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INSINUATIONS OF LIPID PEROXIDATION AND INFLAMMATORY CYTOKINES-INTERLEUKINS AND MATRIX METALLOPROTEINASES IN THE DEVELOPMENT OF OBSESSIVE-COMPULSIVE DISORDER

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ABSTRACT

Background: Some psychiatric diseases have been linked to the pathophysiology of free radicals. We sought to determine whether levels of interleukin (IL)-6, antioxidant vitamins E, C, and A, and matrix metalloproteinases (MMP)-4 and 9 were linked to OCD, in order to investigate the role of free radicals in the etiopathogenesis of OCD.

Methodology: In this study, 48 OCD patients with DSM-IV diagnoses and 48 healthy volunteers served as the control group. While vitamin C was assessed using the phenyl-hydrazine spectrophotometric technique, the serum levels of vitamins E and A were measured using RP-HPLC. ELISA technique was used to assess MMP-2 and MMP-9 and IL-6 levels. AGEs and AOPPs were measured using the UV-vis spectrophotometric method.

Results: In comparison to the control group, Vitamin E levels (p=0.02), vitamin C levels (p=0.15), and vitamin A levels (p=0.25) in both males and females were all significantly lower in the patients than in the control group. In later stages of OCD, antioxidants may reverse because of compensating mechanisms in brain cells. IL-6 levels were also measured, and they were shown to be considerably higher in OCD patients than in control groups (p=0.00). Subjects had significantly higher concentrations of protein oxidation products [AGEs (p=0.00) and AOPPs (p=0.00)] than the control group. Additionally, the levels of MMP-2 (p=0.02) and MMP-9 (p=0.16) were also significantly raised in both male and female patients of OCD.

Conclusion: This indicates that elevated MMP-2 and 9 levels are mediated by elevated IL-6 levels brought on by excessive lipid peroxidation, which encourages the development of OCD. According to a subsequent study, lipid peroxidation, matrix metalloproteinases, interleukins, and increased oxidative and inflammatory status are the main factors for obsessive-compulsive disorder to progress.

Keywords: Inflammation, Cytokines, Peroxidation, Neuropsychiatric disorder, recurrent compulsions

1.0 INTRODUCTION

Numerous neuropsychiatric illnesses, including obsessive-compulsive disorder (OCD), have been linked to the pathophysiology of oxidative stress and toxicity caused by free radicals (Bilici et al., 2001; Ersan et al., 2006; Mahadik et al., 1996). The human body produces free radicals as a result of lipid peroxidation, phagocyte activation, the electron transport system in mitochondria, normal cellular metabolism, and exposure to pollutants, cigarette smoke, and ultraviolet (UV) light (Gutteridge, 1995). Tissue damage could arise from an imbalance between the generation of free radicals and antioxidant defences (Cheeseman and Slater, 1993). Determining the levels of free radicals is challenging because of their incredibly high reactivity and comparatively short half-life. However, the presence of antioxidant molecules may prevent or lessen damage caused by free radicals (Butterfield et al., 2002). Thus, the measurement of certain antioxidant vitamins (Vitamin E, C, and A), lipid peroxidation byproducts, and cytokines like matrix metalloproteinases (MMPs) and interleukins (ILs) can be used to indirectly assess the activity of free radicals in the human body (Leff, 1994; Tezcan et al., 2003). Neurological antioxidant and neuroprotective function depend

on the nonenzymatic antioxidant structures of vitamins E, C, and A (Bates, 1995; Dennert and Lotan, 1978; Diliberto and Allen, 1981; Stephensen, 2001). Numerous studies have demonstrated the importance of vitamins E, C, and A for the central nervous system and the structural and functional harm that results from a drop in their concentrations (Chow, 1991; Harrison and May, 1991). Recurrent obsessions and/or compulsions are hallmarks of obsessive-compulsive disorder (OCD), a neuropsychiatric condition that is chronically debilitating and has a 2%–3% population prevalence (American Psychiatric Association, 2000; Karno et al., 1988). While it is yet unclear how biochemical changes contribute to the pathophysiology of neuropsychiatric diseases, obsessive-compulsive disorder (OCD) may be significantly impacted by variations in antioxidant vitamin levels.

