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Antipsychotic-Treated Schizophrenia Patients Develop Inflammatory and Oxidative Responses Independently From Obesity: However, Metabolic Disturbances Arise From Schizophrenia-Related Obesity

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ABSTRACT

Objective: To define the impact of obesity on inflammatory and oxidative disturbances in antipsychotic-treated schizophrenia patients.

Methods: Several cytokines, inflammatory, metabolic, and oxidative status markers were evaluated in obese ($n = 40$) and non-obese ($n = 40$) antipsychotic-treated patients and compared with age-and BMI-matched controls ($n = 80$).

Results: Schizophrenia patients had higher leptin, TNF-α, adiponectin, visfatin, resistin, P-selectin, NPY, BDNF, CD40-L, MCP-1, and malondialdehyde, and lower IL-6, ghrelin, neopterin, and vitamin E levels compared to their respective controls ($p < 0.001$). Total oxidant status was higher in non-obese patients compared to controls ($p < 0.001$), total antioxidant capacity was higher in obese compared to non-obese patients ($p < 0.01$), but vitamin A and paraoxonase levels were not different. High sensitive-CRP levels were higher in obsese controls relative to non-obese controls ($p < 0.05$) and in obese patients relative to non-obese patients ($p < 0.001$). Fasting glucose, insulin, HbA1c, HOMA-IR, uric acid, total cholesterol, and triglyceride concentrations were higher in obese patients compared to non-obese patients. Insulin concentrations and HOMA-IR were also higher in obese controls than in non-obese controls.

Conclusions: Our results suggest that inflammatory responses and oxidative stress develop independently from obesity in antipsychotic-treated schizophrenia patients. However, schizophrenia-induced obesity causes metabolic disturbances; thereby, obese schizophrenia patients are more liable to cardiovascular events and progress of metabolic syndrome than non-obese patients.

1 | Introduction

Schizophrenia affects 0.5%–1% of the population worldwide and are associated with a higher rate of premature mortality than observed in the general population, with the most common cause of death being cardiovascular diseases (CVD) (Hennekens et al. 2005). The use of antipsychotics is an indispensable approach in treatment, but obesity-inducing side effects of antipsychotics, especially atypical antipsychotics, are common in chronic illness (Hennekens et al. 2005). It is well accepted that

excessive body weight gain is responsible for the development of metabolic disorders, which is a significant risk factor for cardiovascular disease. Increased body weight leads to alterations in cytokine concentrations, which in turn trigger inflammatory responses that eventually end up with metabolic disorders and CVD (Baptista and Beaulieu 2002; Ferreira, Grajales, and Valverde 2020). Numerous studies, including many meta-analyses, demonstrate alterations in blood cytokine levels in schizophrenia patients on antipsychotic treatment (Fonseka, Müller, and Kennedy 2016). Some reports assert that cytokine disturbances are a consequence of obesity (Marcinowicz et al. 2021), and many others suggest that metabolic disorders develop due to the alterations in inflammatory cytokine levels in this patient population (Ferreira, Grajales, and Valverde 2020).

The association between schizophrenia and oxidative stress has been the subject of interest. Besides studies suggesting that toxic free radicals play a role in etiology (Ermakov et al. 2021), there are studies suggesting that pathological processes in schizophrenia cause oxidative stress (Gunes et al. 2017). Either as a cause or a result of illness, oxidative stress is a risk factor for subsequent adverse health outcomes. A wide range of severe clinical conditions, from neurodegenerative disorders to cardiovascular disease, may emerge due to oxidative stress (Buosi et al. 2021). Oxidative stress arises from the imbalance between oxidant and antioxidant molecules, and the resulting increase in reactive oxygen species and decline in antioxidant defense exacerbate the condition. The association between high body mass index (BMI) and increased oxidative stress is demonstrated in chronic patients (An et al. 2018). It is suggested that antipsychotic treatment may induce oxidative stress, which further leads to the development of obesity (Gao et al. 2023). Obesity also induces oxidative stress through biomedical mechanisms (Guan et al. 2024).

Considering the weight-gaining effect of antipsychotics, we aimed to examine the changes in inflammatory, metabolic and oxidative status due to obesity in chronic patients on antipsychotic treatment. We undertook the present study in obese and non-obese patients and age- and BMI-matched healthy controls and examined the levels of several cytokines and various inflammatory and metabolic markers associated with obesity and cardiovascular disease. In addition, to evaluate oxidative stress, we measured plasma malondialdehyde (MDA) levels and serum total oxidant status (TOS), and to evaluate antioxidant status, we analyzed serum vitamin E and vitamin A concentrations, as well as total antioxidant capacity (TAC) and paraoxonase (PON) activity.

2 | Materials and Methods

This study was carried out in patients diagnosed ($n = 80$) according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (12) at Bursa Uludag University School of Medicine, Department of Psychiatry. Forty obese (BMI ≥ 30) patients (OSz) (21 males, 19 females) and 40 non-obese patients (BMI < 30) (NOSz) (27 males, 13 females), all on antipsychotic treatment for at least six months, and 40 obese controls (OC) (22 males, 18 females) and 40 non-obese controls (NOC) (23 males, 17 females), age- and BMI-matched aged between 19 and 63 years, were included in the study. Obese controls were recruited from among volunteers referred to the Endocrinology and Metabolic Diseases Clinics in our University Hospital for obesity, and non-obese controls were volunteers from among University Hospital staff. All participants provided written consent. The study protocol was approved by the Uludag University Faculty of Medicine Ethics Committee (2011-5/3) on 22.02.2011, and was in accordance with the guidelines of the 2002 Declaration of Helsinki.

