Research Article

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Serum chymase levels in obese individuals: the relationship with inflammation and hypertension

Obez bireylerde serum kimaz düzeyleri: enflamasyon ve hipertansiyonla ilişkisi

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Abstract

Background: Inflammation related hypertension is reported in obesity due to synthesis of angiotensin-II (Ang-II) and proinflammatory compounds in obese adipose tissue. Mast cell chymase (MC) also stimulate Ang-II synthesis, and activate transforming growth factor beta-1 (TGF- β 1) and matrix metalloproteinase-9 (MMP-9). The aim of our study is to evaluate the relation of serum chymase levels, a serine protease enzyme secreted from mast cells, in obese patients with hypertension and cytokines that lead to cell damage.

Materials and methods: Three study groups are composed of individuals aged between 19 and 63 with following characteristics; (1) control (n=30): healthy subjects with body mass index (BMI) <25; (2) obese (n=30): patients with BMI >30; (3) obese + HT (n=20): patients BMI >30 and hypertension. Serum Ang-II, MC, TGF- β 1 and MMP-9 are determined by commercial ELISA. Angiotensin converting enzyme (ACE) activity is determined with enzymatic colorimetric assay.

Results: Serum chymase levels did not vary between groups. Chymase levels showed significant negative

correlation with ACE activity ($r = -0.278$, $p = 0.013$) and positive correlation with Ang-II levels ($r = 0.251$, $p = 0.024$). No correlation was evident between chymase levels and hsCRP, TGF- β 1 and MMP-9.

Conclusion: Serum chymase, Ang-II, TGF- β 1 and MMP-9 levels did not change in obese and hypertensive-obese patients despite evident hyperinsulinemia, increased insulin resistance and elevated hsCRP levels.

Keywords: Chymase; Obesity; ACE; TGF- β 1; MMP-9.

Öz

Amaç: Anjiotensin-II (Ang-II) ve pro-inflamatuvar bileşenlerin obez adipoz dokuda sentezlenmesine bağlı olarak obezitede inflamasyonla ilişkili hipertansiyon bildirilmiştir. Mast hücre kimazları da (MC) Ang-II sentezini uyarır, ve transforming growth faktör beta-1 (TGF- β 1) ve matriks metalloproteinaz-9'u (MMP-9) aktive eder. Çalışmamızın amacı, mast hücrelerinden salınan bir serin proteaz enzimi olan kimazın, obez hastalardaki serum düzeylerinin hipertansiyon ve hücre hasarına neden olan sitokinlerin düzeyleriyle ilişkisini değerlendirmektir.

Gereç ve Yöntem: Aşağıdaki özelliklere sahip 19 ila 63 yaş arasındaki bireylerden oluşan 3 çalışma grubu kurulmuştur; (1) kontrol (n=30): vücut kitle indeksi (VKİ) <25 olan sağlıklı bireyler; (2) obez (n=30): VKİ >30 olan hastalar; (3) obez + HT (n=20): VKİ >30 ve hipertansiyonlu hastalar. Serum Ang-II, MC, TGF- β 1 ve MMP-9 düzeyleri ticari ELISA ile tayin edilmiştir. Anjiotensin Dönüştürücü Enzim (ACE) aktivitesi enzimatik kolorimetrik testle tayin edilmiştir.

Sonuçlar: Serum kimaz düzeyleri gruplar arasında değişiklik göstermemiştir. Kimaz düzeyleri ACE aktivitesiyle anlamlı negatif korelasyon ($r = -0.278$, $p = 0.013$) ve Ang-II

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düzeyleriyle pozitif korelasyon ($r=0.251$, $p=0.024$) göstermiştir. Kimaz seviyeleri ve hsCRP, TGF- β 1 ve MMP-9 arasında korelasyon gözlenmemiştir.

Sonuç: Obez ve hipertansif-obez hastalarda aşikar hipersülinemi, artmış insülin direnci ve hsCRP düzeylerine rağmen serum kimaz, Ang-II, TGF- β 1 ve MMP-9 seviyeleri farklılık göstermemiştir.