For example, alteration in serum level of vitamin A (retinol), vitamin E (tocopherol), and vitamin C (ascorbic acid) has been reported in various neuropsychiatric disorders (Maes et al., 2000; Miljevic et al., 1997; Rinaldi et al., 2003). Interleukin-6 (IL-6) is associated with Obsessive-Compulsive Disorder (OCD), with studies showing elevated levels in many patients compared to healthy controls, and this increase is sometimes positively correlated with the severity of the disorder. This suggests a potential role for pro-inflammatory immune responses in the pathophysiology of OCD, although results can be inconsistent across different studies due to factors like medication use and comorbidities (Smith et al., 2000). The association between MMP-9 and 2 and cognitive domains in OCD has not been studied to date; hence the purpose of this study was to assess and test the hypothesis that the severity of symptoms is correlated with antioxidants and serum MMP-2 and 9 and IL-6 levels.

2.0 MATERIALS AND METHODS

2.1 Selection of Patients

This study was conducted by the Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan in collaboration with the The Department of Psychiatry and Behavioral Sciences at UCMD (University College of Medicine and Dentistry, The University of Lahore. The study was approved by the ethical committee of the IMBB, UOL. 55 OCD patients (35 men and 20 women) with diagnoses based on the Diagnostic and Statistical Manual of Psychiatric Disorders, DSM-IV, made up the study group. A expert psychiatrist with DSM-IV training selected these patients at random from UCMD. The 50-person control group was made up of healthy volunteers who were matched by sex, age, and socioeconomic level (30 men and 20 women). Each study participant provided written consent after being informed of the study's objectives. Every participant underwent a neurological and physical evaluation to determine whether any more illnesses existed.

2.2 Inclusion Criteria

These participants had not been treated with any antioxidant agents (such as vitamins E, C, and A), which can affect the concentration of antioxidant vitamins, nor did they have liver or renal failure or other illnesses. The study also did not include patients with co-occurring psychiatric illnesses and mental retardation. gender, age, marital status, educational attainment, socioeconomic level, length of sickness, and severity, as well as experience and information sources. Without concentrating on the content of obsession and compulsion, the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) was utilised to evaluate the severity of OCD (Goodman et al., 1989). Each of the ten items is evaluated by a clinician on a scale of 1 to 4.

2.3 Exclusion Criteria

Exclusion criteria included the following: excessive obesity, the existence of infectious diseases, the presence of epilepsy and severe neurologic disorders, tardive dyskinesia associated with neuroleptics, alcohol and substance misuse or dependency, and the presence of severe organic conditions. A semi-structured questionnaire form organized in compliance with clinical guidelines was used to assess each participant.

2.4 Sample Collection

Each OCD patient and control subject had a total of 10 millilitres of venous blood extracted using a plastic syringe that was fitted with a stainless steel needle. After being drawn into a sterile tube, the blood was centrifuged for 15 minutes at 3000 rpm after being left to clot for 30 minutes at room temperature. Prior to analysis, serum samples were kept at 80°C and shielded from light.

2.6 Determination of Matrix metalloproteinases (MMP-2, 9) and Interleukin (IL-6)

Using singleplex or multiplex electrochemiluminescent-based immunoassays, IL-6 and MMP-2 and 9 were measured through ELISA technique (MesoScale Discovery, Rockville, MD) and OD was measeured at 450nm.

2.7 Determination of Vitamin A, D and E

The phenyl-hydrazine spectrophotometry method was used to assess the serum's vitamin C content (Lowry et al., 1945). The measurement process involved adding 0.3 ml of plasma to 1.2 ml of 5% trichloroacetic acid, followed by a 10-minute centrifugation at 3000 rpm. 0.96 ml of clear supernatant was boiled in a water bath for one hour at 60°C after being treated with 0.4 ml of dinitrophenylhydrazine-thiourea-copper sulphate (DTC) reagent. The sample was immediately refrigerated in ice-cold water following incubation, then 1.6 ml of a solution containing 65% sulphuric acid was gradually added. Both a reagent blank and 0.3 ml of the working standard solution of ascorbic acid were used to repeat the process. At 520 nm, the absorbance of the sample and standard was measured in a spectrophotometer (UV-1201, UVVIS Spectrophotometer, Shimadzu Corporation, Japan) against a reagent blank.

2.8 Measurement of Advanced glycation End products (AGEs) and Advanced Oxidation Protein Products (AOPPS)

Advanced glycation End products (AGEs) and Advanced Oxidation Protein Products (AOPPS) were measured spectrophotometrically and OD was measured at 440nm.