Patients and controls were examined by two independent specialists in psychiatry. The detailed history of the participants was questioned, and psychiatric rating scales were performed in the patient group. All participants were screened for any major health problems. Potential participants who had diabetes, cancer, thyroid gland dysfunction, who were pregnant or lactating, who were taking herbal or illicit drugs, or any medications such as glucocorticoids, nicotinic acid, and beta-blockers, which may cause insulin resistance were all excluded from the study. Volunteers who had first-degree relatives with schizophrenia were not included in the control group.

Anthropometric measurements, blood pressure, and heart rate of all participants were recorded. All participants were questioned about their smoking habits, and the type of antipsychotic drug the patients received was noted. Blood samples were collected from the antecubital vein after 12 h of fasting into EDTA and dry gel tubes, centrifuged at 3000 × rpm for 10 min to obtain whole blood, plasma, and serum, accordingly. Whole blood count, fasting glucose, total protein, albumin, total bilirubin, uric acid, triglyceride, total cholesterol, HDL-cholesterol, thyroid stimulating hormone (TSH), free T4, HbA1c, insulin, ACTH, and cortisol concentrations were measured by commercial kits in routine auto analyzers on the day the blood samples were collected. LDL-cholesterol was calculated by the Friedewald formula. Homa-IR was calculated by the formula: HOMA-IR = fasting serum glucose (mg/dL) × fasting serum insulin value (μU/mL)/405. The rest of the samples were portioned to be stored at −80°C until further analyses. Serum hsCRP, Leptin, TNFα, Adiponectin, Visfatin, Rezistin, P-selectin, NPY, BDNF, CD40L, MCP-1, PAI-1, IL-6, Ghrelin, Neopterin, Protein-S100-B, and AGRP measurements were performed by using commercially available ELISA kits. Plasma MDA levels were measured by high performance liquid chromatography (HPLC) as described by Young and Trimble (1991) and expressed as nmol MDA/mL; serum TOS was evaluated by the colorimetric method as defined by Erel (2005) and expressed as μM H_2O_2 equivalents and serum TAC was evaluated colorimetrically by using OxiSelect TAC assay kit and expressed as μM copper reducing equivalents (CRE); plasma vitamin E and vitamin A concentrations were quantified by the HPLC procedure using UV detection at 292 and 326 nm, respectively, as previously described (Varley 1976) and expressed in μM concentrations, and serum PON activity was determined by the method of Eckerson, Wyte, and Du (1983) and expressed in U/L.

Statistical analyses were performed with IBM SPSS ver.28.0 (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp.). The approximate normal distribution was assessed by the Shapiro-Wilk test.

Results of normally distributed data were presented as mean (± SD), and non-normally distributed data as median (minimum; maximum). The differences between the groups were determined by one-way ANOVA or Kruskal–Wallis tests, where appropriate. The Bonferroni test was used as a multiple comparison test. Spearman's rho correlation analysis was performed to test the relationship between variables. A value of $p < 0.05$ was considered statistically significant.

3 | Results

Demographic characteristics of obese and non-obese controls and patients are given in Table 1. Age, height, and heart rates of female and male subjects were not different between the groups. The rate of smoking was higher in patients. Body weight, neck, waist, and hip circumferences were significantly greater in obese female and male subjects in both patient and control groups.

Fasting glucose, insulin, HbA1c, HOMA-IR, uric acid, total cholesterol, and triglyceride concentrations were higher in obese Sz patients when compared with non-obese patients (Table 2). Insulin concentrations and HOMA-IR values were also significantly higher in obese controls than in non-obese controls. Total protein, albumin, HDL-cholesterol, and LDL-cholesterol concentrations did not differ between the groups.

Changes in cytokine concentrations in obese and non-obese patients and controls are displayed in Figure 1. Leptin levels were significantly high in obese and non-obese Sz patients compared to obese and non-obese controls, respectively ($p < 0.001$). Leptin levels of obese Sz patients were significantly higher than those of non-obese Sz patients as well ($p < 0.05$).

TNF-α, adiponectin, visfatin, resistin, P-selectin, NPY, BDNF, CD40-L, and MCP-1 levels were significantly high in obese and non-obese Sz patients compared to obese and non-obese controls, respectively ($p < 0.001$). These parameters were not different between obese and non-obese subjects in either patient or control groups.

IL-6, ghrelin, and neopterin levels were significantly lower in patients compared to their obese and non-obese control counterparts, respectively ($p < 0.001$). These parameters were not different between obese and non-obese subjects in Sz and control groups, respectively.

High sensitive-CRP levels were significantly higher in obese controls relative to non-obese controls ($p < 0.05$) and in obese patients relative to non-obese patients ($p < 0.001$). hsCRP concentrations of obese controls and obese patients were not statistically different.

No significant difference was observed in S100-B protein and AGRP levels of obese and non-obese control and Sz subjects.