Anahtar Kelimeler: Kimaz; obezite; ACE; TGF- β 1; MMP-9.

Introduction

Obesity has turned into an epidemic, especially in developed countries, mainly due to changes in nutrition habits, decreased physical activity and increased pace of life in modern world, and became a public health concern. Increased adipose tissue mass is an important source of inflammation in obese individuals, causing obesity-related insulin resistance and hypertension by increasing oxidative stress [1]. Hypertension (HT), one of the most common chronic diseases in the whole world, is 90% essential hypertension, and can be defined as elevated blood pressure proceeding with increased stroke volume and/or peripheral resistance [2]. Blood pressure is regulated by a hormonal system, namely the renin-angiotensin system (RAS), which has a key role in acute and chronic regulation of blood pressure along with water and mineral balance. Renin is secreted primarily by the kidneys, and several other tissues such as liver and adipose tissue, catalyzing the synthesis of Angiotensin-I (Ang-I) from Angiotensinogen. Ang-I is processed mainly by angiotensin converting enzyme (ACE) to synthesize Angiotensin II (Ang-II). Increased Ang-II synthesis causes elevated blood pressure [3, 4]. Studies on obese hypertensive patients reported increased regional Ang-II synthesis in adipose tissue [5, 6]. Lakoski et al. reported in 2011 that elevated C-reactive protein (CRP) levels increases risk of hypertension in obese subjects. Interactions between obesity, hypertension and inflammation is extremely complex [1]. It is not clear which one of these factors stimulate the other.

Several studies reported non-ACE pathways for Ang-II synthesis in various physiological and pathophysiological conditions [7, 8]. Tonin, cathepsin-G, trypsin, kallikrein and chymase enzymes are responsible for Ang-II production [8]. Chymase enzyme (EC 3.4.21.29) is a chymotrypsin serine protease showing increased synthesis and secretion in obesity, hypertension and inflammation. Chymase is stored in the form of inactive heparin-proteoglycan complexes in secretory granules of mast cells and

is secreted by their stimulation due to inflammation [9, 10]. It is synthesized as inactive pro-chymase and has no enzymatic activity in pH 5.5 of secretory granules. Optimal pH is reported as 7–9 [11]. Pathologic metabolic processes and stimulants activate chymase. Importance of chymase catalyzed Ang-II synthesis in cardiovascular pathologies such as vascular hyperplasia, aortic aneurysm, angiogenesis, myocardial infarction and cardiac fibrosis is shown with animal studies [12].

Role of chymase in obesity pathogenesis is not clear. Mast cell synthesis also increase in adipose tissue in physiological and pathological conditions [13]. In this respect, increase in tissue chymase levels can be expected in obesity. Increased chymase enzyme activity/expression in hepatic and adipose tissues is reported [14, 15]. However, there are no studies investigating serum chymase activity/amount in obesity. Our primary aim is determination of chymase levels in obese and hypertensive patients.

On the other hand, in addition to converting Ang I to Ang-II, like ACE, chymase enzyme also catalysis conversion of “pro-transforming growth factor β 1” (pro-TGF- β 1) to active TGF- β 1 and “pro-matrix metalloproteinase-9” to active matrix metalloproteinase (MMP-9). These substances are known to have a role in tissue damage [16, 17]; however, knowledge about their levels in obesity is limited. ACE and chymase cause similar effects and synthesize Ang II. Even if ACE-inhibitors and angiotensin receptor blockers suppress Ang-II effects, Ang-II synthesis may continue via chymase pathway; tissue damage can be sustained in hypertensive patients due to cytokines and Ang-II produced by this pathway. Under the light of these findings, we aimed to determine ACE and Ang-II levels in obese and hypertensive obese patients and their relation with serum chymase levels. We also determined TGF- β 1 and MMP-9 levels in these patients in order to understand the changes in these tissue damaging cytokines.

