IJBPAS, April, 2019, 8(4): 815-831

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DIFFERENTIAL EXPRESSION AND SPECIFICITY OF MMP-9, MDA AND 8-OHdG IN NEWLY DIAGNOSED SCHIZOPHRENICS

HAFEEZ Z[5], SHAFAQ M[2], ASLAM N[4], WAQUAR S[1], HUSSAIN F[1], ALI Q[1] AND MALIK A[1*]

1-Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore-Pakistan

2-Bakhtawar Amin Medical and Dental College, Multan-Pakistan

4-Independent Medical College, Faisalabad-Pakistan

***Corresponding Author: Arif Malik (Ph.D): E Mail: arifuaf@yahoo.com; Cell: 0321-8448196; Tel: +92 42-7515460-7, Fax: +92-42-7515519**

Received 25th Nov. 2018; Revised 16th Dec. 2018; Accepted 29th Dec. 2018; Available online 1st April 2019

https://doi.org/10.31032/IJBPAS/2019/8.4.4696

ABSTRACT

INTRODUCTION: Schizophrenia is the sever type of mental disorder that occur due to various type of factors in the late teens or early adulthood. Mostly it is present in the age of 15-35 years. Worldwide it affects approximately 24 million peoples that lead to cause disability. Oxidative biomarkers have the significant role in schizophrenic patients because brain is very sensitive organ in the body as compare to other. Schizophrenia is a mental disorder which involves impairment in perception, abnormal mental function, cognition and abolition resulting in triad of positive, negative and cognitive symptoms results in disturbed behavior. Approximately 1% peoples were suffering from mental disorder though the involvement of various types of factors including social, pharmacological, cognitive, environmental and some biological factors. According to the cardinal symptoms, schizophrenia is categorized into two major types like positive and negative symptoms, deterioration in social functioning, cognitive dysfunction and

occupational functioning. It is a neuro-generative disease either behavioral or structural abnormalities.

MATERIAL AND METHOD: Patients clinically analyzed with schizophrenia were included in present study to evaluate the role of oxidative biomarkers in schizophrenic patients. . In present study, Fifty (50) diagnostic schizophrenic patients were taken as subject in the age limit between 20-50 years and Twenty (20) clinically healthy peoples were included as controls. In present study various types of inflammatory (MMP-9, IL-6) and oxidative biomarkers (8-OHdG, 4-HNE, SOD, GSH and CAT) were determined through commercially available ELIZA kits but the levels of MDA were estimated through spectrophotometery method.

RESULTS: Present study revealed a significant elevation of MDA (3.99±0.013), 8-OHdG (1.29±0.056), isoprostanes (102.26±6.23) and 4-HNE (6.26±1.22) in subjects as compared to control group (0.91±0.0019), (0.13±0.06), (29.65±3.88) and (1.29±0.77) respectively, According to the results of present study levels of inflammatory biomarkers (IL-6 and TNF-α) were significantly increased like IL-6 (6.35±0.55), TNF-α (25.26±1.11) and MMP-9 (103.29±6.35) in subjects as compared to control group (3.88±0.76), (10.16±1.33) and (33.26±2.16) respectively but the levels of antioxidants SOD (1.09±0.33), GSH (6.35±1.55) and CAT (1.23±0.021) were significantly low in subjects as compared to normal individuals (3.08±0.044), (9.66±2.18), (2.88±0.021) respectively. Additionally, levels of vitamin D (16.35±3.08) disturbed in subjects as compare to controls (30.18±8.29).

CONCLUSION: It is concluded that increased oxidative stress in patients with schizophrenia may depends on the inflammation response. These results indicate that, increased oxidative stress in schizophrenic patients is main determinant and increased by oxidative biomarkers (MDA, 8-OHdG, isoprostanes and 4-HNE), nitric oxide and they are not degraded or detoxified by enzymatic antioxidants SOD and CAT and non-enzymatic GSH, Vitamins D. Increased oxidative stress (RNS, ROS) are the major mediators of the neuronal damage and inflammation in the cells of central nerves system. Conclusively, schizophrenia might occur through the up-regulation of oxidative free radicals and down-regulation of antioxidant scavenger activity.

Keywords: Oxidative stress biomarkers, MDA, 8-OHdG, MMP-9, schizophrenia, Antioxidants, Vitamin D