Changes in oxidative status parameters in obese and non-obese patients and controls are shown in Figure 2. Serum MDA concentrations were significantly higher in the obese and non-obese patients compared to obese ($p < 0.01$) and non-obese controls ($p < 0.001$), respectively. Obese control subjects had higher MDA levels compared to non-obese controls ($p < 0.01$). However, no significant difference was observed between MDA levels of obese and non-obese patients. Total oxidant status was significantly higher in the non-obese patients than non-obese controls ($p < 0.01$) but not different between obese and non-obese patients. Neither serum vitamin E nor serum vitamin A concentrations were different between obese and non-obese patients. BY contrast, vitamin E concentrations were significantly lower in obese and non-obese patients compared to obese and non-obese controls, respectively ($p < 0.001$). Total antioxidant capacity was higher in the obese patients compared to non-obese patients ($p < 0.01$). PON activity was not different between the groups.

Since smoking has a considerable effect on oxidative stress, and the smoking rate was significantly higher in patient groups, parameters related to oxidant status were evaluated in terms of the smoking status of the participants, as well, and a summary of results is provided in Table S1. When non-obese and obese controls and patients were further split as non-smokers (NS) and smokers (S), there were no differences among MDA levels of control subgroups. Similarly, there were no differences among MDA levels of patient subgroups. MDA levels of non-smoking-non-obese controls and non-smoking-non-obese patients were comparable, as were non-smoking-obese controls and non-smoking obese patients. However, both smoking-non-obese patients and smoking-obese patients had higher MDA levels compared to smoking-non-obese controls and smoking-obese controls ($p < 0.001$ and $p < 0.05$), respectively. Vitamin E levels in all patient subgroups were lower than in control subgroups (Table S1). TAC was significantly higher in smoking-obese patients compared to those of non-smoking-non-obese patients ($p < 0.01$). Vitamin A, TOS, and PON values were not different in obese and non-obese controls and patients when split as smokers and non-smokers.

BMI was not correlated with any of the inflammatory parameters nor with TOS and PON but was significantly correlated with MDA ($r = 0.271$, $p < 0.001$), vitamin E ($r = 0.211$, $p < 0.01$), vitamin A ($r = 0.225$, $p < 0.01$), TAC ($r = 0.270$, $p < 0.001$) and uric acid levels ($r = 0.332$, $p < 0.001$). The correlation between BMI and MDA was not retained when analyzed in Sz patients only. While MDA levels were correlated with TOS ($r = 0.201$, $p < 0.05$) and TAC ($r = 0.250$, $p = 0.001$), TAC levels were significantly correlated with uric acid levels ($r = 0.662$, $p < 0.001$). Also, vitamin E levels were positively correlated with vitamin A ($r = 0.263$, $p < 0.001$) and negatively correlated with TOS ($r = -0.165$, $p < 0.05$).

There was no difference in BMI between obese patients receiving different antipsychotic treatments. When evaluated in terms of the typical ($n = 10$), atypical ($n = 50$), and typical + atypical ($n = 20$) AP drugs or single ($n = 29$) and multiple ($n = 51$) AP-treatment, there was no significant difference in the inflammatory, metabolic and oxidative status markers in obese or non-obese patients. Levels of the cytokines and the oxidant status parameters measured were not different between female and male subjects in the study groups. Also, no gender difference was observed in these parameters when evaluated according to the type of antipsychotic treatment.

TABLE 1 | Demografic characteristics of male and female subjects in the study groups.