Materials and methods

Study protocol

This study is conducted on 80 phenomena aged 19–63 (mean age: 33 ± 10 years), who presented to Istanbul Bilim University Avrupa Hospital Diet & Nutrition Out-patient Clinic between 01.03.2012 and 01.06.2013; 61 (76%) were women and 19 (24%) were men.

1. **Control group (n=30):** This group included 30 healthy individuals with body mass index (BMI) between 18

and 25 and normal biochemistry test results, who do not use any medications, alcohol or tobacco and do not have any chronic disease. Patients with fasting insulin levels ≥ 10 or HOMA-IR > 2.5 were accepted to be insulin resistant and not included in control group.

2. **Obese group (n=30):** Obese individuals with BMI > 30 and no other chronic health problem, who do not use any medications.
3. **Obese + HT group (n=20):** Obese and hypertensive patients with BMI > 30 and no other chronic disease, receiving only anti-hypertensive treatment. Anti-hypertensive medications of the patients enrolled in obese + HT group were recorded.

Age, gender, weight, height, waist and hip circumference, fat ratio, lean body mass and blood pressure were measured, BMI was calculated. Anthropometric, body composition and biochemical measurements were performed following a 12-h fasting period. HOMA-IR was calculated according to following equation [18].

$$\text{HOMA-IR} = \frac{\text{Glucose (mg/dL)} \times \text{Insulin (mU/L)}}{405} \text{ (insulin resistance).}$$

None of patients were smokers. Subjects with chronic or acute inflammatory diseases, coronary artery disease, cancer, pregnancy, addiction of alcohol and drug were excluded. Subjects who had any hormonal or chronic metabolic diseases such as diabetes mellitus were also excluded. All participants were given information about the study and signed informed consent form was obtained. The study was reviewed and approved by the Human Ethics and Research Committee of Istanbul University (25.01.2012/02).

Total of 10 mL whole blood was drawn to two tubes (two regular) of 5 mL. First tube was received by Avrupa Florence Nightingale Hospital Biochemistry Laboratory for routine biochemistry analyses including fasting blood glucose, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), cholesterol, triglycerides, HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), VLDL-cholesterol (VLDL-C), insulin, hs-CRP and protein. Remaining tubes were centrifuged for 10 min at $600 \times g$ to separate serum samples. Serum samples were transferred to Eppendorf Tubes and were stored at -80°C for experimental analyses. Serum Ang-II, mast cell chymase, TGF- $\beta 1$ and MMP-9 are determined by commercial ELISA immunoassay kits, and ACE activity is determined with colorimetric kit. All experimental analyses were completed in Medical Biochemistry Department, Istanbul Medical Faculty.

Methods

Determination and performance characteristics of serum Ang-II levels

Test are run with serum samples by quantitative sandwich enzyme immunoassay method according to procedures of commercial ELISA kit manufacturer and results are expressed in pg/mL (ASSAYPRO – AssayMax Human Angiotensin-II Elisa Kit/Catalog No: EA3501-1, Lot No: 081151210; MO, USA). Sensitivity: Minimum measurable serum concentration is ~ 0.06 pg/mL; intra-assay CV: 4.9% and inter-assay CV: 7%.

Determination and performance characteristics of serum TGF- $\beta 1$ levels

Test are run with serum samples by sandwich immunoassay method according to procedures of commercial ELISA kit manufacturer and results are expressed in pg/mL (INVITROGEN – Multispecies TGF- $\beta 1$ ELISA Kit/Catalog No: # KAC1688; CA, USA). Sensitivity: minimum measurable serum concentration is < 15.6 ng/mL; intra-assay CV: 5.8%; inter-assay CV: 7.3%; reference range for healthy individuals (serum): 19–71 ng/mL.

