Association of metabolic syndrome components with alterations in oxidative stress and cytokines expression

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ABSTRACT
Metabolic syndrome (MetS) has been associated with a chronic inflammation state; the specific causative etiology to the MetS will need further investigation. The present study aims to explore the levels and roles of pro-and anti-inflammatory biomarkers and antioxidant enzymes in MetS development. Subjects were divided into five groups: healthy controls; patients with dyslipidemia; patients with dyslipidemia and obesity; patients with dyslipidemia, obesity, and hypertension; patients with dyslipidemia, obesity, hypertension, and hyperglycemia. Antioxidant enzyme activities were dramatically decreased in MetS patients, whereas inflammatory marker levels were elevated. The levels of interleukin (IL)-8, IL-23, IL-33, nuclear factor kappa B (NF-κB), resistin, and nitric oxide were positively correlated to triglyceride, low-density lipoprotein-cholesterol, fasting plasma glucose, and glycosylated hemoglobin levels. Therefore, the data indicate that antioxidant enzymes, IL-8, IL-23, IL-33, NF-κB, and resistin might be used as tools to ameliorate and treat metabolic diseases.

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1. Introduction
Metabolic syndrome (MetS) is considered an emerging health issue. Its prevalence increases with age and various diagnostic criteria [1]. A national survey in Egyptian patients by Abd Elaziz et al. [2] reported that the prevalence of MetS was 55% and concerned 85.6% of diabetics and 76.6% of hypertensive patients. In the Egyptian population, the prevalence of obesity is 24.1% when assessed according to the wait circumference or 28.7% if based on the waist-to-hip ratio [3]. The prevalence of hypertension is 26.3% [4] and that of diabetes mellitus (DM) is 15.56% [5].

Chronic low levels of inflammation and oxidative stress are central mechanisms underlying MetS pathophysiology [6]. Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and their elimination by protective mechanisms and can ultimately lead to chronic inflammation. Moreover, oxidative stress activates a variety of transcription factors involved in inflammatory pathways [7]. Hyperlipidemia is often associated with oxidative stress and inflammation [8]. Postprandial hypertriglyceridemia facilitates ROS generation by the nicotinamide adenine phosphate oxidase and plays a vital role in the pathogenesis of obesity-associated MetS aggravating oxidative stress in obese patients [9]. Vona et al. [10] noticed a diminished superoxide dismutase (SOD) activity in obese persons with a significantly high body mass index (BMI), increased triglyceride (TG) levels, and low HDL-c levels. Furthermore, the levels of glutathione peroxidase (GPX) and glutathione S-transferase (GST) are significantly reduced in MetS patients, and a decreased GPX activity is associated with increased obesity [11].

A number of studies have shown that MetS is associated with inflammation, characterized by the abnormal production of proinflammatory cytokines, increased acute-phase reactants, and the activation of a network of inflammatory signalling pathways [12]. Nuclear factor-kappa B (NF-κB) signalling in numerous cell types contributes to the pathology of metabolic disorders. NF-κB-regulated genes direct the differentiation of distinct immune cell types to produce interleukin-1 (IL-1), tumour necrosis factor α (TNF-α) TNF-α, and other proinflammatory cytokines [13]. Moreover, Bruun et al. [14] showed that IL-8 amounts produced by adipocytes depend on the fat mass. Several studies have shown that IL-23 is directly related to the terminal differentiation of Th17 cells. Additionally, the prevalence of Th17 lymphocytes in the adipose tissue is associated with the progression of obesity in humans [15]. Importantly, IL-33, a newly identified member of the IL-1 cytokine family, plays a protective role against inflammation.
of adipose tissue by directly and indirectly regulating T-regulatory cells (Tregs) function. Further investigations are required to determine to which extent IL-33 contributes to metabolic disorders in humans [16].

Although the relationship between oxidative stress and inflammation has been established, further research on how oxidative stress and pro/anti-inflammatory cytokines contribute to the accumulation of different MetS components is currently needed. Notably, one of the current hot topics in MetS research is exploring disease development and complications. The present study surveyed four different Egyptian populations fulfilling different MetS criteria. Moreover, the relation between oxidative imbalance, and IL-8, IL-23, IL-33, NF-κB as well as the development of all MetS components were not addressed completely. Therefore, we aimed to explore the association between MetS components, the levels of pro- and anti-inflammatory biomarkers, and the activity of antioxidant enzymes.