	NOC ($n = 40$)	OC ($n = 40$)	NOSz ($n = 40$)	OSz ($n = 40$)	
Female *n* (%)	17 (49%)	18 (51%)	12 (40%)	18 (60%)	
Male *n* (%)	23 (51%)	22 (49%)	28 (56%)	22 (44%)	
Age (year)	F: 36 (23,60)	F: 44 (19; 55)	F: 35 (21; 47)	F: 37 (26,50)	F: N.S.
	M: 33 (22; 49)	M: 33 (22; 50)	M: 36 (21,63)	M: 40 (22; 57)	M: N.S.
Smoker *n* (%)	29 (36.3)		45 (56.3)		
Height (cm)	F: 163 ± 6.5	F: 159 ± 5.4	F: 157 ± 5.8	F: 159 ± 7.0	F: N.S.
	M: 176.6 ± 8.1	M: 177.9 ± 8.0	M: 171.5 ± 6.4$^{\#}$	M: 173 ± 7.2	M: N.S.
Weight (kg)	F: 65.6 ± 10.2	F: 88.0 ± 10.8***	F: 60.5 ± 10.8$^{\#\#\#}$	F: 90.7 ± 16.6***,$^{+++}$	***Different from NOC, $p < 0.001$
	M: 81.4 ± 11.4	M: 105.9 ± 12.7***	M: 72.0 ± 11.4$^{\#\#\#}$	M: 100.8 ± 14.0***,$^{+++}$	$^{\#\#\#}$Different from OC, $p < 0.001$
					$^{+++}$Different from NOSz, $p < 0.001$
BMI (kg/m^2)	F: 24.4 ± 4.1	F: 34.7 ± 4.4***	F: 24.4 ± 3.9$^{\#\#\#}$	F: 35.7 ± 4.6 ***,$^{+++}$	***Different from NOC, $p < 0.001$
	M: 26.0 ± 2.9	M: 33.8 ± 3.4***	M: 24.4 ± 3.0$^{\#\#\#}$	M: 33.6 ± 2.9***,$^{+++}$	$^{\#\#\#}$Different from OC, $p < 0.001$
					$^{+++}$Different from NOSz, $p < 0.001$
NC (cm)	F: 33 (30; 36)	F: 36 (33; 40)***	F: 33 (30; 36)$^{\#\#\#}$	F: 38 (35; 44)***,$^{+++}$	***Different from NOC, $p < 0.001$
	M: 39 (35; 44)	M: 42 (39; 46)*	M: 38 (33; 43)$^{\#\#\#}$	M: 44 (35; 54)***,$^{+++}$	$^{\#\#\#}$Different from OC, $p < 0.001$
					$^{+++}$Different from NOSz, $p < 0.001$
WC (cm)	F: 82 (63; 101)	F: 102 (85; 126)***	F: 88 (79; 96)$^{\#\#\#}$	F: 112 (84; 151)***,$^{+++}$	***Different from NOC, $p < 0.001$
	M: 96 (75; 112)	M: 113 (95; 137)***	M: 95 (79; 114)$^{\#\#\#}$	M: 117 (99; 133)***,$^{+++}$	$^{\#\#\#}$Different from OC, $p < 0.001$
					$^{+++}$Different from NOSz, $p < 0.001$
HC (cm)	F: 103.4 ± 10.3	F: 121.6 ± 11.1***	F: 101.1 ± 8.4$^{\#\#\#}$	F: 119.9 ± 9.0***,$^{+++}$	***Different from NOC, $p < 0.001$
	M: 106.4 ± 6.8	M: 116.5 ± 8.4***	M: 103.0 ± 5.8$^{\#\#\#}$	M: 116.4 ± 6.3***,$^{+++}$	$^{\#\#\#}$Different from OC, $p < 0.001$
					$^{+++}$Different from NOSz, $p < 0.001$
Heart rate (beats/min)	F: 79 (74; 84)	F: 79 (70; 88)	F: 83 (64,100)	F: 79 (64; 96)	F: N.S.
	M: 78 (70; 84)	M: 78 (70; 990)	M: 82 (60; 128)	M: 83 (60; 100)	M: N.S.
SBP (mmHg)	F: 118 (90; 150)	F: 140 (120; 160)***	F: 110 (90; 145)	F: 116 (95; 130)	***$p < 0.001$, different from NOC, NOSz and OSz
	M: 120 (110; 140)	M: 121 (110; 140)	M: 122 (90; 160)	M: 127 (90; 180)	
DBP (mm Hg)	F: 70 (50; 90)	F: 89 (80; 100)***	F: 65 (50; 80)	F: 74 (50; 100)	***$p < 0.001$, different from NOC, NOSz and OSz
	M: 73 (60; 80)	M: 74 (70; 85)	M: 75 (50; 120)	M: 76 (50; 100)	

Note: Data are presented as mean ± SD when normally distributed and as median (min; max) when non-normally distributed.
Abbreviations: DBP, diastolic blood pressure; F, female; HC, hip circumference; M, male; NC, neck circumference; NOC, non-obese controls; NOSz, non-obese schizophrenia; OC, obese controls; OSz, obese schizophrenia; SBP, systolic blood pressure; WC, waist circumference.

TABLE 2 | Fasting glucose, insulin, HbA1c, HOMA-IR, uric acid, total protein, albumin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, TSH, sT4, ACTH and cortisol levels of obese (OC) and non-obese (NOC) controls, and obese (OSz) and non-obese (NOSz) patients.

	NOC ($n = 40$)	OC ($n = 40$)	NOSz ($n = 40$)	OSz ($n = 40$)	
Glucose (mg/dL)	86 (67; 124)	93 (72; 176)	87.5 (68; 129)	96.5 (72; 154)$^{\#}$	$^{\#}$Different form NOSSD, $p < 0.05$
Insulin (μU/mL)	10.8 (3.2, 147)	17.9 (5.3; 89.8)*	9 (1.4; 171)	16 (1.2; 43.9)$^{\#\#}$	*Different from NOC, $p < 0.05$
					$^{\#\#}$Different form NOSSD, $p < 0.01$
HbA1c (%)	5.4 (4.7; 6.2)	5.55 (4.7; 9.7)	5.35 (4.3; 6.1)	5.65 (4; 7.9)$^{\#}$	$^{\#}$Different form NOSSD, $p < 0.05$
HOMA-IR	2.2 (0.74, 45.01)	3.8 (1.11; 24.92)*	1.95 (0.42; 28.71)	4.01 (0.30; 11.06)$^{\#\#}$	*Different from NOC, $p < 0.05$
					$^{\#\#}$Different form NOSSD, $p < 0.01$
Uric acid (mg/dL)	4.2 (1.9; 7)	5.1 (2.7; 8.5)	4.4 (1.5; 7.2)	5.5 (2.2; 8.9)$^{\#\#}$	$^{\#\#}$Different form NOSSD, $p < 0.01$
T.Protein (g/dL)	7.29 ± 0.40	7.24 ± 0.39	7.20 ± 0.49	7.10 ± 0.63	N.S.
Albumin (g/dL)	4.3 ± 0.27	4.3 ± 0.23	4.3 ± 0.34	4.3 ± 0.35	N.S.
T.Chol (mg/dL)	185.5 (110; 254)	194.5 (125; 347)	180.5 (117; 271)	204.5 (87; 273)$^{\#}$	$^{\#}$Different form NOSSD, $p < 0.05$
HDL-Chol (mg/dL)	44.2 ± 11.3	40.1 ± 8.42	44.0 ± 9.9	44.0 ± 11.9	N.S.
LDL-Chol (mg/dL)	116.5 (53; 185)	121 (34; 230)	111 (55; 166)	124 (20; 187)	N.S.
TG (mg/dL)	89.5 (34; 474)	128.5 (59; 622)	93 (35; 488)	142 (26; 489)$^{\#\#}$	$^{\#\#}$Different form NOSSD, $p < 0.01$
TSH (μIU/mL)	1.6 ± 1.0	1.8 ± 1.1	1.6 ± 1.0	1.8 ± 1.0	N.S.
fT4 (ng/dL)	1.1 ± 0.2	1.3 ± 0.4	1.2 ± 0.2	1.3 ± 1.2	N.S.