Determination and performance characteristics of serum MMP-9 levels

Test are run with serum samples by sandwich immunoassay method according to procedures of commercial ELISA kit manufacturer and results are expressed in ng/mL (eBioscience Human MMP-9 Platinum ELISA/BMS2016/2 and BMS2016/2TEN; Vienna, Austria). Sensitivity: Minimum measurable serum concentration is 0.05 ng/mL; intra-assay CV: 7.3%; inter-assay CV: 10.2%; reference range for healthy individuals (serum): 2.0–139.4 ng/mL.

Determination and performance characteristics of serum ACE activity

Serum ACE activity is determined by kinetic method at 340 nm. ACE in patient's serum samples and the calibrator catalyses the hydrolysis of FAPPG (N-[3-(2-furyl)acryloyl]-L-phenylalanine-glycylglycine) in the reagent, forming furyl-acryloyl-phenylalanine (FAP). Colorimetric kinetic method lasts for 5 min at 340 nm; decrease in absorbance is directly proportional to serum ACE activity. Change in

absorbance during 5 min period in serum (Δ_{serum}) and in standard (Δ_{standart}) were calculated. The following formula was used:

“ACE concentration = $(\Delta_{\text{serum}})/(\Delta_{\text{standart}}) \times 57$ ” (Standard concentration of ACE calibrator is 57 U/L). Sensitivity: minimum measurable concentration is 2.6 U/L; intra-assay sensitivity: 1.1%; inter-assay sensitivity: 2%; reference range for healthy individuals (serum): 20–95 U/L (Angiotensin Converting Enzyme Quantitative Kinetic Colorimetric Assay Kit, Milano-Italy).

Determination and performance characteristics of serum mast cell chymase levels

Test are run with serum samples by sandwich immunoassay method according to procedures of commercial ELISA kit manufacturer and results are expressed in ng/mL (BMASAY BIOMEDICAL; Beijing, China/Human Chymase 1, Mast Cell (CMA1) ELISA Kit–Catalog No: 20538 – 96T. Sensitivity: minimum measurable concentration <0.06 mg/mL; specificity: the Elisa kit shows no cross reactivity with any of the cytokines. Reference range for healthy individuals: 0.312–20 ng/mL).

Statistics

Statistical analyses were evaluated by using Statistical Package for the Social Sciences (SPSS, version 15.0, SPSS Inc, Chicago, IL, USA) program. Compatibility of variables for normal distribution were investigated by analytic methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Definitive analyses were completed by using mean and standard deviation values for variables with normal distribution. The significance between groups for parameters with normal distribution was compared by using one-way ANOVA test. Homogeneity of variances was evaluated with Levene test. In cases of significant difference between groups, two-way post-hoc comparisons were performed with Tukey test. In case of significant difference between groups two-way comparisons were completed by using Whitney-U test and evaluated by using Bonferroni correction. All data except anthropometric and blood pressure values were calculated by using non-parametric Kruskal-Wallis test. Total type-1 error level was 5% for statistical significance. Correlation coefficients and statistical significance were calculated with Pearson test for variables having normal distribution and Spearman test having non-normal distribution.

Results

Four of 61 women in study groups were post-menopausal. Two of these women were in obese group and the other two were in obese + HT group.

Individuals with any condition other than obesity and hypertension were excluded. Blood pressure of patients in obese group were within normal limits and those with uncontrolled hypertension were not included in obese + HT group.

All 20 patients included in obese + HT group were under anti-hypertensive treatment and distribution of anti-hypertensive classes were as follows; ACE-inhibitors: 4 (20%), angiotensin receptor blocker (ARB): 8 (40%), β -blocker: 11 (20%), calcium channel blocker (CCB): 4 (20%), diuretics: 4 (20%).

Eleven patients were receiving monotherapy (55%), while seven patients were under double (35%) and 1 (5%) was under triple anti-hypertensive regimen. One (5%) patient's regimen included four classes of anti-hypertensive medications.

Anthropometric measurements, bioelectrical impedance analysis and blood pressure values

BMI values and bio-impedance analysis results are given in Table 1.