INTRODUCTION

Schizophrenia is a neuropsychiatric ailment manifested as disruptive thinking, effected language, perception and self-sense. It characterized by disturbances in behavior, thinking and feeling and grosses distortion from reality [1]. Approximately 21 million peoples affected in the US of psychiatric disorders in which 1.1% adults suffer from schizophrenia. The identified causative agents for schizophrenia are stress, malnutrition, genetics, drugs and alcohol abuse. The symptoms of schizophrenia are categorized into three groups i.e. general (depression and hostility), positive (delusions and hallucinations) and negative (Anhedonia and Avoilation) [2]. Schizophrenia is a mental disorder, which involves impairment in perception, abnormal mental function, cognition and avolition resulting in triad of positive, negative and cognitive symptoms results in disturbed behavior. Schizophrenia is a very rare disease in world according to the world ranking report approximately 1% peoples were suffer from mental disorder though the involvement of various types of factors including social, pharmacological, cognitive, environmental and some biological factors [3]. According to the cardinal symptoms, schizophrenia categorized into two major types like positive and negative symptoms, deterioration in social functioning, cognitive dysfunction and occupational functioning. It is a neurogenrative disease either behavioral or structural abnormalities [4, 5]. It is well established schizophrenia occur through various abnormality like malfunctioning of some desire genes and non-genetic factors including, alcohol consumption, medications, neonatal infections, ethnicity, drug intake, maternal malnutrition, pre-natal infection during the birth as well as various other types of factors are also involved in schizophrenia. These morphological changes in dendritic spines are more obvious in prefrontal cortical are mediated by Matrix Metalloproteinase 9. So there is prefrontal cortical impairment in schizophrenia [6]. Matrix Metalloproteinase degrade extracellular matrix and is expressed by neurons in brain and also by glial cells. It is released in response to enhanced neuronal activity. Matrix Metalloproteinase plays a role in the conversion of Pro Brain Derived Neurotropic factor into its mature form which contributes in the pathophysiology of schizophrenia. As shown in some prior studies the cellular metabolic stress also involved to increase the development of schizophrenia by increasing oxidative stress and causing molecular damage because

oxidative stress is the common psychiatric disorders [7]. ROS are free radicals e.g super oxide radical ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$) and hydreperoxyl radical ($HO_2^{\cdot}$) and molecules e.g (H_2O_2). Excess of molecular oxygen or its chemical derivatives which are produced in Haber Weiss reaction in which Ferric reacts with Superoxide radical and produce Ferrous and Hydreperoxyl radical which cause lipid peroxidation. Ferrous is recycled in Fenton reaction reacts with hydrogen per oxide and produce hydroxyl radicals. These two types of radicals were produced in cells under stressful condition and cause lipid peroxidation [8]. Oxidative stress is the major cause for the development of different type of disease because the level of oxidative free radicals were increased in biological system as compare to the activity of antioxidant defense scavenger system because the antioxidant have great potential to compete with oxidative free radicals to prevent oxidative damage as well as different types of antioxidants also act as antipsychotic treatment [9]. Central nervous system (CNS) is more sensitive to the high reactive toxic free radicals than the other body organ because the rate of metabolic activity was high in brain cells so it also need the high rate of oxygen for metabolism, so the production of oxidative free radicals also higher in brain as compare to other body organ because oxygen also act as substrate for the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) though the activation of iNOS [10]. The levels of antioxidant enzymes in the cells of CNS also low, neural functional network susceptible to interruption, but high ratio of membrane surface area to cytoplasmic volume. Due to the high surface area the rate of oxidative damage also high results in increased oxidation of membrane polyunsaturated fatty acids (PUFAs) which is the major indicator of oxidative damage which can be measured in biologicals system through the determination of malondialdehyde (MDA), that is the universal oxidative biomarkers. MDA have the directly proportional do the severity of oxidative damage through the production of free radicals in biological system [11, 12]. MMPs are endoproteases which cleave protein components and extracellular matrix [13]. So the signaling molecules are activated or inactivated. 4HNE induces MMP-9 production in Macrophages and production of MMP-2 in vascular smooth muscle cells [9]. As well as vitamin D also have the significant indirect antipsychotic potential. Vitamin D3 (cholecalciferol) and vitamin D2 (Ergocalciferol) are the major type of vitamin

D that can be taken from either endogenous or exogenous source respectively through cascade of reaction [14]. Both of these types are taken up from diet as a exogenous source but can be synthesized in the skin as endogenous source after the exposure of ultraviolet B (UVB) radiation from sunlight because sunlight have direct proportion with the production of vitamin D in normal biological system. Various Other factors also influenced on the activation of vitamin D. Transfer of Vitamin D3 and D2 are transfer from one region to another occurs through binding protein called vitamin D binding protein (VDBP). After binding, it moves towards liver for hydroxylation, process through specific enzyme called 25 hydroxylase and converted to vitamin D (25-OH). Commonly it is used to determine the status of vitamin D in biological system because it is storage form of vitamin D [15]. McGrath stipulated that low prenatal vitamin D (especially during the third trimester) may be a risk factor for the development of schizophrenia in offspring. Similarly, a lack of vitamin D supplementation during the first year of life in Finnish boys correlated with an increased risk of developing schizophrenia because it has the ability for the regeneration of neuron and multifactorial effect in biological system [16].

AIM AND OBJECTIVE

- The aims and objective of present study was to evaluate the role of oxidative biomarkers like MDA, 4-OHdG, 4-HNE and inflammatory biomarkers including MMP-9, IL-6 in schizophrenia subject.
- As well as also determine the role of antioxidants like SOD, CAT and GSH in schizophrenia subject and also determine how antioxidants reduce the activity of free radicals damaging process.

