. 589–604, 2006.

[42]Collins C. E. and Rampton D. S. , "Review article: platelets in inflammatory bowel disease—pathogenetic role and therapeutic implications," Alimentary Pharmacology & Therapeutics, vol. 11, no. 2, pp. 237–247, 1997.

[43]Nakata M., Yada T., Soejima N., and Maruyama I., "Leptin promotes aggregation of human platelets via the long form

of its receptor," *Diabetes*, vol. 48, no. 2, pp. 426–429, 1999.

[44]Konstantinides S., Sch¨afer K., Koschnick S., and Loskutoff D. J., "Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity," *Journal of Clinical Investigation*, vol. 108, no. 10, pp. 1533–1540, 2001

[45]Frohlich M. , Imhof A. , Berg G. , Association between C-reactive protein and features of the metabolic syndrome: a population-based study. Diabetes Care. 2000; 23:1835-1839.

[46]Schultz D. R., Arnold P. L., Properties of four acute phase proteins: C-reactive protein, serum amyloid A protein, a1-acid glycoprotein and fibrinogen. Semin Arthritis Rheum. 1990; 20:129–47.

[47]Yeo E. S., Hwang J. Y., Park J. E., Choi Y. J., Huh K. B. , Tumor necrosis factor (TNF-alpha) and C-reactive protein (CRP) are positively associated with the risk of chronic kidney disease in patients with type 2 diabetes. Yonsei Med J. 2010 Jul; 51(4):519-25.

[48]Hansen D., Dendale P., Beelen M., Jonkers R. A., Mullens A., Corluy L. et al, Plasma adipokine and inflammatory marker concentrations are altered in obese, as opposed to non-obese, type 2 diabetes patients. Eur J Appl Physiol. 2010 Jun; 109(3):397-404.

[49]Biasucci LM, Liuzzo G, Grillo RL, Caligiuri G, Rebuzzi AG, Buffon A, Summaria F, Ginnetti F, Fadda G, Maseri A Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability. Circulation 1999; 99 :855–860.

[50]Pasceri V, Cheng JS, Willerson JT, Yeh ET, Chang J (2001) Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by antiatherosclerosis drugs. Circulation 103:2531–2534.

[51]Torzewski M, Rist C, Mortensen RF, Zwaka TP, Bienek M, Waltenberger J, Koenig W, Schmitz G, Hombach V, Torzewski J Creactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. Arterioscler Thromb Vasc Biol 2000;20 :2094–2099.

[52]Ferri C, Desideri G, Valenti M, Bellini C, Pasin M, Santucci A, DeMattia G. Early upregulation of endothelial adhesion molecules in obese hypertensive men. Hypertension 1999; 34(Pt 1): 568–573.

[53]Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997; 336: 973–979.

[54]Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. BMJ 1996; 312: 1061– 1065.

[55]Freedman DS, Joesoef MR, Barboriak JJ, Stallone DD, Byers T. Correlates of leukocyte counts in men. Ann Epidemiol 1996; 6: 74–82.

[56]Tesfamariam B: Free radicals in diabetic endothelial cell dysfunction. Free Radic Biol Med 1994, 16(3):383–391.

[57]Wild SH, Roglic G, Green A, Sicree R, King H: Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004, 27(10):2569.

[58]Betteridge DJ: What is oxidative stress? Metabolism 2000, 49(2):3–8.

[59]Maritim AC, Sanders RA, Watkins JB 3rd: Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003, 17(1):24–38.

[60]Ahmed R: The physiological and biochemical effects of diabetes on the balance between oxidative stress and antioxidant defense system. Med J Islamic World Acad Sci 2005, 15(1):31–42.

[61]Rahimi R, Nikfar S, Larijani B, Abdollahi M: A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother 2005, 59(7):365–373.

[62]Brownlee M: The pathobiology of diabetic complications. Diabetes 2005, 54(6):1615.

[63]Soliman GZA: Blood lipid peroxidation (superoxide dismutase, malondialdohyde, glutatione) level in Egyptian type 2 diabetic patients. Singapore Med J 2008, 49(2):129–136.

[64]Reaven G: Insulin resistance and its consequences: type 2 diabetes mellitus and coronary heart disease. Diabetes mellitus: a fundamental and clinical text Philadelphia. Circulation 1996, 93:1780–1783.

[65]Gonzalez-Quintela A. , Alende R., Gude F. , Campos J., Rey J. , Meijide L. M., Fernandez-Merino C. and Vidal C.. Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. Journal compilation.2007 British Society for Immunology, Clinical and Experimental Immunology, **151:** 42–50.

[66]Ebtesam, A. AL-Suhaimi, Fadwa, M. AL-Kulaifi and Adeeb Shehzad., 2013. Serum Adipokines Metabolic Function and Immunological Correlations in Treated Type 2 *Diabetes mellitus* and Obese Subjects. World Applied Sciences Journal 22 (7): 933-938

[67]Okamatsu Y, Matsuda K, Hiramoto I, Tani H, Kimura K, Yada Y, Kakuma T, Higuchi S, Kojima M, Matsuishi T: Ghrelin and leptin modulate immunity and liver function in overweight children. Pediatr Int 2009, 51:9–13.

