Relationship between serum YKL-40 and BMI

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Abstract: Objective: Obesity is a chronic condition recognized as a low-grade inflammatory process. YKL-40 is a protein secreted by activated macrophages, was found to be elevated in conditions that are characterized by inflammation like obesity and its complications. The circulating level of YKL-40 in obesity has not been adequately studied. Therefore, this study was designed to investigate the relationship between the level of YKL-40 and different levels of increased body mass index (BMI) in Egyptian subjects. **Research Design and Methods:** Serum levels of YKL-40, C-reactive protein (CRP), insulin and other parameters were assessed in 60 subjects of different levels of increased body mass index(>25 kg/m^2) compared to 20 subjects of normal body mass index (>18.5 up to 25 kg/m^2) with matched age and sex. Serum YKL-40 and insulin levels were measured by enzyme-linked immunosorbent assay (ELISA) whereas CRP levels were measured by turbidimetric immunoassay. **Results:** Serum YKL-40 levels were found to be significantly elevated in overweight, obese and morbid obese subjects when compared with normal control subjects. Its levels were 121.7, 121.3 and 131.6 ng/mL among overweight, obese and morbid obese respectively and 88.2 ng/mL for normal BMI. This level of YKL-40 is significantly higher compared to normal subjects at $P< 0.05$. YKL-40 level was found to be also significantly positively correlated with BMI, body fat percentage (BF %), index of central obesity (ICO) and CRP. CRP level was found to increase significantly with the rise of BMI. The level was 4.09 mg/L among overweight rises to 4.85 mg/L among obese and shoots up to 10.86 mg/L among morbid obese while normal control subjects CRP was 2.40 mg/L. **Conclusion:** The study suggests that YKL-40 and CRP levels are elevated in overweight, obese and morbid obese subjects in relation to BMI.
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Key words: BMI= body mass index and CRP= C reactive protein

1. Introduction

Obesity can be described as the "New World Syndrome". Its prevalence is on continuous increase in all ages of many developed countries [(1)]. The problem of obesity has been nearing an epidemic level in Egypt nearly about 70% of adult women and 48% of men in Egypt are overweight or obese [(2)].

Obesity is a chronic disease characterized by a condition of low-grade inflammation. It is defined by an excess of adipose tissue, is often associated with development of various metabolic disorders such as insulin resistance, hypertension, cardiovascular disease in addition to chronic disease such as osteoarthritis, stroke, sleep apnea, some cancers, and inflammation-bases pathologies [(3, 4)].

Adipose tissue (AT) is formed of mature adipocytes and stromal vascular fraction (SVF) cells which include pre-adipocytes, fibroblasts, endothelial cells, infiltrating macrophage. SVF cells including pre adipocytes and macrophages are considered a source of inflammation related molecules, both pro-inflammatory cytokines and acute phase proteins have been found elevated in the obese [(4, 5)].

YKL-40, was known as an inflammatory mediator, could possibly play a role in obesity related inflammation[(5)].YKL-40 is a 40 KDa heparin and chitin-binding glycoprotein also known as chitinase-3-like protein 1(CHI3L1). The abbreviation YKL-40 is based on the one letter code for the first three N-terminal amino acids, tyrosine (Y), lysine (K) and leucine (L) and the molecular mass of 40 KDa. Its gene is localized in a highly conservative area on chromosome 1q31- q32[(6)]. It belongs to the family 18 of glycosyl hydrolases comprising chitinases from various species but without any enzymatic properties and it is secreted by activated macrophages and neutrophiles in different tissues with inflammation, vascular smooth muscle cells, cancer cells and arthritic chondrocytes[(7)]. It a probably has a role in cell proliferation and differentiation, inflammation, and protection against apoptosis. It may have a role in angiogensis by stimulating the migration and reorganization of vascular endothelial cells and it also control mitogensis by initiating mitogen-activated protein kinase (MAPK) and phosphoinoside-3 kinase (PI3K) signaling cascades in fibroblasts. It has been

regarded as an autoantigen capable of inducing T-cell responses [5, 6, 8].

Therefore, the aim of this study was to assess the serum level of YKL-40 in different levels of obesity.

2. Research Design and methods

Cohort study

Using a cross-sectional, case-control design, the participants in this study were divided into four distinct groups according to BMI (>18.5 kg/m^2). Participants were carefully screened, and exclusion criteria were diabetes, renal or hepatic disease, acute or chronic apparent inflammatory disease, thyroid dysfunction, cardiovascular disease, endocrine disorders, alcohol or drug abuse, recent or ongoing infection, history of malignant disease, smoking and treatment with anti-inflammatory drugs. Participants (n = 80) were given both oral and written information about the experimental procedures before giving their written informed consent.