MATERIAL AND METHOD

SOURCE OF DATA

The present work was conducted in university of Lahore to determine the key role of Matrix metalloproteinase-9 (MMP-9), inflammatory cytokines like IL-6, oxidative biomarkers (MDA, 8-OHdG, 4-HNE) and antioxidants (SOD, GSH, CAT) in diagnosed schizophrenic individuals. All these selected patients diagnosed at fountain house Lahore. In present study, fifty (50) diagnostic schizophrenic patients were taken as subject in the age limit between 20-50 years and Twenty (20) clinically healthy peoples were taken as controls. All of these experimental protocols were officially approved from the Research, Ethical Committee of The Institute

of molecular biology and biotechnology (IMBB), The University of Lahore.

INCLUSION CRITERIA

Patients clinically analyzed with schizophrenia were included in present study to evaluate the role of oxidative biomarkers in schizophrenic patients.

EXCLUSION CRITERIA

The individuals those were clinically analyzed other diseases like cancer, diabetes, hepatitis etc or taking drugs of respective diseases were excluded from this experiment.

CHEMICALS

All chemical reagents used for analytical grades were bought from sigma/Invitrogen Chemical Co. (St. Louis, Mo, USA).

BLOOD SEPARATION

Serum was separated after centrifugation for 10 minutes at 3000rpm and stored at -80^{o}C.

EVALUATION OF GLUTATHIONE (GSH)

Blood GSH was evaluated through Moron *et al*., 1979 method by using Ellman's reagent (5, 5-dithio bis (Nitrobenzoic acid) or DTNB) to produce a chromophore TNB with maximal absorbance at 412nm and oxidized glutathione GSSG.

EVALUATION OF CATALASE (CAT)

Catalase was also evaluated through the method of Aebi, et al. and this method was done through spectrophotometry method [19].

EVALUATION OF MALONDIALDEHYDE (MDA)

The estimation of Lipid peroxidation in blood samples was done through calorimetrically method by measuring Thiobarbituric acid reactive substances (TBARS) with the help of spectrophotometer by the method of Ohkawa *et al*., [18]. Because the TBARS is directly proportional to MDA that is the universal oxidative biomarkers.

ESTIMATION OF SUPER OXIDE DISMUTASE (SOD)

The determination of superoxide dismutase (SOD) activity was also determined through Kakkar, 1972 method [17].

DETERMINATION OF NITRIC OXIDE

It is well established that the Griess assay was applied to determine the nitrite (NO) concentration according to colorimetric principle [20].

ESTIMATION OF MMP-9 ELISA

Estimation of matrix metallo-proteinases (MMPs) is impotent to evaluate its role in schizophrenic patients because it is well established that the specific type of

MMPS have significant role in various disorders and is directly proportional with the severity of disease. The MMPs were estimated through commercially available ELIZA kits.

ESTIMATION OF IL-6 ELISA

As shown above inflammatory biomarkers especially IL-6 was determined through human ELISA kit (Bio Vendor).

ESTIMATION OF VITAMIN D

Vitamin D was also estimated by using human ELISA kit (Bio Vendor).

ESTIMATION OF OXIDATIVE BIOMARKERS

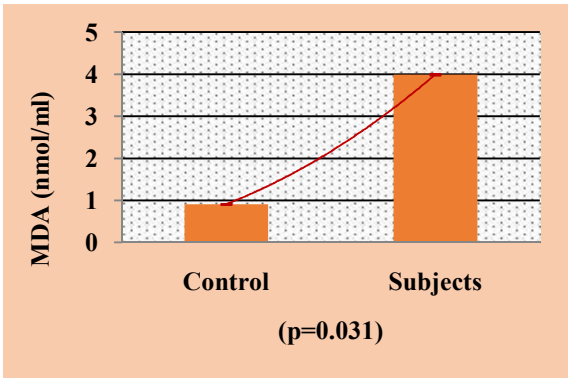
The estimation of oxidative biomarkers including 8-OHdG and 4-HNE were determined through commercially available ELIZA kits method.

RESULTS

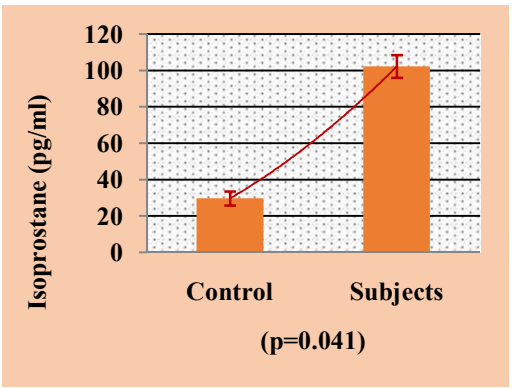
As shown in results the weight has no relation with disease because schizophrenia is the neuronal disorder that take place due to different physical and chemical factor but according to the demographical data age might have significant effect on the health of patient and the values of RBCs and WBCs was not differed between control and subjects. Similarly BMI might have positive correlation with disease, and was increased in subjects as shown in table 01. In consistence with many other observations present study revealed a significant elevation (P=0.031) of MDA (3.99±0.013) in subjects as compared to control group (0.91±0.0019). Malondialdehyde (MDA) is a universal oxidative biomarker that bind with DNA and form DNA adduct that is another oxidative biomarker in subjects so according to the results of present study the levels of 8-OHdG (1.29±0.056) was high in schizophrenic patients as compared to normal person (0.13±0.06) and the P values were highly significant (P=0.041). Like 8-OHdG the levels of isoprostanes (102.26±6.23) and 4-HNE (6.26±1.22) also increased in subjects as parallel to normal healthy group (29.65±3.88), (1.29±0.77) respectively, and the P values also more significant (P=0.041), (P=0.030) respectively.