2. Materials & methods

2.1. Study design and participants

The study participants included 125 individuals, who were distributed as follows: 25 subjects in the healthy group (G.I) and 100 patients diagnosed with MetS components distributed into four groups (each containing 25 patients). The MetS patients were subdivided according to different criteria as follows: G.II: dyslipidemia (low HDL-c + hypertriglyceridemia; 2 criteria); G.III: dyslipidemia and obesity (3 criteria); G.IV: dyslipidemia, obesity, and hypertension (4 criteria) and G.V: dyslipidemia, obesity, hypertension, and T2DM (5 criteria) [17].

2.1.1. Ethical approvals & participation

The study protocol and written informed consent were approved by the ethical committee of NNI (Ref # NNI/FS/16/4). Data from randomly selected patients aged 18–65 years were collected at the OPD of Endocrinology & Metabolism, National Nutrition Institute, Ministry of Health, Cairo, Egypt from a paper questionnaire and clinical laboratory reports. The questionnaire was used to assess demographic data (age, weight, and height). Three blood samples were taken from participants after overnight fasting. One sample in Na-fluoride for measuring the fasting blood sugar (FBS) from participants after overnight fasting. One sample in EDTA for RT–PCR analyses. Pr & anti-Inflammatory cytokines

Patients were considered to have MetS when they presented 3 or more of the joint statement criteria: 1) abdominal obesity (BMI > 30/kg/m² or waist circumference > 102 cm in males and > 88 cm in females), 2) serum TGs > 150 mg/dL (1.7 mmol/L) or patients receiving treatment for hypertriglyceridemia, 3) serum HDL-c < 40 mg/dL (1.03 mmol/L), 4) arterial blood pressure > 130/85 mmHg, and 5) fasting blood glucose (FBG) ≥ 110 mg/dL [17]. Exclusion criteria included autoimmune disorders as well as respiratory, kidney, liver, cerebrovascular, and heart diseases. Furthermore, pregnant and lactating women as well as patients with medical conditions such as malignancies and alcohol abuse were not enrolled in the study.

2.2. Biochemical assays

The levels of plasma glucose, triglyceride, and HDL-c were measured using reagent kits from Human Diagnostics (Germany) according to the manufacturer’s protocol. In addition, nitric oxide (NO) and resistin as well as the activity of antioxidant enzymes (SOD, GPX, GST, and CAT) were determined using ELISA reagent kits purchased from Bio-Diagnostics (Giza, Egypt) following the manufacturer’s protocol.

2.3. RNA isolation and QRT-PCR for PRO- and anti-Inflammatory cytokines

Total RNA was isolated from blood samples using the Qiagen tissue extraction kit (MBI Fermentas, Germany), then IL-8, IL-23, IL-33, and NF-κB DNA sequences were amplified by step one plus PCR using PCR kit (Invitrogen, Germany) according to the manufacturer’s instructions. Real-time PCR was conducted in 20 μL comprising 10 μL of 1x SsoFast EvaGreen Supermix (Bio-Rad, Hercules, CA, USA), 2 μL of cDNA, 6 μL of RNase/DNase-free water, and 500 nM of the primer sets (Table 1), whereas all primers were purchased from Eurogentec (Belgium). The thermal cycler conditions were as follows: 30 s at 95°C, 40 cycles of 5 s at 95°C, and 10 s at 60°C. For each reaction, a melting curve study was performed with a 65°C–95°C ramp. The number of cycles at which the fluorescent signal exceeded an arbitrarily defined threshold close to the middle of the log-linear amplification step was estimated, and the relative amount of mRNA was calculated. The amplification data were analyzed following the 2−ΔΔCt method of Livak and Schmittgen [18] using the software provided with the thermocycler.