BMI, waist-hip circumference and fat ratio, fat mass and lean mass were significantly higher in obese and obese + HT groups ($p < 0.001$) in comparison with control group.

Systolic blood pressure values were increased in both patient groups with respect to control group. This increase was highly significant in obese group ($p = 0.007$); however, since significance limit in two-way group comparisons was accepted as $p = 0.017$ according to Bonferroni correction, the increase in obese + HT group did not reach statistically significant level ($p = 0.018$).

There was no significant difference in height and diastolic blood pressure values among three groups. There was no difference in anthropometric and blood pressure values between obese and obese + HT groups.

Biochemical markers

The increase in serum glucose, insulin, HOMA-IR, TG, VLDL, hsCRP and GGT levels in obese group with respect to control group were highly significant, while the

Table 1: Distribution of anthropometric measurements, bio-impedance analysis and blood pressure values among control, obese and obese + HT groups (mean $\pm$ SD).

	Control (n = 30)	Obese (n = 30)	Obese + HT (n = 20)
Height (cm)	167.30 $\pm$ 7.71	162.9 $\pm$ 9.28	167.6 $\pm$ 9.29
Weight (kg)	58.03 $\pm$ 8.31	87.16 $\pm$ 10.65 ^a	88.2 $\pm$ 8.51 ^a
BMI (kg/m ²)	20.42 $\pm$ 1.54	32.9 $\pm$ 2.88 ^a	31.5 $\pm$ 1.36 ^a
Waist circumference (cm)	70.18 $\pm$ 8.63	102.81 $\pm$ 8.22 ^a	102.50 $\pm$ 14.07 ^a
Hip circumference (cm)	94.33 $\pm$ 7.21	115.06 $\pm$ 8.17 ^a	116.70 $\pm$ 11.35 ^a
W/H ratio	0.73 $\pm$ 0.07	0.89 $\pm$ 0.07 ^a	0.87 $\pm$ 0.08 ^a
Fat ratio (%)	0.19 $\pm$ 0.04	0.36 $\pm$ 0.06 ^a	0.37 $\pm$ 0.06 ^a
Fat mass (kg)	11.30 $\pm$ 3.21	32.08 $\pm$ 7.52 ^a	33.15 $\pm$ 5.25 ^a
Lean mass (kg)	44.57 $\pm$ 9.81	54.90 $\pm$ 9.25 ^a	55.13 $\pm$ 8.91 ^a
SBP (mmHg)	114.00 $\pm$ 8.34	119.67 $\pm$ 7.30 ^b	119.75 $\pm$ 7.86
DBP (mmHg)	74.67 $\pm$ 6.00	76.33 $\pm$ 5.56	77.25 $\pm$ 5.49

^ap < 0.001; ^bp = 0.007 significance with respect to control group. BMI, Body mass index; W/H, waist/hip; SBP, systolic blood pressure; DBP, diastolic blood pressure.

increase in LDL-C was moderately significant between these groups.

Serum HDL-C values were severely decreased in obese group with respect to control group. There was 14.63% increase in HDL-C levels in obese + HT group with respect to obese group; however, it was not statistically significant.

The increase in insulin, HOMA-IR, TG, TC, VLDL, ALT and GGT levels was highly significant in obese + HT group with respect to control group, while the increase in glucose, hsCRP levels and AST activity was moderately significant in obese + HT group with respect to control

group. Total protein levels did not change between study groups. There was no change in other parameters between two obese groups (Table 2).

Obesity and obesity + hypertension treatment did not have any significant effect on serum Ang-II, TGF- β 1, MMP-9, chymase levels and ACE activities (Figures 1–5).

Serum chymase levels were positively correlated with Ang-II levels (p = 0.024, r = 0.251; n = 80) and negatively correlated with ACE activity (p = 0.013, r = -0.278; n = 80). There was highly significant (p < 0.001) positive correlation (r = 0.629) between serum MMP-9 and TGF- β 1 values (n = 80) (Table 3).