All these parameters were directly proportional with the severity of oxidative damage through the production of free radicals in biological system and represent to specific site of damage. Therefore, measuring of oxidative biomarkers in serum is an important indicator of lipid peroxidation in severe schizophrenic patients. According to the results of present study in graphs the levels of inflammatory biomarkers (IL-6 and TNF-α) also significantly increased in subjects as compared to normal healthy individuals like levels of IL-6 (6.35±0.55)

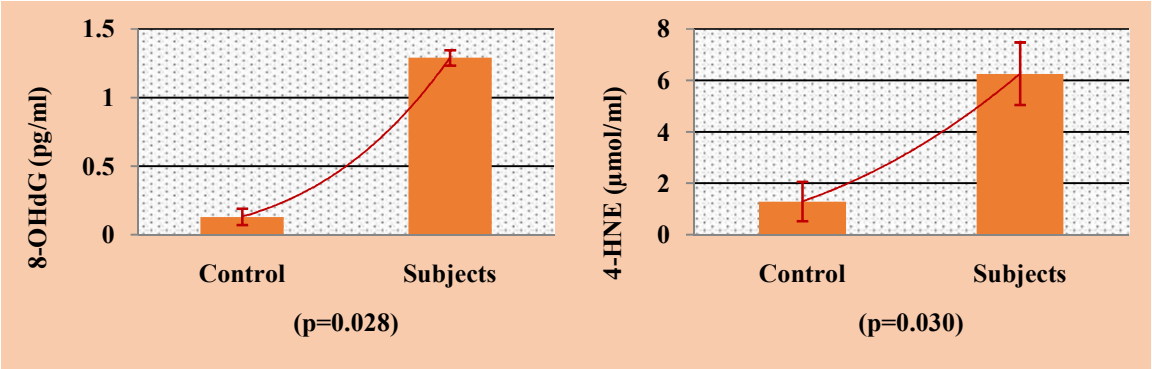
was significantly (P=0.034) increased in subjects as compare to control group (3.88±0.76). As well as the levels of TNF-α (25.26±1.11) and MMP-9 (103.29±6.35) were high in patients as compared to normal individuals (10.16±1.33) and (33.26±2.16) respectively and the P values were highly significant for both variables (P=0.00) and (P=0.037) respectively. The low levels of antioxidants were the indicator of oxidative damage because during the oxidative stress the levels of free radicals were increased but the levels of antioxidants were decrease as shown in table 01. So according to present study the levels of SOD (1.09±0.33), GSH (6.35±1.55) and CAT (1.23±0.021) were significantly low in subjects as compared to normal health patients (3.08±0.044), (9.66±2.18), (2.88±0.021) respectively, and the P values also highly significant (P=0.00), (P=0.042), (P=0.018) respectively. As shown is table 01 in schizophrenic patients thyroid profile also disturbed that might have the positive correlation with the severity of neuronal disorder development. So the levels of T3 (4.56±1.26) and T4 (9.09±2.19) were decreased in subjects as compared to normal person (6.35±2.16), (11.26±3.29) respectively and the P values were highly significant (P=0.027), (P=0.041) respectively but the levels of TSH (1.35±0.92) were high in schizophrenic patient Vs control (0.63±0.056). Like some prior studies the levels of vitamin D (16.35±3.08) disturbed in subjects as compared to control (30.18±8.29) because vitamin D has great preventive potential against oxidative stress condition.