Table 1. The primer pair sequence of studied genes.

| Primer | Forward sequence base pairs | Reversed sequence base pairs |
|--------|----------------------------|-----------------------------|
| IL-8   | 5′-GTCAAGTTTGGCCAGAGGATT-3′ | 5′-TTATGAAATCTCGACCCCTCTCAACAAAACTCTCTC-3′ |
| IL-23  | 5′-TGGCAAGATTCTGGAGGAGGCT-3′ | 5′-TAGTGGCAACTCCGAGGGCTGCTG-3′ |
| IL-33  | 5′-GCCACTTTTGGGAAGATACAACT-3′ | 5′-GGGGATTTCACGACCTGCA-3′ |
| NF-κB  | 5′-TACCTGGGAGAATAAGTTC-3′ | 5′-CTGTCACGGAGTCGTCAATTAGC-3′ |
| β-actin| 5′-GCCACCACCTTCTCAATAGG-3′  | 5′-TGACTGCTGAACATACCTG-3′    |
The values were normalized to those obtained for β-actin.

2.4. Statistical analysis

Data were analyzed using the Tukey–Kramer method for post-hoc analysis to compare the groups with each other. A simple linear correlation analysis was performed with Pearson’s method to measure the degree of dependency between variables. The results were expressed as mean ± SD. Statistical significance was considered for \( P < 0.05 \). All results were analyzed using the Statistical Package for Social Science ver. 20 software.

3. Results

The analysis of the demographic distribution (Table 2) revealed a significant variation \( (P < 0.05 \) to \( P < 0.001 \)) in-between MetS groups (i.e. G.II to G.V) when compared with data from healthy controls for all demographic data such as age (years), weight (kg), and BMI (kg/cm\(^2\)). Both body weight and BMI showed a significant increase \( (P < 0.001) \) in the G.III, G. IV, and G.V in comparison with that of healthy controls.

The systolic (SBP) and diastolic (DBP) blood pressure were significantly elevated \( (P < 0.001) \) in G.IV (dyslipidemia + obesity + hypertension + DM) compared with that of healthy controls. Furthermore, the SBP was not significantly different \( (P > 0.05) \) between G.II and G.III. On the other hand, The SBP of G.V patients was significantly increased \( (P < 0.05) \) compared with that of the G.II and G.III. The DBP of G.V and G.IV populations was significantly higher \( (P < 0.001) \) than that of the healthy group.

Furthermore, a significant TG level increase \( (P < 0.001) \) was recorded in all MetS groups compared with that of healthy controls (Table 3). The production of HDL-c was significantly \( (P < 0.001) \) decreased in all MetS groups compared with that of healthy controls. Higher FBG levels were detected in G.V compared with those in other MetS groups and healthy controls. SBP and DBP were significantly elevated \( (P < 0.001) \) in G.IV and G.V compared with those of G.II, G.III, and healthy controls (Table 3).

Our analysis revealed a dramatic decrease in all antioxidant enzymes \( (P < 0.001) \) in MetS groups (Figure 1). NO levels were significantly \( (P < 0.001) \) elevated in all MetS groups compared to those of healthy controls.

| Table 2. Changes in the demographic data, gender, age, weight, body mass index among different population groups according to their criteria’s distribution. |
|---|
| | Healthy G1* 0 criteria | G.II** 2 Criteria | G.III* 3 Criteria | G.IV* 4 Criteria | Group V* 5 Criteria | F value | P value |
| Gender n (%) | | | | | | | |
| M | 16 (64) | 12 (48) | 16 (62) | 12 (48) | 14 (56) | – | – |
| F | 9 (36) | 13 (52) | 9 (38) | 13 (52) | 11 (44) | – | – |
| Age (Years) | 36.56±3.30a | 48.44±6.36b | 50.88±6.41b | 43.68±11.37b | 50.00±4.26b | F=17.635 | P=0.000 |
| Height (Cm) | 168.32±5.65ab | 168.70±5.11ab | 165.12±6.57ab | 163.96±5.13ab | 164.92±8.46ab | 3.014 | 0.005 |
| Body Weight (kg) | 75.84±8.10a | 78.89±10.60d | 87.74±9.60b | 84.83±8.33c | 92.58±8.09b | 3.967 | 0.000 |
| Body Mass Index (BMI) (kg/m²) | 26.21±2.54a | 27.70±3.26a | 34.03±4.17a | 31.60±3.32a | 34.21±3.70a | 25.00 | 0.000 |
| Mean not sharing a common superscript symbol(s) differ significantly at \( P < 0.05 \). Values were represented as Mean±SD & n = 25 patients. G.I (Healthy group with 0 criteria); G.II (Dyslipidemia; i.e. 2 criteria, Low HDL & Hyper-triglycerides); G.III (Dyslipidemia + Obesity i.e. 3 criteria); G.IV (Dyslipidemia + Obesity + Hypertension; i.e. 4 criteria), and G.V (Dyslipidemia + Obesity + Hypertension + DM; i.e. 5 criteria). |