Table 2: Distribution of serum glucose, insulin, HOMA index, triglycerides, total cholesterol, hsCRP, ALT, AST, GGT, and total protein between groups.

	Control (n = 30)	Obese (n = 30)	Obese + HT (n = 20)
Glucose (mg/dL)	81.5 (7.2–103)	94 (77–114) ^a	94 (73–114) ^b
Insulin (mU/L)	5.5 (2.78–8.87)	12.28 (3.41–26.79) ^a	12 (8–24) ^a
HOMA-IR index	1.13 (0.51–2.25)	2.77 (0.64–7.07) ^a	2.69 (1.5–5.10) ^a
Triglycerides (mg/dL)	60.5 (38–137)	110 (42–348) ^a	123.5 (57–227) ^a
Total cholesterol (mg/dL)	171 (110–272)	201 (133–325)	204 (143–275) ^c
HDL-C (mg/dL)	61 (29–86)	46.5 (26–69) ^a	51 (34–81)
LDL-C (mg/dL)	104.45 (50.9–193)	126 (67.4–234) ^d	128.15 (72.7–172)
VLDL (mg/dL)	12 (8–34)	22 (8–70) ^a	31 (11–51) ^a
hsCRP (mg/L)	0.5 (0.1–3.8)	2.05 (0.1–33.6) ^a	1.55 (0.1–4.1) ^e
ALT (U/L)	13.5 (5–40)	16 (7–61)	25 (5–49) ^a
AST (U/L)	17.5 (6–31)	17 (11–33)	26.5 (10–62) ^f
GGT (U/L)	10.6 (5.4–25.2)	15.8 (6.4–59.4) ^a	18.75 (8–50) ^a
Total protein (g/dL)	7.16 (6.29–7.75)	7.16 (6.42–8.25)	7.05 (5.87–7.97)

Values are expressed as median (min–max). ^ap < 0.001; ^bp = 0.032; ^cp = 0.009; ^dp = 0.023; ^ep = 0.010; ^fp = 0.014 significance with respect to control group. HOMA-IR, Homeostatic model assessment-insulin resistance; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL, very low density lipoprotein; hsCRP, high-sensitive C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase.

Figure 1: Distribution of serum Ang-II levels according to study groups. Values are expressed median (25th and 75th percentile).

Figure 3: Distribution of serum MMP-9 levels according to study groups. Values are expressed median (25th and 75th percentile).

Figure 2: Distribution of serum TGF- β 1 levels according to study groups. Values are expressed median (25th and 75th percentile).

Figure 4: Distribution of serum chymase levels according to study groups. Values are expressed median (25th and 75th percentile).

Discussion

Scientific research within the last decade suggests that hypertension increase inflammation. Markers such as acute-phase proteins, CRP and complement factor C3 are associated with incidence of hypertension and increased arterial blood pressure [19–21]. Obesity is related with high BMI and increased adipose tissue weight is also a risk factor for hypertension. Obesity, inflammation and hypertension have a complex relationship. Metabolic problems caused by each of these factors interact and it is not clear which one is induced by the other [22]. There are also several studies indicating that long-term benefits of anti-hypertensive treatment go far beyond blood pressure lowering, and anti-inflammatory effects of medications may have a major role

in beneficiry effects [23–26]. Regarding the relation between obesity and inflammation, meta-analysis of prospective studies stated that increased inflammation may possess risk for more weight gain in the future regardless of initial weight and smoking habits and other factors [22]. Based upon this finding, development of obesity may be closely associated with inflammation [27, 28]. Recent studies report endocrine function of adipose tissue and increase adipose tissue mass may stimulate low level inflammation leading to augmented secretion of pro-inflammatory cytokines [29]. Lakoski et al. reported that elevated CRP levels increase the risk of hypertension in obese individuals [1].

In this study, we investigated hsCRP levels in obese and obese + hypertensive patient groups. hsCRP levels were significantly elevated in obese group with respect to

Figure 5: Distribution of serum ACE activity according to study groups.