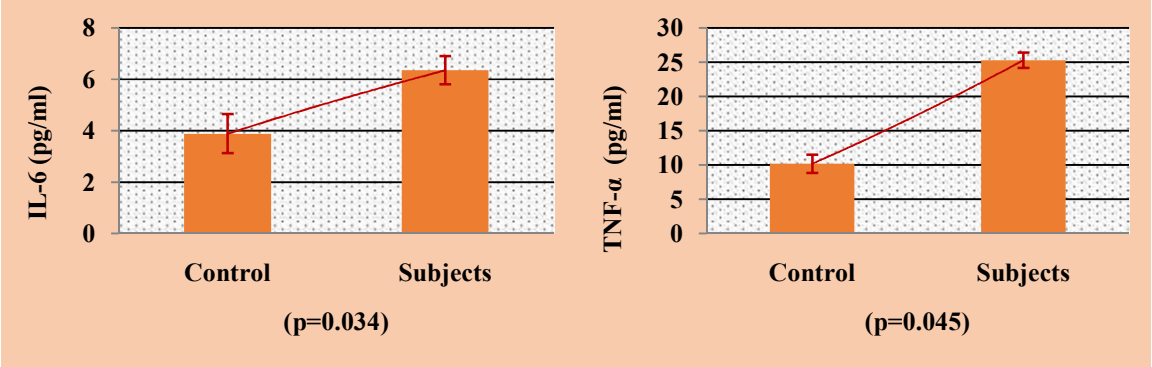
A



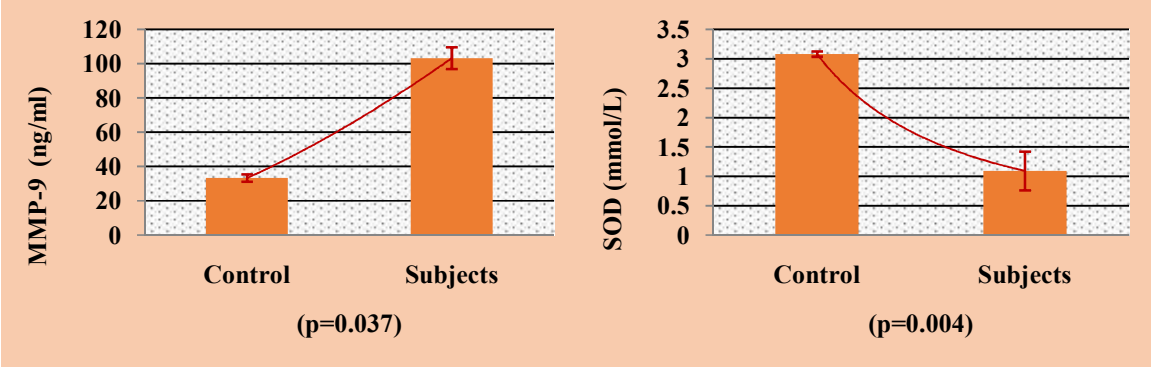
B


8-OHdG (pg/ml)
1.5
1
0.5
0
Control
Subjects
(p=0.028)
4-HNE (µmol/ml)
8
6
4
2
0
Control
Subjects
(p=0.030)

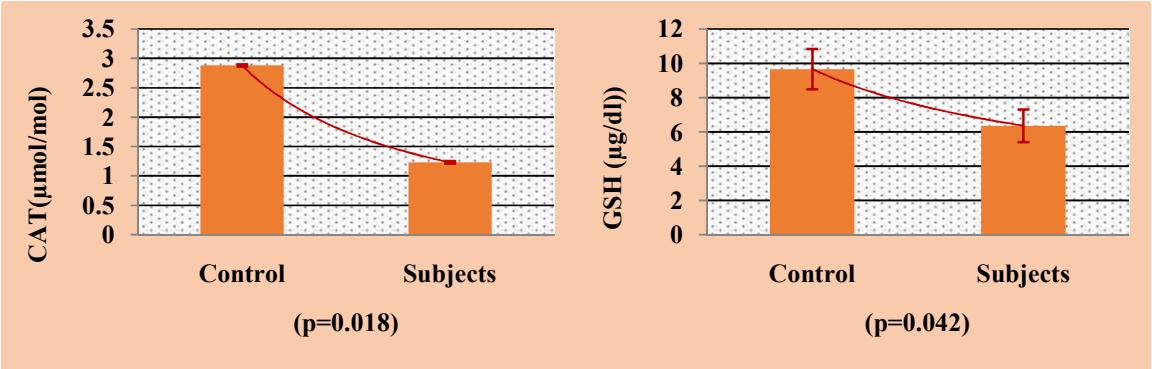
C D


IL-6 (pg/ml)
8
6
4
2
0
Control
Subjects
(p=0.034)
TNF-α (pg/ml)
30
25
20
15
10
5
0
Control
Subjects
(p=0.045)

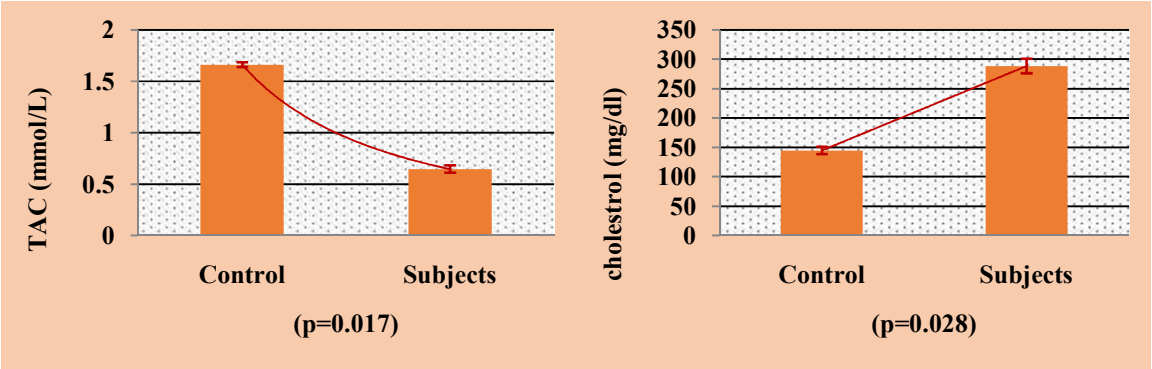
E F


MMP-9 (ng/ml)
120
100
80
60
40
20
0
Control
Subjects
(p=0.037)
SOD (mmol/L)
3.5
3
2.5
2
1.5
1
0.5
0
Control
Subjects
(p=0.004)