| Table 3. Changes in the lipid profile [Triglycerides (TG) and high-density lipoprotein (HDL-c)]; fasting blood glucose (FBG); blood pressure and resistin among different population groups according to their criteria’s distribution. |
|---|
| | Blood Pressure |
| Group Characteristic | (mg/dl) | (mg/dl) | (mg/dl) | (mmg/Hg) | (mmg/Hg) | Resistin (ng/dl) |
| G.I Healthy Control | 116.56±20.77a | 56.72±5.20b | 89.20±9.32a | 121.60±6.07a | 79.60±5.75a | 6.53±0.73a |
| G.II (Dyslipidemia) | 217.30±37.52b | 46.48±4.13a | 85.93±10.56a | 125.67±10.47a | 80.07±9.34a | 26.37±5.91b |
| G.III (Dyslipidemia + Obesity) | 190.00±42.72b | 45.05±9.58b | 79.24±11.57a | 127.28±6.56a | 79.40±9.37a | 28.78±5.72b |
| G.IV (Dyslipidemia + Obesity + Hypertension) | 190.44±50.32b | 47.33±4.26a | 84.00±120.43a | 144.68±12.9b | 89.40±5.46b | 34.90±6.67c |
| G.V (Dyslipidemia + Obesity + Hypertension + DM) | 204.28±70.13b | 46.76±8.173a | 260.92±41.00b | 149.24±7.9b | 89.48±4.79b | 37.55±9.96c |
| F value | 17.635 | 12.271 | 365.037 | 45.751 | 13.638 | 88.235 |
| P value | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Mean not sharing a common superscript symbol(s) differ significantly at \( P < 0.05 \). Values were represented as Mean±SD & n = 25 patients. G.I (Healthy group with 0 criteria); G.II (Dyslipidemia; i.e. 2 criteria, HDL & Triglycerides); G.III (Dyslipidemia + Obesity i.e. 3 criteria); G.IV (Dyslipidemia + Obesity + Hypertension; i.e. 4 criteria), and G.V (Dyslipidemia + Obesity + Hypertension + DM; i.e. 5 criteria). |
controls, and this elevation was higher with the increase in MetS component number. SOD activity was significantly ($P < 0.001$) decreased in sera of G.III and G.V patients compared with that in healthy controls. Likewise, GPX and GST activities were significantly ($P < 0.001$) decreased in all MetS groups compared with those in healthy controls, and this depletion was higher with the increase in the number of MetS components.

Regarding the correlation between obesity marker (BMI) and oxidative stress (Figure 2.A-D), Pearson’s $r$ analysis revealed a significant positive correlation between the alterations in BMI and NO ($r = 0.616, P < 0.001$). In contrast, a significant ($P < 0.001$) negative correlation was found between BMI and the antioxidant enzymes SOD ($r = -0.366$), GPX ($r = -0.512$), and GST ($r = -0.530$). In addition, the data showed a significant positive correlation between hypertriglyceridemia and NO ($r = 0.373, P < 0.001$) and a strong negative correlation ($P < 0.001$) between hypertriglyceridemia and SOD ($r = -0.243$); GPX ($r = -0.410$), and GST ($r = -0.522$) (Figure 3.A-D).

