JBAAR
Journal of Basic And Applied Research
ISSN 2413-7014

Pro-inflammatory Cytokines Levels in Obese and Diabetic Egyptians & its Correlation with Cardiometabolic Syndrome.

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Received: 20-4-2017
Revised: 1-5-2017
Published: 7-5-2017

Keywords:
CRP, IL-6, TNF – α, Obesity, Diabetes Mellitus, Cardiometabolic Syndrome

Abstract: Background: To date, the mechanisms linking components underlying the cardiometabolic syndrome (also known metabolic syndrome) remain unclear and other investigators have suggested that inflammation plays a primary pathogenic role. Pro-inflammatory cytokines including IL-6 and tumor necrosis factor alpha (TNF- α) are released from macrophages within the vessel wall during an inflammatory response. These cytokines mediate distant inflammatory effects, including activation of hepatic genes encoding acute phase reactant CRP. **Purpose:** This study aimed to evaluate the levels of these pro-inflammatory markers in Egyptian obese and diabetic subjects and their correlation with metabolic syndrome. **Results:** Our results showed that there were statistically significant differences among studied groups in the inflammatory markers (hs- CRP, IL-6 & TNF- α), being higher in obese and diabetics. Significant positive correlation was seen between inflammatory markers and most of metabolic syndrome markers. **Conclusion:** Our results are confirming the role of pro-inflammatory markers in the development and pathogenesis of metabolic syndrome in Egyptians.

Cite this article as: Barakat , L.A.A., Abd elbaky , A. A., Abdel-Aziz, T. and Abd El-Samee, E. (2017) Pro-inflammatory Cytokines Levels in Obese and Diabetic Egyptians & its Correlation with Cardiometabolic Syndrome. Journal of basic and applied Research, 3(2): 77-81
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INTRODUCTION

Metabolic syndrome (MetS) has rapidly become a household name since its introduction a few years ago by the World Health Organization (WHO), and later the United States National Cholesterol Education Program (NCEP), as an operational clinical entity associated with increased cardiovascular risk. It refers to a constellation of metabolic abnormalities associated with increased risk for type 2 diabetes and cardiovascular disease (CVD) (David et al 2006).

The common characteristics of the cardiometabolic syndrome among all groups include abdominal obesity (large waist circumference), insulin resistance, dyslipidaemia and increased blood pressure (Erik and Klein, 2009).

One candidate factor of metabolic syndrome is low grade systemic inflammation, itself a known participant in the development of obesity, insulin resistance and atherosclerosis. To date, the mechanisms linking components underlying the metabolic syndrome remain unclear and other investigators have suggested that inflammation plays a primary pathogenic role (Reilly et al., 2003). Pro-inflammatory cytokines including IL-6 and tumor necrosis factor (TNF-α)are released from macrophages within the vessel wall during an inflammatory response. These cytokines mediate distant inflammatory effects, including activation of hepatic genes encoding acute phase reactant CRP (Anna et al., 2010).

This study aimed to evaluate the levels of these pro-inflammatory markers in Egyptian obese and diabetic subjects and its correlation with metabolic syndrome.

MATERIALS AND METHODS

The study was conducted on one hundred sixty five unrelated Egyptian subjects selected matching in age, divided into three groups: group1 (control group), it consists of fifty five non obese non diabetic healthy volunteers, group 2 consists of fifty five obese, non-diabetic subjects and group 3 consists of fifty five obese, diabetics .

Diabetes mellitus was diagnosed based on the WHO criteria, as reported in 2000 (WHO, 2000). All subjects were informed of the purpose of the study and their consent was obtained. Physical data for each subject, including weight, height and waist circumferences were recorded. Blood pressure was measured for all subjects. Non diabetic volunteers were judged to be in good health according to their medical history and their fasting blood glucose (<100mg/dL). Laboratory investigations including,

fasting blood glucose level by colorimetric method and fasting serum insulin level (Pal et al., 2008). Insulin resistance was measured using the homeostasis model assessment of insulin resistance (HOMA-IR), a reliable marker for insulin resistance, was calculated as fasting insulin X glucose level/22.5 (Katz et al., 2000). Hb A1c also was measured as a marker of long term control in blood glucose (Miedema, 2005).

Serum hs-CRP was measured (Ridker et al., 2002), Serum TNF-*α* and IL-6 levels were measured by enzyme-linked immunosorbent assay (ELISA) technique (enzyme-amplified sensitivity immunoassay (EASIA) kits, BioSource Europe SA). Subjects also were assayed for Lipid profile (Al-Omar et al.,2010) & (Mehrotra et al.,2009). Non HDL-C was calculated as following: Non HDL-C = total cholesterol minus HDL-C (Salim, 2011). The Port Said University Ethics Committee approved the study.

Statistical analysis: The collected data were tabulated and analysed using SPSS version 16 software. Categorical data were presented as number and percentages, Chi square test (X2) was used as a test of significance while quantitative data were expressed as mean and standard deviation. Comparison of variables among groups of the study was made by one way analysis of variance (ANOVA). Bonferroni's correction was also applied to analyses. Differences were considered statistically significant at $p<0.05$ and highly significant at $p<0.01$.