Subjects

Participants reported to the laboratory between 8 and 10 A.M. after an overnight fast. A general health examination was performed; blood samples were drawn from an antecubital vein.

Sixty subjects classified equally to 3 groups each of 20 according to their body mass index (BMI) as overweight having BMI (25-29.9 kg/m^2), obese having BMI (30-34.9 kg/m^2), morbid obese having BMI $\geq$ 35 kg/m^2 were enrolled in the study to be compared with 20 age and sex matched normal subjects having BMI < 25 kg/m^2.

Standing height and body weight were measured in normal clothing without shoes. BMI was calculated as weight divided by squared height (in kilograms per square meter), also index of central obesity (ICO) and waist-to-hip ratio (WHR) [9].

Blood sampling

After overnight fasting, venous blood samples were collected for the assay of fasting plasma glucose, $HbA_{1C,}$ lipid profile, routine liver and renal function tests (ALT, AST, urea and creatinine), C-reactive protein (CRP), insulin and YKL-40. Samples were divided into aliquots for serum preparation and then stored at -20 ^{0}c until time of analysis for determination of insulin and YKL-40.

Laboratory analyses

Fasting plasma glucose, lipid profile, liver and renal function tests were measured using Mindray-BS380 auto-analyzer (*BIOLABO*, Maizy, France) while low density lipoprotein (LDL-C) was calculated by the formula of Friedwald [10].The HbA_{1C} was measured in whole blood colormetrically using Glycohaemoglobin kit (*Stanbio*, USA). CRP was measured by Turbidimetric Immunoassay in serum (*Spinreact*, Spain). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to Matthews's equation [11].

Serum YKL-40 and fasting insulin levels were determined by ELISA technique using commercially available kits: Quantikine Human CHI3L1 Immunoassay kit (*R&D systems*, USA) and DRG Insulin enzyme Immunoassay kit (*DRG*, GmbH, Germany) respectively using Automated ELISA system.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). All statistical analysis and correlations were performed using Graph pad prism (windows version 5; Graph pad software 2007). Difference between means was assessed by unpaired student t test. Differences were considered statistically significant at $P < 0.05$.

3. Results

Anthropometric characteristics of all studied groups are shown in table (1). All studied group did not differ with regard to age and sex.

The clinical characteristics as well as the levels of fasting insulin, HOMA-IR, CRP, YKL-40 of all studied groups are shown in table (2).

Concerning serum YKL-40 levels, they were found to be significantly elevated at p < .05 in overweight (121.7 ± 10.2), obese (121.3 ± 9.06) and morbid obese (131.6 ± 7.95) subjects when compared with normal (88.2 ± 8.0) subjects. While insulin levels were found to be significantly elevated in obese (11.8 ± 0.78) and morbid obese (14.8 ± 1.44) subjects when compared with normal (9.22 ± 0.36) subjects also they were found to be significantly elevated in morbid obese (14.8 ± 1.44) subjects when compared with overweight (10.6 ± 0.68) subjects.

$HOMA_{IR}$ indices were found to be significantly elevated in obese (2.75± 0.19), morbid obese (3.69 ± 0.34) subjects when compared with normal (2.05 ± 0.09) subjects also they were found to be significantly elevated in morbid obese (3.69 ± 0.34) subjects when compared with overweight (2.36± 0.18) and obese (2.75± 0.19) subjects.

Concerning CRP levels, they were found to be significantly elevated in overweight (4.09 ± 0.64 mg/L), obese (4.85 ± 0.55 mg/L) and morbid obese (10.86 ± 2.28 mg/L) when compared with normal control subjects (2.40 ± 0.3 mg/L) also CRP levels were found to be significantly elevated in morbid obese (10.86 ± 2.28 mg/L) when compared with overweight (4.09 ± 0.64 mg/L) and obese (4.85 ± 0.55 mg/L) subjects.

YKL-40 levels were found to be significantly positively correlated with BMI (r = 0.302), BF% (r = 0.266), ICO (r = 0.246), and CRP (r = 0.236) (Figure 1).