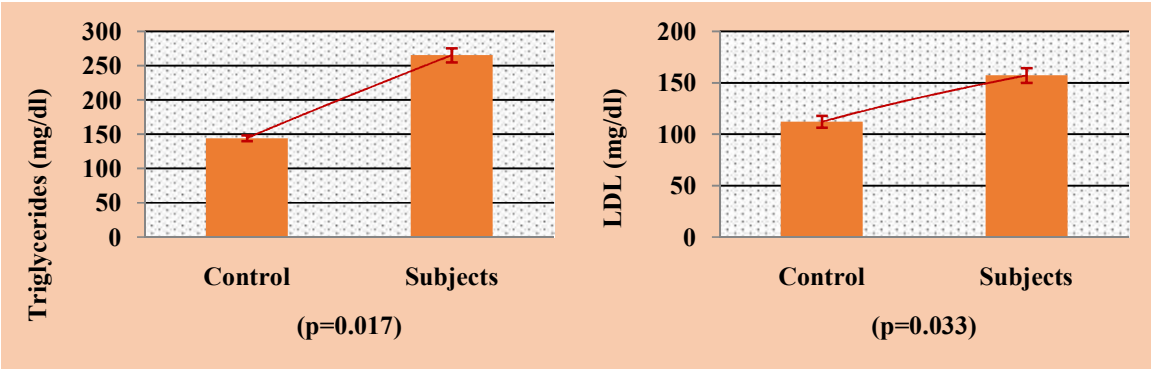
G H


CAT(µmol/mol)
3.5
3
2.5
2
1.5
1
0.5
0
Control
Subjects
(p=0.018)
GSH (µg/dl)
12
10
8
6
4
2
0
Control
Subjects
(p=0.042)

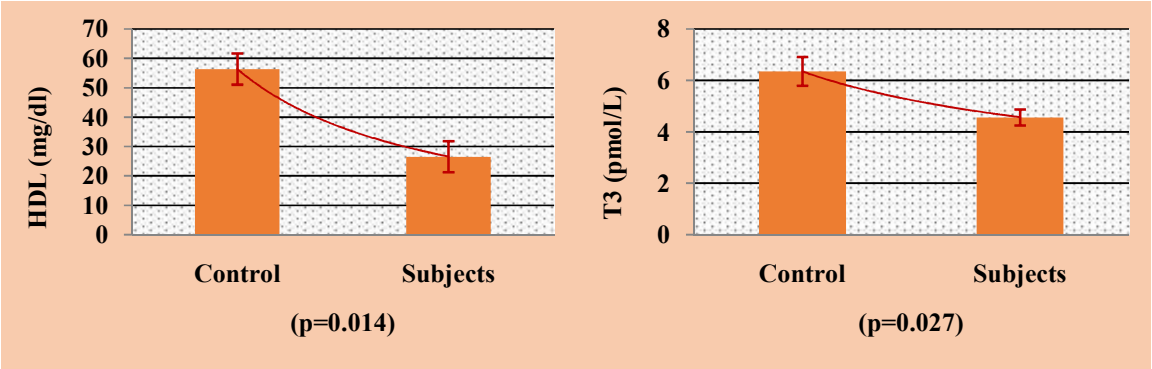
I J


TAC (mmol/L)
2
1.5
1
0.5
0
Control
Subjects
(p=0.017)
cholestrol (mg/dl)
350
300
250
200
150
100
50
0
Control
Subjects
(p=0.028)

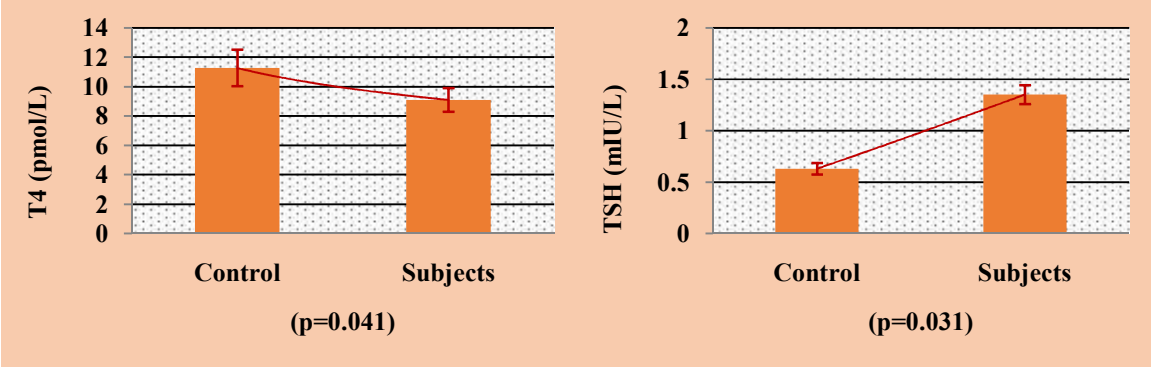
K L


Triglycerides (mg/dl)
300
250
200
150
100
50
0
Control
Subjects
(p=0.017)
LDL (mg/dl)
200
150
100
50
0
Control
Subjects
(p=0.033)