RESULTS

There were no statistically significant differences between cases and control in age or gender distribution. There were statistically significant differences among studied groups in FBG, HbA1C and HOMA-R. Post hoc &p values by (Bonferroni test) showed no significant difference in FBG, HbA1c between group I &group II (p >0.05), significant difference in HOMA-R (p <0.05) between group I & group II, group II & group III and between group I & group III (p <0.05).

There were statistically significant differences among studied groups in inflammatory markers (hs- CRP, IL-6 & TNF- α). Post hoc &p values by (Bonferroni test) applied on significant values showed no significant difference between group I &group II (p >0.05), and significant difference between group I & group III and group II & group III (p <0.05).(table1)(figure1)

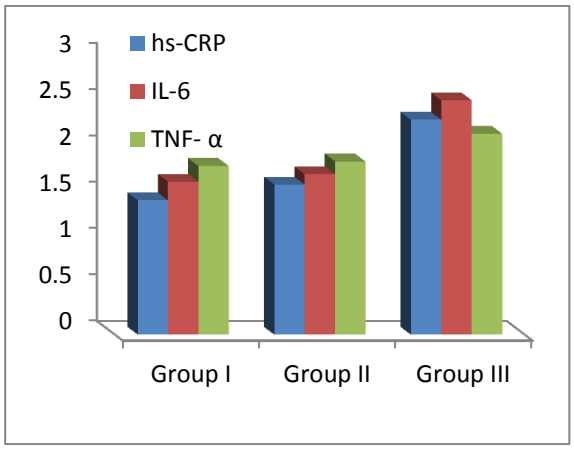
Significant positive correlation was seen between hs-CRP & BMI, WC, diastolic blood pressure, HbA1c, HOMA-IR, TG, non HDL-C , between IL-6 & BMI, WC, diastolic blood pressure, HbA1c, HOMA-IR, non HDL-C and between TNF-α & WC, diastolic blood pressure, HbA1, TG, non HDL-C.

There were non-significant positive correlation between hs-CRP & systolic blood pressure, between IL-6 & systolic blood pressure, non HDL-C and between TNF-α & systolic blood pressure, BMI, non HDL-C. There was significant negative correlation between hs-CRP and IL-6 with HDL-C while it was non-significant between TNF-α and HDL-C.(table2).

Table (1): Mean of high-sensitivity CRP,interleukin-6 & Tumour necrosis factor *α*, among the studied groups.

Variable	Group I (*n*=55)	Group II (*n*=55)	Group III (*n*=55)	F	p
hs-CRP (mg/l)	1.45 ± 0.73	1.62 ± 0.99	2.32 ± 1.11	7.23	<0.01
IL6 (pg /ml)	1.65 ± 1.01	1.73 ± 1.14	2.53 ± 1.34	4.85	<0.01
TNF- α (pg /ml)	1.82 ± 0.81	1.87 ± 1.09	2.17 ± 0.89	20.18	<0.01

P<0.05 significant and P<0.01 highly significant.NS : non-significant.



Figure(1): Mean of hs-CRP, IL-6& TNF-αamong the studied groups:

Table (2): Correlations between hs-CRP, Interleukin- 6 and Tumour Necrosis factor- α & cardio-metabolic risk factors in the studied groups.

Variable	Hs-CRP		IL6		TNF- α	
	r	P	r	P	r	P
BMI	0.25	<0.01	o.33	<0.01	0.13	>0.05
WC	0.21	<0.05	0.29	<0.01	0.17	<0.05
SBP	0.14	>0.05	0.07	>0.05	-0.02	>0.05
DBP	0.29	<0.01	0.40	<0.01	0.19	<0.05
Hb A1c	0.18	<0.05	0.41	<0.01	0.23	<0.05
HOMA-R	0.25	<0.01	0.21	<0.05	0.15	>0.05
Non HDL-C	0.4	<0.01	0.32	<0.01	0.24	<0.01
TG	0.21	<0.05	0.1	>0.05	0.03	>0.05
HDL-C	-0.3	<0.01	-0.33	<0.01	-0.13	>0.05

P<0.05 significant, P<0.01 highly significant. NS: non-significant.

DISCUSSION

Obesity is associated with an increased risk of developing insulin resistance. In obese individuals,

adipose tissue releases increased amounts of non-esterified fatty acids, glycerol, hormones, pro-inflammatory cytokines and other factors that are involved in the development of insulin resistance. When insulin resistance is accompanied by dysfunction of pancreatic islet β-cells — the cells that release insulin, failure to control blood glucose levels and T2DM results (Khan et al., 2006).