Table (1): Anthropometric parameters in all studied groups ($\bar{\chi}$±SEM)

Factor	Normal	Overweight	Obese	Morbid obese
Number	20	20	20	20
Age	26.05 ± 1.09	26.95 ± 1.17	28.60 ± 1.27	27.50 ± 1.35
Weight (kg)	66.65 ± 1.48	88.20 ± 2.09	97 ± 1.67	120.2 ± 3.50
Height (cm)	174.2 ± 1.19	177.4 ± 1.62	174.9 ± 1.23	174.8 ± 1.28
BMI	21.94 ± 0.37	27.95 ± 0.26	31.67 ± 0.21	39.32 ± 1.06
Waist (cm)	80.15 ± 1.02	96.60 ± 1.44	103.7 ± 1.46	119.4 ± 2.44
Hip (cm)	96.30 ± 0.88	106.8 ± 1.28	112.1 ± 0.92	124.6 ± 2.06
ICO	0.46 ± 0.006	0.54 ± 0.007	0.59 ± 0.007	0.68 ± 0.012
WHR	0.83 ± 0.008	0.90 ± 0.010	0.93 ± 0.014	0.96 ± 0.012

Table (2): Clinical and laboratory characteristics in all studied groups

Factor	Normal	Overweight	Obese	Morbid Obese
Creatinine (mg/dl)	0.92± 0.03	0.88 ± 0.02	0.98 ± 0.03[b]	0.92 ± 0.02
Urea (mg/dl)	28.1 ± 2.10	26.1± 1.34	28.0 ± 2.13	26.4± 1.16
ALT (U/L)	15.9 ± 1.33	23.5 ± 2.53[a]	28.1 ± 3.47[a]	38.5 ± 3.87[a,b]
AST (U/L)	26.2 ± 1.49	28.9 ± 1.68	30.5 ± 2.14	36.6 ± 2.68[a,b]
FBG (mg/dl)	89.8 ± 2.05	89.5 ± 2.99	93.7 ± 2.68	102 ± 2.85[a,b,c]
HbA_{1c}%	4.89 ± 0.10	5.07 ± 0.11	5.12± 0.11	5.63± 0.14[a,b,c]
Insulin (µU/ml)	9.22 ± 0.36	10.6 ± 0.68	11.8 ± 0.78[a]	14.8 ± 1.44[a,b]
$HOMA_{IR}$	2.05 ± 0.09	2.36± 0.18	2.75± 0.19[a]	3.69 ± 0.34[a,b,c]
BF%	16.1 ± 0.54	23.5± 0.39[a]	28.4± 0.39[a,b]	37.3± 1.26[a,b,c]
TAG (mg/dl)	64.6 ± 6.32	107.4 ± 8.84[a]	135.9 ± 13.9[a]	141 ± 13.9[a,b]
TC (mg/dl)	166.9± 3.99	191.4 ± 9.37[a]	193.1 ± 10.6[a]	225.2 ± 11.7[a,b,c]
HDL-C (mg/dl)	51.2 ± 2.09	37.9 ± 1.42[a]	36.8 ± 1.54[a]	36.8± 0.86[a]
LDL-C (mg/dl)	102.8 ± 4.04	132 ± 9.37[a]	129.1 ± 10.1[a]	160.3 ± 10.7[a,c]
Risk ratio 1	3.33± 0.11	5.12 ± 0.25[a]	5.25 ± 0.19[a]	6.20± 0.36[a,b,c]
CRP (mg/L)	2.40 ± 0.3	4.09 ± 0.64[a]	4.85± 0.55[a]	10.86 ± 2.28[a,b,c]
YKL-40 (ng/ml)	88.2 ± 8.0	121.7 ± 10.2[a]	121.3 ± 9.06[a]	131.6 ± 7.95[a]

Results were expressed as mean ± SEM, **a**: significantly different from normal group; **b**: significantly different from overweight group; **c**: significantly different from obese group.