Note: Data are presented as mean ± SD when normally distributed and as median (min; max) when non-normally distributed.

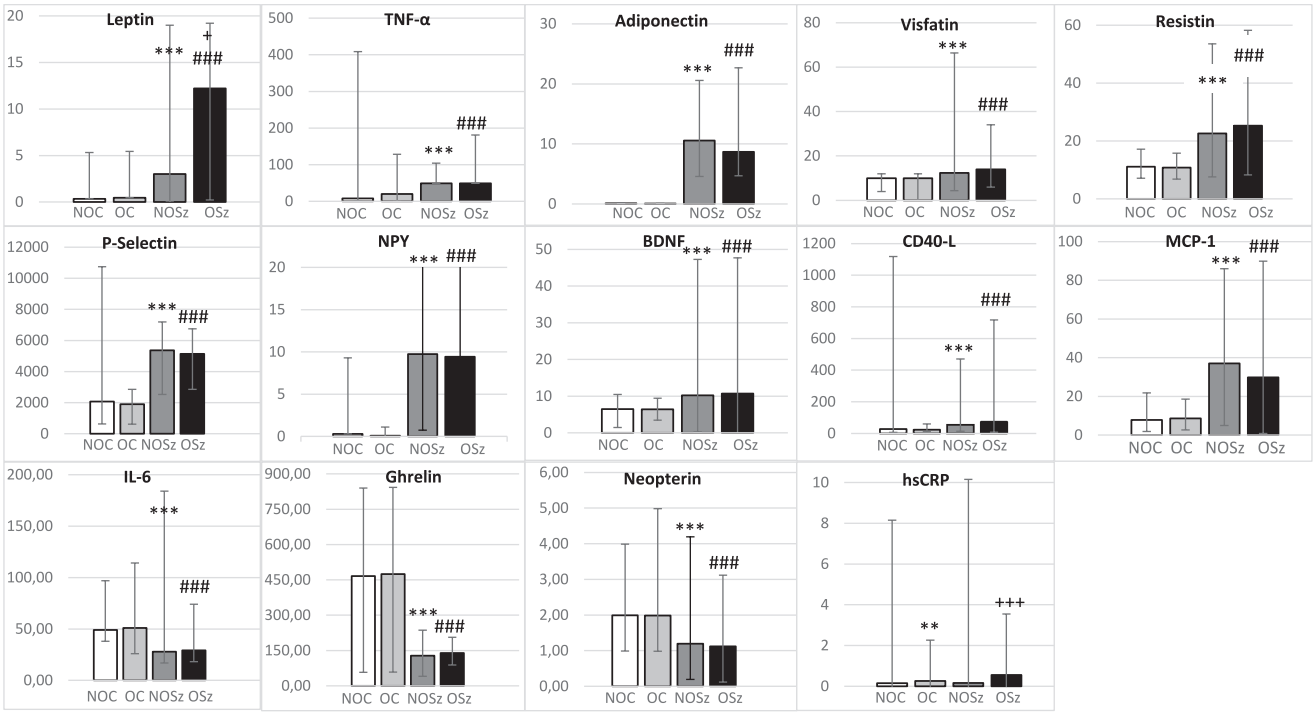

FIGURE 1 | Leptin (ng/mL); TNF-α (pg/mL); adiponectin (ng/mL); visfatin (ng/mL); resistin (ng/mL); P-selectin (pg/mL); NPY(ng/mL); BDNF (ng/mL); CD40-L (ng/mL); MCP-1 (ng/mL); IL-6 (pg/mL); ghrelin (ng/mL); neopterin (ng/mL); hsCRP (mg/dL) levels of non-obese controls (NOC), obese controls (OC), non-obese schizophrenia (NOSz) and obese schizophrenia (OSz) patients (bars represent medians; error bars represent min–max values). **·***Different from NOC, $p < 0.01$ and $p < 0.001$, respectively; $^{\#\#\#}$different form OC, $p < 0.001$; $^{+,+++}$different form NOSz, $p < 0.05$ and $p < 0.001$, respectively.