[68]Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.

[69]Bourdon E, Loreau N, Blache D. Glucose and free radicals impair the antioxidant properties of serum albumin. FASEB J 1999;13:233-44.

[70]Austin GE, Mullins RH, Morin LG. Non-enzymic glycation of individual plasma proteins in normoglycemic and hyperglycemic patients. Clin Chem 1987;33:2220-4.

[71]Rondeau P, Bourdon E. The glycation of albumin: Structural and functional impacts. Biochimie 2011;93:645-58.

[72]Morris MA, Preddy L. Glycosylation accelerates albumin degradation in normal and diabetic dogs. Biochem Med Metab Biol 1986;35:267-70.

[73]Bhonsle HS, Singh SK, Srivastava G, Boppana R, Kulkarni MJ. Albumin competitively inhibits glycation of less abundant proteins. Protein Pept Lett 2008;15:663-7.

[74]Bhonsle HS, Korwar AM, Kote SS, Golegaonkar SB, Chougale AD, Shaik ML, et al. Low plasma albumin levels are associated with increased plasma protein glycation and HbA1c in diabetes. J Proteome Res 2012;11:1391-6.

[75]Rodriguez-Segade S, Rodríguez J, Mayan D, Camiña F. Plasma albumin concentration is a predictor of HbA1c among type 2 diabetic patients, independently of fasting plasma glucose and fructosamine. Diabetes Care 2005;28:437-9.

[76]Bishop ML, Fody EP, Schoeff L. Clinical Chemistry: Principles, Procedures, Correlations.Philadelphia, Lippincot Williams and Wilkins, 2005.

[77]Xue G. B., Zheng W.L, Wang L. H., Lu L. Y. **Alpha 1-antitrypsin.**A novel biomarker for obesity in humans. Saudi Med J 2013; Vol. 34 (1)**.**

[78]James K, Merriman J, Gray RS, Duncan LJ, Herd R. Serum alpha 2-macroglobulin levels in diabetes. J Clin Pathol 1980;**33**:163–166. 1987;**139**:4122–4128.

[79]Brissenden JE, Cox DW. alpha 2-Macroglobulin in patients with obstructive lung disease, with and without alpha 1-antitrypsin deficiency. Clin Chim Acta 1983;**128**:241–248.

[80]Baumann H, Richards C, Gauldie J. Interaction among hepatocyte-stimulating factors, interleukin 1, and glucocorticoids for regulation of acute phase plasma proteins in human hepatoma (HepG2) cells. J Immunol.,1987. 139:4122-4128.

[81]Mackiewicz A, Schooltink H, Heinrich PC, Rose- John S. Complex of soluble human IL-6- receptor/IL-6 up-regulates expression of acute-phase proteins. J Immunol 1992;**149**: 2021–2027.

[82]Sun, T., Lien, Y. Y.,and Gross, S., Clinical application of a high-resolution electrophoresis system, Ann. Clin. Lab. Sci., 8, 219, 1978.

[83]Festa, A., D'Agostino, Jr, R., Tracy, R. P. and Haffner, S.M.(2002) Elevated levels of acute phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes **51**, 1131–1137

[84]Pickup, J. C. (2004) Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care **27**, 813–823

[85]Sjoholm, A. and Nystrom, T. (2006) Inflammation and the etiology of type 2 diabetes. Diabetes Metab. Res. Rev. **22**,4–10.

[86]Ardawi M. S, Nasrat HA, Bahnassy AA. Serum immunoglobulin concentrations in diabetic patients. Diabet Med 1994; 11: 384-87.

[87]Ford ES. Body mass index, diabetes, and C-reactive protein among U.S. adults. Diabetes Care 1999; 22: 1971–1977.

[88]Pickup JC, Crook MA. Is type 2 diabetes mellitus a disease of the innate immune system? Diabetologia 1998; 41: 1241-48.

[89]Lindsay RS, Krakoff J, Hanson RL, et al. Gamma globulin levels predict type 2 diabetes in the Pima Indian Population. Diabetes 2001; 50: 1598-603.

[90]Rodriguez-Segade S, Camiña MF, Carnero A et al. High serum IgA concentrations in patients with diabetes mellitus: agewise distribution and relation to chronic complications. Clin Chem 1996; **42**:1064–7.

[91]Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006; **444**:860–7.

[92]Singh B, Mallika V, Goswami B. Metabolic syndrome: diagnosis, potential markers and management - an update. Clin Chim Acta 2007; **380**:4–12.

[93]Ronti T, Lupattelli G, Mannarino E. The endocrine function of adipose tissue: an update. Clin Endocrinol 2006; **64**:355–65.

[94]Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab 1998; **83**:847–50.

[95]Beagley KW, Eldridge JH, Lee F et al. Interleukins and IgA synthesis.Human and murine interleukin 6 induce high rate secretion in IgA-committed B cells. J Exp Med 1989; **169**:2133–48.

[96]Browning LM, Krebs JD, Jebb SA. Discrimination ratio analysis of inflammatory markers: implications for the study of inflammation in chronic disease. Metabolism 2004; **53**:899–903.