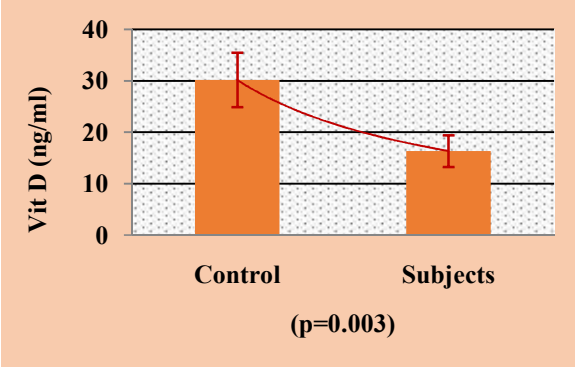
M N



O **P**



Q **R**



S

DISCUSSION

Schizophrenia is the sever type of mental disorder that occur due to various type of factors in the late teens or early adulthood. Mostly it is present in the age of 15-35 years. Worldwide it affects approximately 24 million peoples that lead to cause disability. According to the results of present study the oxidative biomarkers have the significant role in schizophrenic patients

because brain is very sensitive organ in the body as compared to others. As shown in prior studies the rate of metabolism was high in the brain because brain need more energy in the presences of oxygen every time so after the occurrence of any abnormality in oxygen metabolism leads to induce oxidative enzyme activation that triggers to the production of free radicals and cause oxidative stress. Oxygen reacts with unsaturated lipids and form oxidation products mainly lipid hydroperoxides. Secondary products are different aldehydes like Malondialdehyde (MDA), propanal, hexanal, 4-Hydroxy nonenal (4-HNE) [21, 22]. It is the most mutagenic product of lipid peroxidation and is more convenient and reliable biomarker for lipid peroxidation of omega 3 and omega 6 containing lipids. It can be tested with thiobarbituric acid to produce a chromogen fluorescent red adduct to evaluate auto oxidative degradation of fats and oils [22, 11]. It is a cytotoxic product originating from lipid peroxidation of live microsomal lipids [23]. 4-HNE produced in biomembrane lipid peroxidation and produce free radicals and chemicals which has genotoxic effects [24]. It is produced in large amounts and is very reactive aldehyde which act as second messenger. It is involved in the regulation of several transcription factors for example nuclear factor erythroid 2 related factor, Activating Protein 1 and peroxisome proliferator activated receptors [25]. These are involved in cell proliferation, differentiation, cell survival, autophagy, senescence, apoptosis, and necrosis. According to the results of present study free radicals were bind with membrane bilayer and initiate the process of oxidation because membrane is consist of lipid bilayer that is very sensitive to free radicals. Lipids in the form of PUFAs is the main component of plasma membrane. These PUFAs are separated through the unconjugated double bonds in the presences of methylene groups. Free radicals generated in response to oxidative stress binds with double bond and initiate the process of oxidation results in membrane damage by oxidizing into small fragment called lipid peroxidation (LPO) through cascade of chemical reaction and induce the loss of integrity of membrane through brake the double bond of phospholipids.

After the breakdown of lipid bilayer the levels of malondialdehyde (MDA) were increased that is the major universal oxidative biomarkers. MDA was analyzed to determine the severity of oxidative damage. Additionally, MDA further move toward the nucleic acid and bind with DNA and made

the DNA adducts that analyzed in the form of 8-OHdG. This biomarkers are used to check the severity of DNA damage in biological system. According to the literature it is well established that the oxygen free radicals causing damaging of macromolecules including carbohydrates, proteins, lipids and nucleic acid through the production and activation of hydroxyl radicles ($OH^{\cdot}$) and this free radicle are produce through different mechanism especially through Fenton reaction of hydrogen peroxide that further attacks to the cellular and mitochondrial DNA stand through cascade of reaction and cause modification. Interaction between $OH^{\cdot}$ free radicals and DNA nucleobases such as guanine cause the C8-hydroxyguanosine (8-OHGua) or deoxyguanosine (8-hydroxy-2-deoxyguanosine). Initially hydroxyl radicles interact with DNA and form DNA adducts that isolated in the form of 8-OHdG to check the stage of DNA damage through hydroxyl free radical binding in oxidative stress condition in schizophrenic patient. As shown Matrix metalloproteinase-9 (MMP-9) is the major inflammatory biomarker that have the direct correlation with schizophrenic severity. MMP-9 belongs to Zn dependent proteases. It is secreted as inactive proenzyme which is activated outside the cell upon proteolytic cleavage. MMPs degrade extracellular matrix like proteases, growth factors, extracellular domains of transcellular proteins for example cell adhesion molecules and receptors [26]. According to the prior studies few reports were established to evaluate the role of vitamin D in schizophrenic patients because within the passage of time scientists are focused to the activity of vitamin D because it have the great potential to reduce the expression or development of different disorders including brain related disease, infertility, various types of cancer, bone related disease. so as shown in present study results the levels of vitamin D were significantly decrease in schizophrenic patients as compared to normal healthy individuals and shows an inverse correlation between the disease activity and vitamin D concentration according to CGI-S and PANSS scores [27].