Values are expressed median (25th and 75th percentile).

Table 3: The significant correlation values between serum chymase, Ang-II, ACE activity, MMP-9, TGF- β 1, hsCRP, HOMA-IR and BMI levels (n = 80).

	p-Value	r
Chymase vs. Ang-II	0.024	0.251
Chymase vs. ACE	0.013	-0.278
MMP-9 vs. TGF- β 1	<0.001	0.629
hsCRP vs. BMI	<0.001	0.460
hsCRP vs. HOMA-IR	0.001	0.377

Ang-II, Angiotensin II; ACE, angiotensin converting enzyme; MMP-9, matrix metalloproteinase; TGF- β 1, transforming growth factor; hsCRP, high sensitive C-reactive protein; BMI, body mass index; HOMA-IR, homeostatic model assessment-insulin resistance.

healthy control group. Increased inflammation in obese patient group can be related with increased adipose tissue mass as well as elevated levels of some cytokines and proteins exhibiting pro-inflammatory effects. American Heart Association Guidelines define hsCRP levels >3 mg/L as high risk for coronary heart disease. Elevated hsCRP forms complex with plasma LDL and VLDL, causing pro-inflammatory state due to activation of classical complement pathway. Our correlation findings show no relation between hsCRP and serum LDL. We can deduce that inflammation in our obese patient group, who has no clinical abnormality other than obesity, did not reach adequate levels to possess disease risks. hsCRP levels in obese + HT group showed statistically significant increase with respect to control group and statistically insignificant decrease with respect to obese group, which can be associated with anti-inflammatory actions of anti-hypertensive regimens. These findings are consistent with

results of studies reporting decreased CRP levels in individuals receiving medications for treatment of metabolic risk factors (e.g.: statins, fibrates, ACE-inhibitors, nicotinic acid, etc.) [23–26].

Human and animal studies indicated local angiotensin II synthesis in adipose tissue [30–32]. Human studies reported increased plasma renin activity, angiotensinogen, Ang-II and aldosterone levels in response to increased obesity. Consequently, blood pressure increases due to synthesis of Ang-II from angiotensinogen. This mechanisms have two pathways:

1. ACE-dependant pathway
2. ACE independent pathway: Ang-II synthesis by catalytic activity of chymase [13].

The second mechanism differs from catalysis by ACE. Chymase activity is very low in healthy tissue; in case of inflammation, chymase activation by stimulation of mast cells results in angiotensin II synthesis.

Adipose tissue is an important source of mast cells in physiological and pathological conditions. Human and animal studies reported a correlation between obesity and increased mast cell and mast cell proteases [33, 34].

In this study, we investigated the change in serum Ang II, ACE activity and chymase levels secondary to inflammation in two obese study groups and their interactions with cytokines that cause tissue damage, namely TGF- β 1 and MMP-9. No significant change was observed in any of the parameters between study groups. These results suggest that increased inflammation due to obesity and hypertension was not high enough in measured serum samples to effect mast cell chymase levels. The lack of any correlation between chymase and hsCRP levels supported this notion. A study on obese and obese + diabetic patients investigated adipose tissue biopsy samples and immunohistochemical evaluation revealed no significant increase in mast cells in control and obese groups; however, number of mast cells was significantly higher in obese + diabetic group [13]. Another study in 2010 on pre-eclamptic pregnant women in 20th gestational week reported significantly increased plasma chymase activity in pre-eclamptic pregnant women with respect to normal pregnancy [35].