4 | Discussion

The use of antipsychotics is one of the suggested causes of obesity in schizophrenia (Baptista and Beaulieu 2002), and obesity has been associated with the development of metabolic disturbances and increased oxidative stress in individuals with Sz (Ferreira, Grajales, and Valverde 2020; Gao et al. 2023). In this study, we questioned whether obesity has a specific

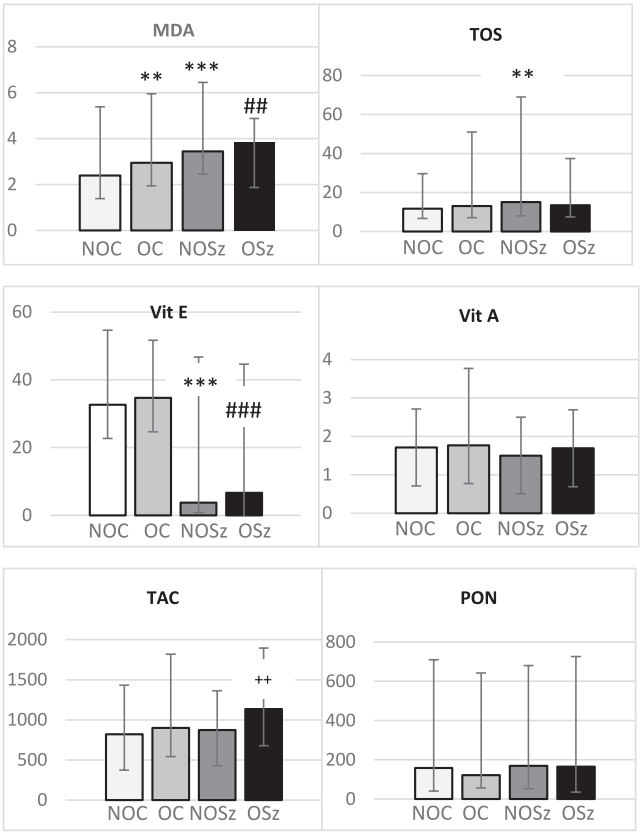

FIGURE 2 | Malondialdehyde (MDA) (nmol/mL); total oxidant status (TOS) (μM H2O2 equivalents); vitamin E (μmol/L); vitamin A (μmol/L); total antioxidant capacity (TAC) (μM copper reducing equivalents) and Paraoxonase (PON) (U/L) levels of non-obese controls (NOC), obese controls (OC), non-obese schizophrenia (NOSz) and obese schizophrenia (OSz) patients (bars represent medians; error bars represent min–max values). **:***Different from NOC, $p < 0.01$ and $p < 0.001$, respectively; $^{\#,\#\#,\#\#\#}$different form OC, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; $^{++}$different form NOSz, $p < 0.01$.

influence on metabolic and inflammatory disturbances in antipsychotic-treated patients. Also, we examined if obesity has any impact on increased oxidative stress in these patients. According to the findings of this study, inflammatory markers leptin, adiponectin, visfatin, resistin, TNF-α, P-selectin, NPY, BDNF, CD40-L, and MCP-1 levels significantly increase, and IL-6, ghrelin, and neopterin levels decrease in obese and non-obese patients compared to those of obese and non-obese controls, respectively. These peptides are cytokines or mediators that are valuable indicators of the inflammatory status of the body. Their alterations have been strongly associated with future metabolic or cardiovascular disturbances (Fonseka, Müller, and Kennedy 2016). The changes in these parameters, except that of leptin, are at the same level in obese and non-obese patients taking antipsychotics in the present study, suggesting the changes are independent of obesity (Figure 1). Unlike the other cytokines, leptin levels were significantly higher in obese schizophrenic subjects than those of the non-obese patients, and this was in accordance with the findings of Sirota et al. (2015). Lis et al., relying on the observations from different meta-analyses, pointed out that changes in body weight gain influence leptin levels, however direct effects of antipsychotics on adipose tissue should also be taken into consideration (Lis et al., 2020). In line with their suggestions, we found higher leptin concentrations in the obese patient group compared to the non-obese patients, other than higher leptin concentrations in obese and non-obese patients compared to healthy controls. While leptin levels have been associated with AP-treatment (Lis et al. 2020), some studies indicate that increments are related solely to weight gain, not directly to medical treatment (Juge-Aubry et al. 2003). Our results reveal that patients on antipsychotic treatment exhibit elevation in leptin levels, and this is more pronounced in patients with higher BMI. These results support the impact of obesity on leptin levels in schizophrenia.

Studies comparing the cytokine levels in obese and non-obese schizophrenic patients undergoing antipsychotic treatment are limited. Sirota et al. found that, in AP-treated Sz patients, TNF-α concentrations were higher in obese compared to non-obese subjects (Sirota et al. 2015). They stated that previous reports of higher TNF-α levels in obese but otherwise healthy subjects compared to non-obese healthy individuals confirmed their findings, indicating the impact of obesity but not Sz on TNF-α levels. In our study, TNF-α levels were significantly higher in both obese and non-obese patients compared to their respective controls and were statistically comparable. Accordingly, our results show that changes in this inflammatory marker are influenced in patients who are on AP-treatment, independently from obesity. In the same study, Sirota et al. found that adiponectin levels were lower in obese patients than in non-obese patients (Sirota et al. 2015). The unaltered adiponectin concentrations in obese and non-obese patients observed in our study contradict with Sirota et al.'s results. Although the results of the two groups in their study were very close, a statistical significance of $p = 0.025$ was established, contrary to the identical adiponectin levels in obese and non-obese patients in our study. The groups were divided as BMI < 24 and BMI > 27 in their study, whereas BMI was < 30 in non-obese and ≥ 30 in obese groups in the present study. The difference in cut-off value for defining obesity and the exclusion of the patients with BMI greater than 24 and less than 27 in their study might be the reason for the discrepancy between the results of the two studies. In a study designed with healthy male volunteers, adiponectin, leptin, and TNF-α levels were elevated, and ghrelin levels were decreased after AP, namely olanzapine treatment, and parallel with ours, these results were not correlated with adiposity (Ferreira, Grajales, and Valverde 2020). Likewise, leptin, ghrelin, AGRP, and visfatin levels were not correlated with BMI in the study of Basoglu et al., where they evaluated the effects of 6 weeks of olanzapine treatment in non-obese male first-episode psychotic patients (Basoglu et al. 2010). A similar result for IL-6 levels was reported by Sirota et al. (2015), showing identical IL-6 levels in obese and non-obese Sz subjects, supporting our suggestions that obesity does not have a significant impact on the alteration of IL-6 in Sz patients on AP-treatment. In the study of Beumer et al., the presence of metabolic syndrome and its components did not influence TNF-α and IL-6 levels of patients with Sz (Beumer et al. 2012). However, they resulted in higher leptin values parallel to our findings. On the other hand, unlike our results, the presence of metabolic syndrome caused statistically a more significant elevation in adiponectin levels in patients. They noted that these immune compounds were found to be associated with gender or