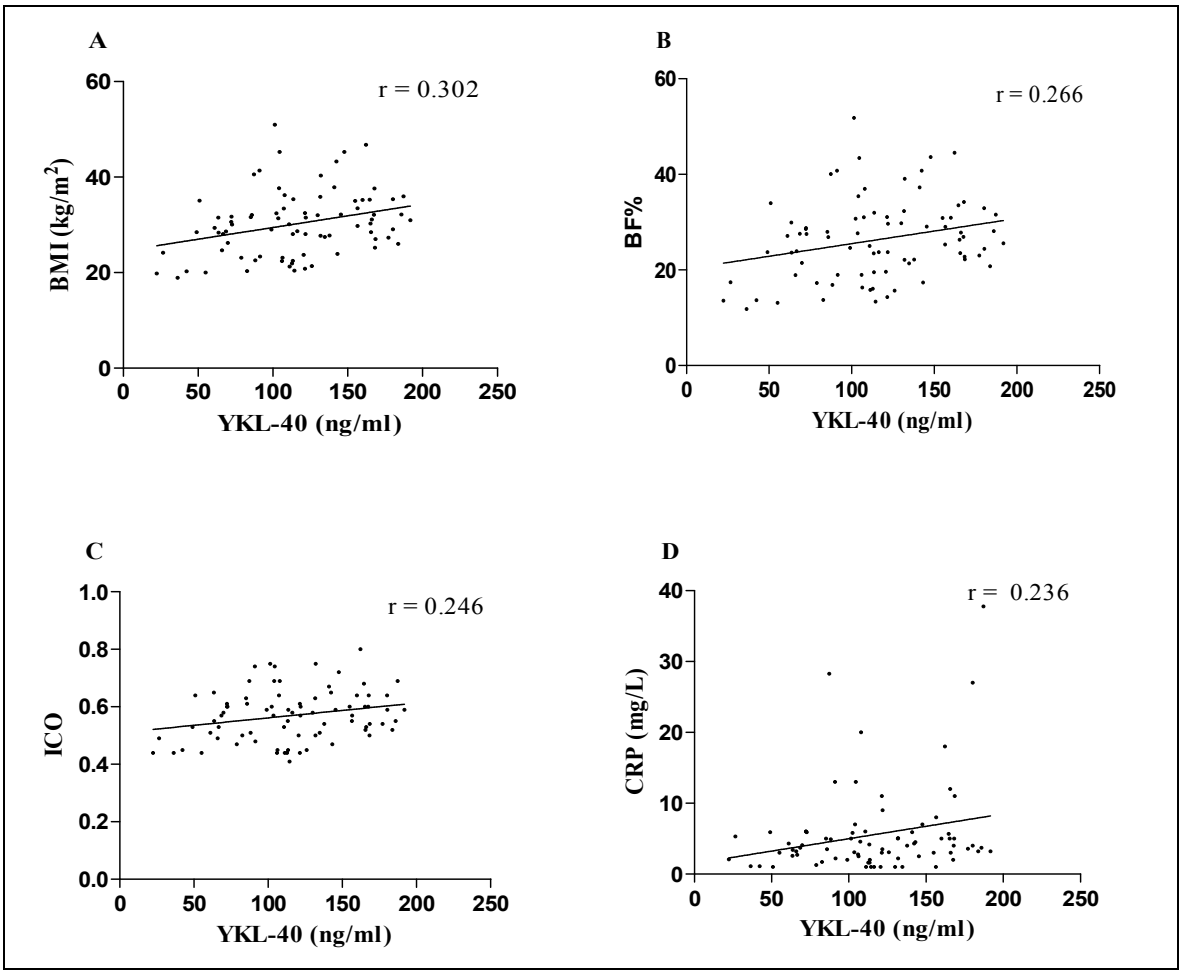

Figure (1) A: correlation between serum YKL-40 and BMI; **B**: correlation between serum ykl-40 and BF%; **C**: correlation between serum Ykl-40 and ICO; **D**: correlation between serum YKL-40 and serum CRP.

4. Discussion

In the present study**,** serum YKL-40 was measured as an inflammatory marker in normal, overweight, obese and morbid obese subjects. This study demonstrated that overweight, obese and morbid obese subjects have elevated serum YKL-40 in comparison with normal control subjects. In addition it revealed a significant positive association of YKL-40 with BMI, ICO and BF%.

These results may be due to the fact that obesity is accompanied by a low grade inflammatory condition through an increase of macrophages infiltration in adipose tissues which represent the source of many of the circulating inflammatory molecules [(12)] and suggest that YKL-40 play a role in obesity related inflammation.

In this study, higher YKL-40 levels were associated with greater BF% and adiposity, in agreement with **Kyrgios *et al.*** [(5)] and **Schaller *et al.*** [(13)] but in contrast to **Rathcke *et al.*** [(14)], **Nielsen *et al.*** [(15)] and **Hempen *et al.*** [(16)]. These discrepancies may be due to variations in subject characteristics, sample size, age matching and analytical procedures. Moreover, higher YKL-40 levels were associated with higher ICO and this association may be due to the fact that an excess visceral adiposity is related to a more pro-inflammatory state due to the greater infiltration of macrophages taking place in the abdominal fat [(17)].

CRP was elevated in overweight, obese and morbid obese subjects. These findings may be due to that CRP is an acute phase reactive protein produced by the liver under transcriptional control by interleukin-6 (IL-6) which especially elevated in obesity and CRP is also produced by adipose tissue [(18)]. In addition YKL-40 levels were significantly in a positive correlation with CRP levels and this correlation may be due to a sub-inflammatory state in obesity.

5. Conclusions

YKL-40 and CRP are significantly elevated in overweight, obese and morbid obese subjects in relation to BMI compared with normal control subjects. YKL-40 was found to be significantly in a positive correlation with CRP. These results indicate that low grade inflammation in obesity may contribute to this relation.

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