3.0 STATISTICAL ANALYSIS

Data analysis was done using SPSS software (Version 11.0). Every variable was subjected to descriptive statistics. Standard deviation ± mean was used to express the values.

Using an independent sample t-test, the mean values for the main variables in the patient and control groups were compared. Significant p-values were defined as less than 0.05.

4.0 RESULTS

Analysis Of Antioxidants And Lipid Peroxidation Biomarkers In Serum Of Obsessive Compulsive Disorder Patients

Vitamin E levels (p=0.02), vitamin C levels (p=0.15), and vitamin A levels (p=0.25) were all significantly lower in the patients than in the control group, regardless of gender. Antioxidants may reverse in later stages of OCD due to brain cell compensatory mechanisms. The OCD group's vitamin A, C, and E levels were somewhat lower than those of the control group, but the difference was not statistically significant (p>0.05). AOPPS, AGEs levels were measured in the control and OCD groups to assess the degree of lipid peroxidation. AOPPS levels were 2.12±0.02 IU/mol in males and 1.92±0.03 IU/mol in the females of the OCD group and 0.52±0.07 IU/mol in the control group. Table 2 shows that the OCD group's AGEs were substantially greater than those of the controls (p<0.01). Studies indicate a connection between obsessive-compulsive disorder (OCD) and high levels of matrix metalloproteinases (MMPs), specifically MMP-2 and 9. OCD patients both males and females show high levels of MMPs 2 and 9 as cpmared to controls (Table 3). The blood-brain barrier (BBB) disruption and neuroinflammation linked to these enzymes can impact the cortico-striato-thalamo-cortical (CSTC) circuits, which are crucial for obsessive behaviors. OCD patients have been shown to have elevated levels of the inflammatory cytokine IL-6, which may indicate a connection between inflammation and the pathophysiology of the condition. IL-6 in our study, also depicted crucial role in OCD patients showing significant elevation in serum levels as compared to controls (p=0.00) (Table 3). Higher IL-6 levels have been linked in some studies to longer disease duration and more severe OCD, while other research indicate IL-6 may be more of an inflammatory marker that isn't always correlated with symptom intensity. Although further research is needed to completelyunderstand the impact of IL-6, results suggest that OCD is influenced by the immune system.

Table 1: Response of antioxidative Vitamins in Obsessive Compulsive Disorder

Parameters	**Control (IU/mol)**		**OCD Patients (IU/mol)**		**p-value**
	Male	Female	Male	Female	
Vitamin E	27.96±1.19	32.02±0.12	20.82±1.26	21.92±0.18	0.02
Vitamin C	95.37±0.019	85.06±0.05	52.8±0.02	66.28±0.02	0.15
Vitamin A	1.57±0.15	1.62±0.12	0.51±0.07	0.45±0.01	0.25

Table 2: Response of Lipid peroxidation products in Obsessive Compulsive Disorder

Parameters	**Control (IU/mol)**		**OCD Patients (IU/mol)**		**p-value**
	Male	Female	Male	Female	
AOPPS	0.52±0.07	0.47±0.01	2.12±0.02	1.92±0.03	0.00
AGEs	0.54±0.05	0.41±0.01	4.74±0.16	2.95±0.12	0.00

Table 3: Response of Inflammatory Cytokines in Obsessive Compulsive Disorder

Parameters	**Control**		**OCD Patients**		**p-value**
	Male	Female	Male	Female	
MMP-2 (mg/dl)	0.46±0.02	0.86±0.05	0.81±0.03	1.63±1.02	0.28

MMP-9 (mg/dl)	80.75±3.03	92.32±2.12	55.61±4.09	68.32±2.12	0.16
IL-6 (pg.ml)	38.5±0.14	39.2±0.03	66.2±0.23	69.05±0.15	0.00