components of metabolic syndrome; however, the most outstanding association was the state of the disease itself. The differences in disease states of the patients in their study and our study may explain the dissimilarities between the results.

P-selectin, an adhesion molecule stored in platelets and endothelial cells that are activated by inflammatory stimuli, is associated with atherosclerosis, and high levels of P-selectin are also determined in patients (Bourassa et al. 2020; Mohite et al. 2017). Buarassa et al. found a positive correlation between P-selectin and TG levels in obese Sz patients but not in normal-weight patients (Bourassa et al. 2020). However, their work did not discuss the relation between P-selectin and body weight. On the other hand, parallel with our findings, Mohite et al. demonstrated that P-selectin levels were not associated with BMI in either patients and controls (Mohite et al. 2017). These results show that the elevation in P-selectin levels in antipsychotic treated patients is also separate from obesity.

The chemokine MCP-1 is important for its influence on the infiltration of monocytes/macrophages into adipose tissues, which could further increase the inflammatory properties of adipose tissues. In their report, Steiner et al. stated that serum MCP-1 levels were not associated with Sz but were positively influenced by BMI (Steiner et al. 2010). Their findings were contrary to our results; however, in a cross-sectional and meta-analytic study, Frydecka et al. suggested that MCP-1 acts as a trait marker in Sz since it is found to be elevated in both first-episode and multi-episode Sz patients (Frydecka et al. 2018). In line with their observation, the elevation in MCP-1 levels in AP-treated patients in the present study may represent a subclinical pro-inflammatory state in schizophrenia.

Protein S100-B, which has autocrine and paracrine actions on neurons, is considered the CRP of the brain (Morera-Fumero et al. 2023). Morera-Fumero et al., based on different findings from several studies reporting higher, lower, or unchanged S100-B concentrations in Sz, studied the evolution of the protein in the course of the disease (Morera-Fumero et al. 2023). Their findings pointed out that three months of AP treatment normalized S100-B levels, which were elevated at admission. They interpreted this normalization as being due to the reversal of the acute inflammatory state after treatment. In an earlier study we observed that S100-B concentrations were decreased after six weeks of AP-treatment (Sarandol et al. 2007). In line with these results, the unchanged S100-B levels in our patients who have been on antipsychotic treatment for at least six months can be attributed to the anti-inflammatory effects of AP drugs. Our results also confirm the findings of Steiner et al. (2010), indicating the inefficacy of obesity on the concentration of this protein.

Unlike the other inflammatory markers investigated in this study, hsCRP was significantly higher in obese individuals in both control ($p < 0.05$) and patient ($p < 0.001$) groups compared to their non-obese counterparts. Increased CRP concentrations were reported in patients compared to healthy controls, and these findings were related to immune dysregulation associated with the illness (Fernandes et al. 2016). Contrary to these data, hsCRP concentrations were not different between patients and healthy controls in our study when obese and non-obese subjects were evaluated together. However, our results are in accordance with that of Boozalis et al., stating significantly elevated hsCRP levels in obese compared to non-obese patients (Boozalis, Devaraj, and Okusaga 2019). CRP is considered a marker and predictor of CVD and metabolic syndrome. Our results emphasize the negative impact of weight gain on this matter, and the higher level of significance of the difference in the obese patients indicates that such patients are more liable to cardiovascular events and the progress of metabolic syndrome.

According to the results of this study, levels of the inflammatory cytokines studied changed in Sz patients on AP-treatment, regardless of obesity. There are previous reports in line with or opposing our results (Ferreira, Grajales, and Valverde 2020; Patlola, Donohoe, and McKernan 2023). Patlola et al. explained this discrepancy by the varying effects of antipsychotics on different cytokines (Patlola, Donohoe, and McKernan 2023). As indicated in their meta-analysis, antipsychotics cause different responses in peripheral cytokine concentrations (Patlola, Donohoe, and McKernan 2023). For instance, while risperidone treatment significantly decreases IL-6 and TNF-α levels, clozapine causes elevation in IL-6 and TNF-α levels. Also, Patola et al. underlined the importance of the treatment duration and the patient's state when evaluating the results (Patlola, Donohoe, and McKernan 2023). Parallel to Patlola et al.'s remarks, Ferreira et al. indicated that alterations in adiponectin expression and secretion might occur progressively (Ferreira, Grajales, and Valverde 2020). The duration of therapy, as well as the type of drug used, affects cytokine expression and secretion (Ferreira, Grajales, and Valverde 2020). Also, the direct effect of antipsychotics on adipose tissue should be taken into consideration. It is stated that second-generation antipsychotics modify the endocrine function of white adipocytes; this may be the reason for altered cytokine levels in antipsychotic-treated schizophrenic patients but not in controls in our study.