The analysis of IL-8, IL-23, and NF-κB gene expression showed a significant ($P < 0.001$) elevation in all MetS groups compared with the expression levels in healthy controls, and this elevation was higher with the increase in the number of MetS components. In contrast, IL-33 gene expression was significantly ($P < 0.001$) decreased in all MetS groups compared with that in healthy controls, and this decrease was higher with the increase in MetS criteria (Figure 4.A-D). Resistin levels were significantly ($P < 0.001$) elevated in all MetS groups compared with that in healthy controls, and this elevation was higher with the increase in MetS criteria (Table 2).

A significant ($P < 0.001$) positive correlation was observed between BMI and the levels of IL-8 ($r = 0.472$), IL-23 ($r = 0.530$), NF-κB ($r = 0.519$), and resistin ($r = 0.538$). However, a significant negative correlation was found between IL-33 expression and BMI ($r = -0.547$).
Figure 2. The correlation between body mass index (BMI) with oxidative stress markers; whereas; (A): nitric oxide (NO); (B): superoxide dismutase (SOD); (C): glutathione S-transferase (GST) and (D): glutathione peroxidase (GPX) in healthy controls and MetS populations. 

(TG levels were positively correlated ($P < 0.001$) with the expression of IL-8 ($r = 0.507$), IL-23 ($r = 0.401$), NF-$\kappa$B ($r = 0.599$), and resistin ($r = 0.403$), whereas they were negatively correlated with IL-33 levels ($r = -0.369$) (Figure 6,A-E). Similarly, FBG measurements were positively correlated with IL-8 ($r = 0.272$, $P < 0.001$), IL-23 ($r = 0.457$, $P < 0.01$), NF-$\kappa$B ($r = 0.321$, $P < 0.01$), and resistin ($r = 0.407$, $P < 0.001$) levels and negatively correlated with IL-33 expression ($r = -0.261$; $P < 0.03$) (Figure 7,A-E).

4. Discussion

MetS is considered a worldwide health issue for individuals presenting a cluster of cardiovascular risk factors including obesity, dyslipidemia, hypertension, and impaired glucose tolerance (hyperglycemia) [19]. Importantly, understanding the pathophysiology of MetS is essential for the development of effective preventative strategies and intervention tools that can help to reduce the disease’s rising incidence and comorbidities. Herein we investigated the relationship between pro- and anti-inflammatory biomarkers and antioxidant enzyme activities with the development and accumulation of MetS components. The present data are in accordance with Al-Bachir and Bakir [20], who reported a positive correlation between BMI and MetS criteria in overweight and obese adolescent. Moreover, Fan et al. [21] reported that the hypertriglyceridemia is linked to the reduced HDL-c through the generation of small dense-LDL (sdLDL), which originates from the delipidation of larger atherogenic very small density lipoprotein (vLDL) and large LDL-c. This conversion established proatherogenic lipoprotein phenotype as a generic predictor for DM and MetS.

In the present study, NO levels were significantly increased in all MetS groups compared with those in healthy controls, and this elevation was higher with the increase in MetS component number (diabetic and hypertensive groups were higher than obese and dyslipidemia groups). Asl et al. [22] reported higher NO levels associated with an increase in other metabolic components such as BMI, FBG, TG, and higher blood pressure. This was confirmed by our data as NO is significantly raised in MetS populations as a result of hyperglycemia [23]. The observed overproduction of...
ROS in the obesity and hyperglycemic groups might contribute to mitochondrial dysfunction. This might be particularly the case for the superoxide anion (O$_2^-$), which is dismutated to produce H$_2$O$_2$ [24]. Rajendran et al. [25] evidenced that NO levels increased in hypertensive patients as a compensatory mechanism against high blood pressure increasing NO secretion from the vascular endothelium. ROS overproduction was related to obesity, hypertension, diabetes, vascular disease, atherosclerosis, many metabolic disorders, inflammation, and cardiovascular diseases [26]. Notably, an imbalance of NO bioavailability and increased production of ROS, mainly superoxide, might promote endothelial dysfunction. A cause of the increased prevalence of hypertension with aging might be the advancing endothelial dysfunction associated with aging and induced by increased oxidative stress [27]. Evidence suggests that NO plays a major role in regulating blood pressure and impaired NO bioactivity is an important component of hypertension. Impaired NO is also implicated in arterial stiffness, a major mechanism of systolic hypertension [28].