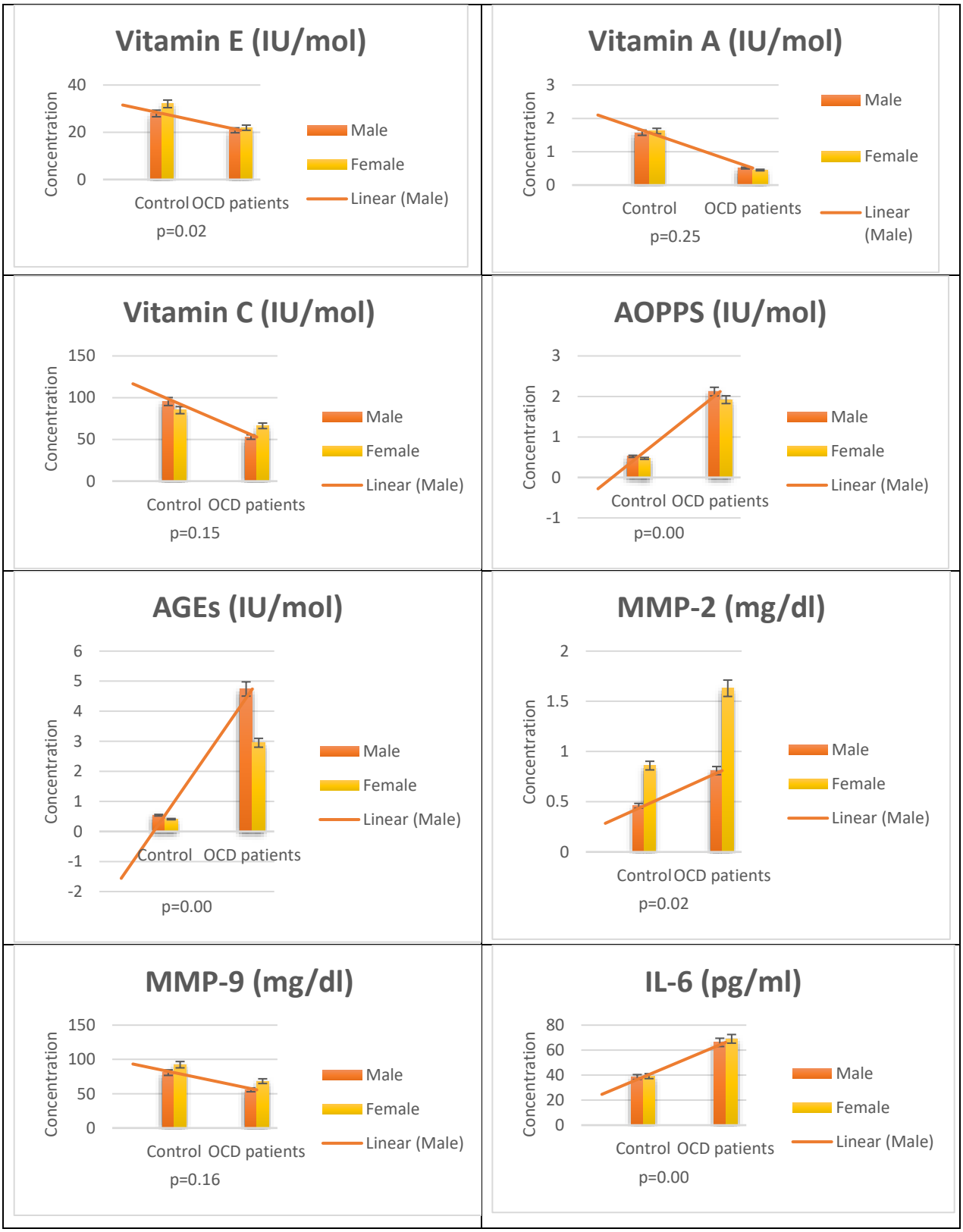

Figure 1: Response of antioxidants and inflammatory cytokines in obsessive-compulsive disorder

5.0 DISCUSSION

Neurological processes depend on vitamins E and C. The antioxidant capacity of tissues is enhanced by vitamin A (Kartha and Krishnamurthy, 1977; Ricciarelli et al., 2007). This fact, along with an increasing amount of evidence, suggests that changes in vitamin antioxidant levels are linked to oxidative stress, which causes a number of neuropsychiatric illnesses. The pathophysiology of OCD may be directly linked to changes in serum antioxidant levels, according to mounting data in recent years (Ersan et al., 2006; Kuloglu et al., 2002; Maes et al., 2000; Selek et al., 2008). According to the current study, OCD patients had significantly lower vitamin E and C levels than controls ($p<0.05$). Additionally, Ersan et al. discovered that OCD patients had noticeably reduced vitamin E levels (Ersan et al., 2006). Increased free radical burden is linked to oxidative stress, and antioxidant vitamins E, C, and A, glutathione (GSH), specific trace elements, and metalloenzymes like iron-containing catalase, selenium-containing glutathione peroxidase, and superoxide dismutase (SOD) play a protective role in shielding the body from damage caused by free radicals (Machlin and Bendich, 1987). Numerous studies have discovered a strong correlation between OCD and oxidative stress, indicating that the antioxidant defence system and free radicals are involved (Behl et al., 2010; Chakraborty et al., 2009; Özdemir et al., 2009; Selek et al., 2008). Antioxidant defence against oxidative free radical attack is provided by vitamins A, E, and C (Bates, 1995). Vitamin A levels in OCD patients have not been investigated in any prior research. OCD patients in our study had lower vitamin A levels than controls, although the difference was not statistically significant. According to this study, OCD patients had considerably greater serum levels of MMP-2, 9, and IL-6 than controls ($p<0.05$). These findings point to increased metabolism of free radicals in OCD, which suggests some degree of oxidative stress-induced tissue damage (Figure 2). These results aligned with previous research that found greater MDA levels in OCD patients (Behl et al., 2010; Ersan et al., 2006; Kuloglu et al., 2002; Selek et al., 2008).