Anti-inflammatory effects of antipsychotics are postulated by several authors (Beumer et al. 2012). The immune suppressive effects of antipsychotics cause a decrease in pro-inflammatory cytokines and an increase in anti-inflammatory cytokines. Lower concentrations of pro-inflammatory cytokines IL-6, neopterin, and ghrelin observed in this study may be due to the anti-inflammatory force of these drugs. As proposed by Fonseka et al., state marker cytokines such as IL-6 and trait markers such as TNF-α exert different responses to AP treatment (Fonseka, Müller, and Kennedy 2016); this may explain the lower IL-6 versus higher TNF-α and MCP-1 levels observed in patients in our study.

Despite the conflicting data about the influence of antipsychotics on many cytokines, data on visfatin, NPY, neopterin, AGRP, and BDNF levels are relatively limited. Indeed, the influence of obesity on these cytokines has not been studied before. As far as we know, our results indicating that obesity does not have an impact on serum concentrations of these proteins in Sz patients on AP-treatment is the first report on this matter.

Fasting glucose, insulin, HbA1c, HOMA-IR, uric acid, total cholesterol, and triglyceride measurements are important parameters to monitor metabolic disturbances and CVD risk.

These parameters were significantly higher in obese patients than non-obese patients in the present study. A significance was not achieved for these parameters between obese and non-obese controls, except for insulin and HOMA-IR values. The increase in these parameters in obesity has been well documented before (Li et al. 2021). Our results show that obesity-associated carbohydrate and lipid metabolism disturbances are more pronounced in patients on AP-treatment. These findings underline the outstanding role of obesity in metabolic disturbances leading to related cardiovascular and metabolic diseases in schizophrenia. In the study of Koricanac et al. (2022), the influence of various antipsychotics on metabolic variables such as lipid status, glucose, and insulin concentrations was different: significantly lower HDL-cholesterol and higher glucose levels were detected in only the clozapine-treated patients compared to controls. In the present study, the alterations in the metabolic markers were not statistically different between the drugs used (data not shown). In addition, the results of Koricanac et al. were not evaluated in terms of BMI or body weight; therefore, a comparison with our study on this aspect is not possible.

Higher MDA levels observed in patient groups in the present study are in accordance with the previous reports stating increased oxidative stress in chronic illness (Ermakov et al. 2021; Gunes et al. 2017; Sarandol et al. 2007). According to our results, obesity did not further influence oxidative stress parameters studied in chronic patients on antipsychotic treatment. Although MDA levels were higher in obese controls than non-obese controls, there was no difference in obese and non-obese patients. An et al. have suggested that primarily, antipsychotics induce oxidative damage, and then this leads to the development of obesity in chronic schizophrenia (An et al. 2018). Higher MDA levels in patient groups, regardless of obesity, may be explained by the oxidative influence of AP treatment. Another factor influencing oxidative stress may be lower vitamin E concentrations in patient groups. Vitamin E, one of the most efficient exogenous antioxidants, was significantly lower in patients regardless of obesity. Lower vitamin E levels may be because of increased consumption due to higher oxidative stress or inadequate intake due to the poor dietary habits that are very common in schizophrenia (Dadheech et al. 2006; McCreadie 2003). Our results indicate that obesity does not further influence serum vitamin E concentrations in the study groups. TOS is elevated in non-obese patients but not in obese patients. The significantly higher TAC in the obese patients may be effective in lower TOS in this group. Also, higher TAC in obese patients can be explained by significantly higher uric acid concentrations in this group. Elevated uric acid levels are widespread in obese individuals, and this is more pronounced in schizophrenia (Godin et al. 2015; Li et al. 2021).

Our results indicate that although obesity does not have an impact, smoking aggravates oxidative stress. While MDA levels in non-obese patients were higher than those of non-obese controls (Figure 2), MDA levels in non-smoking-non-obese patients were not different from those of non-smoking-non-obese controls, further divided as smoking and non-smoking (Table S1). Similarly, MDA levels in non-smoking obese patients were comparable with those of non-smoking obese controls. However, smoking-non-obese patients had higher MDA levels compared to smoking-non-obese controls, and smoking obese patients had higher MDA levels compared to smoking obese controls (Table S1).

The present work was designed to observe the impact of obesity on metabolic, oxidative stress, and inflammatory markers in patients on antipsychotic treatment. A limitation of this study is that the number of patients on different treatments is not large enough to make a strong statistical evaluation of the influence of different drugs on the changes observed. Future studies with larger number of patient groups on different treatments would provide data to better compare the impact of different antipsychotics on the changes observed.

5 | Conclusions

Our results suggest that while antipsychotic-treated schizophrenia patients develop inflammatory and oxidative responses independently from obesity, metabolic disturbances arise from obesity during treatment. However, the significant negative impact of weight gain on CRP levels indicates that obese patients are more liable to cardiovascular events and progress of metabolic syndrome than non-obese patients. As far as we know, this study is original in providing data on the changes of several cytokines and oxidative stress markers in obese and non-obese patients on antipsychotic treatment compared to BMI-matched controls. Since disease state, type of medication, and duration of treatment may influence the alterations in inflammatory markers, future studies with homogeneous patient and treatment groups would provide better insight into this context.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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