The present data revealed that SOD, GPX, and GST activities were significantly decreased in all MetS groups compared with those in healthy controls, and this decrease was higher with the increase in MetS criteria (the diabetic group was lower than other groups). MetS was also negatively correlated with all antioxidant enzyme activities. Recently, high BMI, high TG levels, low HDL-c levels, hypertension, and DM, as well as diminished SOD, GPX, and GST activities were reported in obese persons [10,29,30]. Altered adipose organ function might play a fundamental pathogenetic role once fat accumulation has occurred. Modulation of insulin sensitivity appears to be, at least in part, related to changes in redox balance, oxidative stress, and inflammation, with a relevant underlying role for mitochondrial dysfunction that may exacerbate these alterations [31]. Moreover, SOD, GPX, GST, catalase (CAT), and NO synthase are antioxidant enzymes scavenging reactive metabolites in normal individuals. Therefore, oxidative stress in obesity inducing lipogenesis and steatosis leads to an impaired antioxidant defense system [32]. Additionally, SOD and GPX downregulation and NADPH
The expression of IL-8, IL-23, and NF-κB mRNAs was significantly increased in all MetS groups compared with the healthy controls, and the diabetic group showed the higher increase. The level of the proinflammatory markers IL-8, IL-23, and resistin as well as NF-κB were positively correlated with TG, LDL, and FBS levels and were negatively correlated with HDL-c amounts. Importantly, IL-8 levels were linked to visceral adiposity, fasting glucose and insulin levels, and lipid profile in an Egyptian population with different grades of obesity [33]. The role of elevated IL-8 protein levels in hyperlipidemia was confirmed by the positive correlation with lipids markers of visceral adiposity [35]. The positive correlation between IL-8 and BMI is consistent with the findings of Juncal-Ruiz et al. [36] showing that serum IL-8 levels are higher in overweight individuals. There is also evidence supporting IL-8 involvement in the establishment of the inflammatory microenvironment of the vascular wall and cardiovascular disease [37]. Simonini et al. [38] concluded that, in human coronary atherosclerosis, IL-8 is an important mediator of angiogenesis by contribution to plaque formation. Also, IL-8 is a potent chemoattractant and may be responsible for the recruitment of neutrophils and T lymphocytes into the subendothelial space, inducing adhesion of monocytes to endothelium, and migration of vascular smooth muscle cells which lead to intimal thickening and atherosclerosis [39]. Macrophage infiltration in the adipose tissue promotes the release of IL-8, triggering systemic and local inflammatory responses in metabolic inflammation, insulin resistance, and T2DM [40]. In addition, hyperglycemia promotes monocyte adhesion to endothelial cells, leading to the release of IL-8. Hyperglycemia also significantly correlates with FBG and HbA1c levels [41]. Importantly, IL-8 is a major adipocytokine producing insulin resistance via the inhibition of insulin-induced Akt phosphorylation in adipocytes [42]. Elevated serum IL-8 levels were found in diabetic subjects, and it is suggested
Figure 5. The correlation between body mass index (BMI) and cytokines (A): IL-8; (B): IL-23; (C): IL-33; (D): NF-κB and (E) Resistin in healthy and metabolic syndrome population’s groups. Whereas; Interleukin (IL). Nuclear Factor-kappa-B (NF-κB).

that this cytokine might contribute to the development of diabetic macroangiopathy [43]. The activity of IL-8 can be a therapeutic target for perverting and ameliorating visceral adiposity, atherosclerosis, diabetes, and complications.