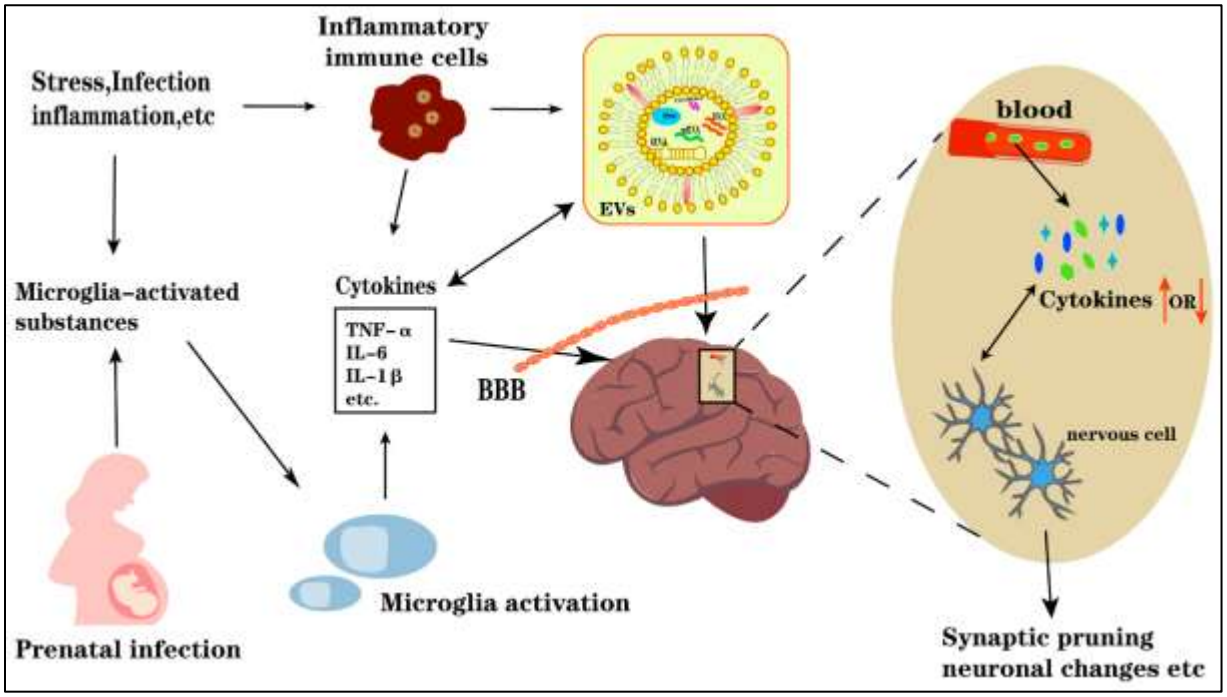

Figure 2: Mechanistic approach of role of cytokines in the etiopathogenesis of OCD

6.0 CONCLUSION

According to the study, people with OCD have a pro-inflammatory cytokine profile, which suggests that their brains are more prone to inflammation. We discovered a general vitamin imbalance in antioxidants, which could play a significant part in the etiopathogenesis of the illness. Studies have shown that certain cytokines, including as MMP-2, 9 and IL-6 have changed levels, which may have a pro-inflammatory effect on OCD patients. Although the results of various studies vary, these alterations might be related to the intensity of the symptoms.

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DISCLOSURE OF CONSENT: The authors declare no conflict of interest

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