Kotsis et al. reported that tissue RAS and circulatory RAS are in constant interaction [36]. Systolic and diastolic blood pressure values were within 70–120 mmHg reference range in all groups of our study. Unchanged ACE, chymase and Ang-II values can be associated with normal blood pressure values. The weak positive correlation between chymase and Ang-II indicate that chymase levels in our study do not make a significant contribution to

Ang-II elevation. The weak negative correlation between ACE and chymase emphasize that their levels are reversely proportional; however, the low correlation coefficient suggest results can be more significant, if sample size is increased. Previous studies reported increased Ang-II and insulin levels in obesity [36]. In the same manner, insulin levels in our study were significantly higher in the obese group. However, there was no correlation between Ang-II and insulin levels. The relation between hyperinsulinemia and hypertension is well established. Lack of change in ACE, Ang-II and chymase levels in our study groups suggest an unknown mechanism preventing inflammation originating from adipose tissue and formation of atherosclerotic plaques; on the other hand, a major drawback of our study was that we are not able to rule out the effects anti-hypertensive medications on serum Ang-II levels in obese + HT patient group. ACE-inhibitors, ARBs, diuretics and CCBs cause increase in Ang-II, while β -blockers decrease it [37, 38]. Lack of correlation of any parameters with Ang-II can be attributed to effect of anti-hypertensive regimens of patients. Mean HDL-C and LDL-C were >45 mg/dL and ≤ 130 mg/dL in both obese groups, respectively, indicating that study groups had low risk for disease and protective effect of antihypertensive medications in obese + HT group may have prevented the rise in chymase levels.

Chymase, an endo-peptidase secreted by mast cells, also converts TGF- β 1 and MMP-9 to their active metabolites [39, 40]. Human studies demonstrated the positive correlation between TGF- β 1 and blood pressure and TGF- β 1 regulating blood pressure via endothelin and RAS pathways. TGF- β 1 is a cytokine that directly stimulates matrix molecules causing tissue damage. MMP-9 is a matrix metalloproteinase known to stimulate angiogenesis and increase in several pathological conditions. There was no change in TGF- β 1 and MMP-9 levels in our study; however, they were strongly and significantly inter-correlated. Sepúlveda-Flores et al. conducted a study on obese patients and found no relation between fibrotic markers and TGF- β 1 [41]. Another study on pre-eclamptic pregnant women reported no elevated serum TGF- β 1 levels in comparison with normal pregnant women [42]. Laviades et al. reported elevated serum TGF- β 1 levels in hypertensive patients with microalbuminuria and left ventricular hypertrophy with respect to uncomplicated hypertensive patients, which was associated with tissue damage [43]. There was no elevation in TGF- β 1 serum levels in our study, which can be related to lack of tissue damage, especially cardiac damage. The findings of Laviades et al. are consistent with our

study. Same evaluation can be done for MMP-9 values. Gkaliagkousi et al. reported increased serum MMP-9 values in untreated hypertensive patients and proposed that determination of MMP-9 levels in essential hypertension may predict cardiovascular disease risk and vascular dysfunction in early stages of essential hypertension [44]. Our study revealed a positive correlation between serum MMP-9 levels and body fat ratio/fat mass. MMP-9 is more sensitive to increase in adipose tissue weight and may qualify as a stronger marker than TGF- β 1 for tissue damage. The direct proportion of MMP-9 levels and adipose tissue weight suggest that determination of MMP-9 serum levels in regular intervals may indicate the success of weight-loss diets and exercise programs in suppressing the pro-inflammatory risk of obese and overweight patients. We undertook an in-depth review of PubMed database and found no study investigating chymase, TGF- β 1 and MMP-9 levels in obesity.

Increased fat mass and BMI in obesity are known to be source of inflammation. In our study, the significant correlations between hsCRP vs. BMI and also vs. HOMA-IR emphasize this knowledge. However, lipid levels, well-known markers of cardiovascular disease risk, are within acceptable limits, and according to our findings, compliance to effective hypertensive treatment regimen prevents inflammation and delays tissue damage. Our obese patient group had hyperinsulinemia and elevated hsCRP levels, thereby inflammation; however, this level of inflammation was not adequate enough to increase chymase levels and blood pressure. In conclusion, conduction of long-term and comparative clinical studies and association of their results with inflammation factors are necessary to determine the conditions that stimulate secretion of chymase from adipose tissue.

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