As one of IL-12 family cytokines, IL-23 is mainly considered as a proinflammatory and stimulatory cytokine with key roles in the development of Th1 and Th17 subsets of helper T cells [44]. IL-23 is produced by macrophages and is markedly elevated in obese patients [45]. The present data show that IL-23 expression was positively correlated with LDL and TG levels. IL-23 levels were inversely correlated with HDL-c [46]. Additionally, an imbalance in IL-23 was found in cases with high BMI, SBP, DBP, TG, LDL-c, HDL-c, and FBG levels [46,47]. Moreover, IL-23 promotes the release of other proinflammatory cytokines like IL-1, IL-6, and TNF-α, and was linked with end-organ damage [48]. IL-23 has been associated with atherosclerosis in humans and animal models and might be associated with hyperinsulinemia and insulin resistance in premature Coronary artery disease (CAD) patients [49]. Lee et al. [50] reported that injection of IL-23 increased Th17 lymphocytes in peripheral blood mononuclear cells as well as serum levels of IL-17A in Dahl salt-sensitive rats, leading to a significant elevation of systolic blood pressure. This study supports the link between IL-23/IL-17 axis and hypertension. Interleukin-23/IL-17 axis is stimulated in obese women independently of the increase in abdominal fat and insulin resistance [51]. Furthermore, Abdel-Moneim et al. [52] reported that IL-23 augments the differentiation of Th17 cells and
Figure 6. The correlation between hypertriglyceridemia and cytokines (A): IL-8; (B): IL-23, (C): IL-33; (D): NF-κB and (E) Resistin in healthy and metabolic syndrome population’s groups. Whereas; Interleukin (IL). Nuclear Factor-kappa-B (NF-κB).

initiates inflammation by enhancing the production of proinflammatory cytokines (TNF-α, IL-1β, and IL-6), chemokines, and induces the release of inducible nitric oxide synthase (iNOS); and in turn, causes an increase in free radicals which may induce β-cell destruction and subsequently hyperglycemia. Therefore, the possible involvement of IL-23/Th-17 proinflammatory cytokine axis in hypertension and CVD related to MetS seems worthy of investigation.

Our study found that IL-33 mRNA expression was significantly decreased in all MetS groups compared with that in healthy controls, and this decrease was higher with the increase in MetS criteria (diabetic and hypertensive groups were lower than obese and dyslipidemia groups). Previous studies investigated the link between IL-33, an anti-inflammatory cytokine, and obesity [16]. IL-33 has a crucial role in lipid metabolism and exhibits a protective role in the pathogenesis of atherosclerosis and acute cardiovascular events [53]. Moreover, IL-33 prevents cardiomyocyte apoptosis, decreases myocardial fibrosis and myocyte hypertrophy, and impedes the atherosclerotic process [53]. Thus, IL-33 plays critical role in cardiovascular events and is protective against obesity and diabetes by inhibiting lipid accumulation and storage and metabolic gene expression in white adipose tissue [54]. Notably, IL-33 protects against obesity-induced inflammation by promoting Th2 cytokine accumulation and macrophage M2 polarization [55]. Additionally, a large-scale screen also indicated that IL-33 plays a crucial role in alleviating lipoprotein accumulation; thereby having a protective role against atherosclerosis and hypertension [56]. Because of the negative correlation between insulin resistance and IL-33 in DM, IL-33 might be involved in glucose
Figure 7. The correlation between FBG levels and some pro-inflammatory and anti-inflammatory cytokines (A): IL-8; (B): IL-23, (C): IL-33; (D): NF-κB and (E) Resistin in healthy and metabolic syndrome population’s groups. Whereas; Interleukin (IL), Nuclear Factor-kappa-B (NF-κB).