In accordance, the current study revealed significant increase of FBG, HbA1C and insulin resistance in the obese diabetic group compared to the non-diabetic groups, HOMA-IR seen significantly increased in obese group in comparison with non-obese group. This coincides with the study of Rao (2001) and the study of Azab et al (2016) who reported that T2DM is associated with insulin resistance and compensatory hyperinsulinemia (Rao, 2001 &Azab et al., 2016). Also, insulin resistance was significantly increased in the healthy obese group compared to the healthy non obese group in the study of Despres (2001) who reported insulin resistance increased in obese subjects especially those with abdominal (visceral obesity) (Despres, 2001).

An increased number of macrophages resident in human adipose tissue has been reported in obesity that may contribute to the inflammatory process by secreting pro-inflammatory cytokines such as TNF-α and IL-6. In addition to increased infiltration of macrophages in adipose tissue, obesity is associated with changes in the phenotype of macrophages from alternatively activated toward a more classical and pro-inflammatory cell as the source of pro-inflammatory mediators (Rasouli and Philip, 2008).

Interleukin-6 (IL-6) is highly expressed in adipose tissue and positively correlated with obesity in humans. Peripheral administration of IL-6 interrupts insulin signaling due to enhance expression of SOCS3 in hepatocytes suggesting that obesity-induced IL-6 expression mediates insulin resistance (Senn et al., 2003). As central administration of IL-6 enhances energy expenditure and decreases obesity, IL-6 can also influence obesity and insulin sensitivity through a central nervous system mechanism. Interleukin-6 increases inflammation directly or by stimulating hepatic C - reactive protein production (Park et al., 2004).

Tumor Necrosis Factor alpha (TNF-α) was originally identified as an endotoxin-induced serum factor that mediates tumor necrosis and cancer cachexia. TNF-α is mainly expressed in monocytes and macrophages as a 26 kDa transmembrane protein and then is converted to active trimer by TNF-α converting enzyme. TNF-α is a typical pro-inflammatory cytokine that is increased in obese humans and rodents suggesting that TNF-α contributes to insulin resistance. TNF-α stimulates the phosphorylation of insulin receptor substrate (IRS) on Ser-307 residues that suppresses insulin-induced IRS1 tyrosine phosphorylation and activation of downstream targets. TNF-α also suppresses the expression of phosphodiesterase 3B (PDE3B) and perilipin. As PDE3B reduces cAMP after insulin stimulation, and perilipin regulates the access of hormone-sensitive lipase in adipocytes, TNF-α induces lipolysis in adipocytes to release free fatty acid. Free fatty acid in turn binds to Toll-like receptor (TLR), and pro-inflammatory factors are expressed through NF-κB activation (Kwon and Pessin, 2013).

Elevated levels of C-reactive Protein (CRP) are associated with insulin resistance, BMI and hyperglycemia, and are increased with the number of the MetS components. It is more likely to be elevated in obese insulin-resistant, but, not in obese insulin-sensitive subjects. Because the MetS has been linked with a greater chance of future CVD events, CRP levels may be an important independent predictor of unfavorable outcomes in the MetS (Despre, 2006).

According to the results of the current study, there was an increase of the inflammatory markers (hs-CRP, IL-6 and TNF-α) in obese group in comparison with controls. However, this increase was non-significant. There were statistically significant increase in same markers when comparing diabetic group to non-diabetic groups, this was in agreement with previous studies (Zhao et al., 2011& Goyal et al., 2012) who stated that inflammation as measured by serum inflammatory markers have been shown to be increased in people with diabetes.

There were significant correlation between hs-CRP, IL-6 & TNF- α with measures of obesity as BMI and WC. The clear relationship that we observed between inflammatory markers concentration and BMI states the role of adipose tissue in initiating and sustaining sub minimal inflammation. This agrees with results of other studies as the study of Bastard et al (2000) in which 14 obese women were studied again after 3 weeks of very low caloric diet. The diet resulted in a mean reduction of 2.1 kg/m2 in BMI and a mean reduction of 3 kg in adipose tissue mass and was associated with significant decrease in IL-6 & TNF- α (Bastard et al., 2000). However, the clear demarcation between these markers levels in obese diabetics in comparison to obese non diabetics provides evidence for a positive association between hs-CRP, IL-6 & TNF- α levels and type 2 diabetes mellitus among our studied population and also that this association was independent of BMI

and WC suggesting that this elevation might not be limited to obesity alone in this population. Similar observations have been made in previous studies (Barzilay et al., 2001 & Pradhan et al., 2001). However, in contrast, other studies have demonstrated that controlling for BMI and/or WC completely abolished association between hs-CRP and T2D (Pankow et al., 2003 &Festa et al., 2002).
Our study showed significant correlation between hs-CRP and most of the metabolic syndrome components as BMI, WC, DBP, HbA1C, HOMA-IR, non HDL-C, triglycerides and HDL-C. IL-6 was significantly correlated with BMI, WC, DBP, HbA1C, HOMA-IR, non HDL-C and HDL-C. TNF- α significantly correlated with WC, DBP, HbA1C and non HDL-C. These results were in concordance with many previous studies (Garg et al., 2012, Agarwal et al., 2011 &Dandona et al., 2005).

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