Overexpression of proinflammatory cytokines enhances NF-κB activation, involving adipose tissue inflammation with systemic metabolic risk factors (e.g. obesity, endothelial dysfunction, insulin resistance, and DM) [59]. NF-κB is a key transcription factor of M1 macrophages and is required for the induction of a large number of inflammatory genes, including those encoding TNF-α, IL-1β, and IL-6 [60]. The present findings show significantly higher NF-κB mRNA expression levels in MetS populations. The low grade inflammation with elevated inflammatory cytokines was linked with adipocyte metabolic dysfunction in obesity, accounting for the higher gene expression of the transcriptional factor NF-κB in obese subjects [61]. Furthermore, the activation of NF-κB is triggered by the production of advanced glycation on proteins and lipids.
following the inflammatory responses in case of hyperlipidemia and hyperglycaemia with increased expression of TNF-α as well as adipocyte or macrophage infiltration [13]. However, a positive correlation between NF-κB mRNA expression and FBS and HbA1c levels in MetS patients was observed. In vascular endothelial cells, NF-κB mediates induction of pro-inflammatory cytokines, chemotactic factors, and adhesion molecules which in turn promote monocyte recruitment and atherosclerosis progression [62]. Moreover, NF-κB over-expression triggers calcification of endothelial cells leading to endothelial dysfunction and further vascular complications [63]. The NF-κB signalling pathway might also influence brown adipogenesis leading to the preferential enlargement of visceral adipocytes. Chronic inflammation and adipocyte dysfunction might alter endothelial function leading to hypertension [52]. Furthermore, Malle et al. [65] reported that NF-κB was significantly associated with pancreatic β-cell dysfunction and insulin resistance in MetS. Notably, NF-κB stimulates the production of IL-1β, IL-6, and TNF-α, which are contributing to the induction of insulin resistance. TNF-α has directly inhibited insulin signalling, resulting in insulin resistance by activating c-Jun amino-terminal kinase and inhibitor of NF-κB kinase, leading to serine phosphorylation of insulin receptor substrate-1, which in turn induced insulin resistance and T2DM [52]. Therefore, NF-κB pathway, a ubiquitous transcription factor, was associated with the progression of MetS components like obesity, hypertension, and hyperglycaemia. Moreover, NF-κB may cause the production of cytokines and other immune factors and can be used as a potential therapeutic target for perverting or ameliorating metabolic diseases.

The adipose tissue secretes adipocytokines, e.g., leptin, adiponectin, and resistin, which regulate key physiological processes [66]. The present study focused on the changes in resistin levels as an adipocyte-specific protein that displayed a pivotal function in inflammation related to MetS. Resistin levels were significantly elevated in all MetS groups compared with those in healthy controls. In accordance with these data, resistin was positively correlated with BMI [67] and lipid profile markers [68], whereas it was negatively correlated with HDL-c in abdominally obese patients. Resistin induced TNF-α, IL-1β, IL-6, IL-8, and IL-12 that stimulate the proinflammatory response and NF-κB activation [69]. Notably, resistin is positively associated with metabolic disorders and increasing coronary artery calcification, which is a quantitative measure of atherosclerosis along with other inflammatory markers [70]. Furthermore, resistin is involved in the development of atherosclerosis via promoting the formation of foam cells and the proliferation and migration of vascular endothelial and smooth muscle cells [71]. Importantly, plasma resistin levels significantly correlated with IL-6, soluble TNF receptor 2, intercellular adhesion molecule 1, vascular adhesion molecule 1, and E-selectin, and resistin levels independently associated with hypertension [72]. Human resistin causes inflammation and contributes to insulin resistance, obesity, and T2DM [73]. Also, resistin may interfere with insulin signalling by stimulating the expression of phosphatase and tensin homolog deleted on chromosome ten, which dephosphorylates 3-phosphorylated phosphoinositide [71].

The current investigation is particularly relevant as it explores the relationship between MetS components and the inflammatory markers IL-8, IL-23, IL-33, NF-κB, and resistin as well as the activities of the antioxidant enzymes SOD, GPX, and GST and might help predict the development of MetS. However, there are a few limitations such as the low number of patients in each group and the significant difference in gender and age. An analysis of the results based on age, sex, and disease duration might also be relevant. Finally, the protein levels of inflammatory and anti-inflammatory biomarkers and the patients’ medication data need to be determined as they might influence the inflammation or metabolism in MetS.

5. Conclusion

The current study revealed a molecular association between the expression levels of cytokines (IL-8, IL-23, IL-33, and resistin) with the different components of MetS. Also, NF-κB expression levels as well as the activities of SOD, GPX, and GST were linked to the components of MetS. The severity of inflammatory biomarkers was mostly elevated with the increase in the number of MetS components. These data also indicated that several cytokines can be used as a potential therapeutic tool to ameliorate and treatment of metabolic disorders. The mechanism of action by which the pro- and anti-inflammatory biomarkers exert their effects on MetS is not fully understood and needs further investigation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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