This result remained after the determination of different type of factors including sun exposure, sex, skin color, dietary life style as well as ethnicity. The present study result show that negative correlation between the severity of psychotic symptoms and vitamin D concentration. A cross section studies established that vitamin D have the negative relationship between the somatic complaints, physical energy and psychomotor activity. Normal levels of

vitamin D also prevent the production of free radicals in oxidative stress because vitamin D have the great ability to maintain the calcium levels in biological system through cascade of reaction either influx of calcium in rough endoplasmic reticulum (RER) and efflux in mitochondrial membrane. Calcium trigger cell death through the stimulation of apoptosis that depends upon different type of caspases. In schizophrenic patients the mortality rate of neuronal cell were increased as compared to regeneration that leads to the development of different type of mental disorders especially schizophrenia. Deficiency of calcium through vitamin D triggers iNOS production to cause oxidative stress through the production of free radicals. As shown in results these free radicals were directly bind with macromolecules and accelerate the damaging process through lipid peroxidation. In this results the levels of oxidative biomarkers were increased that represent to the severity of disease in schizophrenic patients [16]. It has been observed that IL-6 has a significant positive correlation with the antipsychotic dose. There is a contradiction in the relationship of antipsychotic dose with cytokines, various studies showed strong correlation, some literature shows negative association and on the other hand various literature articles shows no relation among each other [28]. Antipsychotics, such as atypical antipsychotic leads to increase the cases of diabetes, obesity which in turn up-regulate IL-6 and leptin. It has been observed through various studies and literature that leptin has neuroprotective role. IL-6 moreover plays a key role in inflammation and stress profile. Increase inflammatory cytokines leads to increased level of ROS resulting depletion of antioxidants, generating the condition of oxidative stress [29-31].

CONCLUSION

It is concluded that increased oxidative stress in patients with schizophrenia may be depends on the inflammation response. These results indicate that, increased oxidative stress in schizophrenic patients is the main determinant and estimated by oxidative biomarkers (MDA, 8-OHdG, isoprostanes and 4-HNE), nitric oxide and they are not degraded or detoxified by enzymatic antioxidants SOD and CAT and non-enzymatic GSH, Vitamins D. Increased oxidative stress (RNS, ROS) are the major mediators of the neuronal damage and inflammation in the cells of central nerves system. Conclusively, schizophrenia may occur through the up-regulation of oxidative

free radicles and down-regulation of antioxidant scavenger activity.

CONFLICT OF INTEREST

Authors declare no conflict of interest

ACKNOWLEDGEMENTS

Authors are highly thankful for of all the staff and students of lab-313, The University of Lahore-Pakistan for their valuable contributions in the preparation of this manuscript. Here authors are also acknowledged the financial support by the Institute of Molecular biology and Biotechnology, The University of Lahore-Pakistan.

REFERENCES

[1] Baumann C, D Sevinsky, and DS Conklin (2013). Lipid biology of breast cancer .Biochimica et Biophysica Acta. 1831: 1509-1517.

[2] Castellani K, D Honda and X Zhu (2004) "Contribution of redox-active iron and copper to oxidative damage in Alzheimer disease. Ageing Research Reviews. 3:319-326.

[3] Del Rio A, J Stewart and N Pellegrini (2005).A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, Metabolism and Cardiovascular Diseases. 4: 316-328.

[4] Fragkouli A, C Papatheodoropoulos and S Georgopoulos (2012). Enhanced neuronal plasticity and elevated endogenous sAPP levels in mice over-expressing MMP9.J Neurochem.121.239-251.

[5] Gkogkas CG, A Khoutorsky, R Cao (2014). Pharmacokinetic inhibition of elF4E-dependent Mmp9 mRNA translation reverses fragile X syndrome-like phenotypes. Cell Rep.9: 1742-1755.

[6] Halliwell and JMC Gutteridge (1984). Oxygen toxicity, oxygen radicals, transition metals and disease: Biochemical Journal. 219: 1-14.

[7] Kadiiska B, C Gladen, DD Baird (2005).Biomarkers of oxidative stress study III. Effects of the nonsteroidal anti-inflammatory agents indomethacin and meclofenamic acid on measurements of oxidative products of lipids in CCl4 poisoning: Free Radical Biology and Medicine. 38: 711-718.

[8] Kanner J, B German and JE Kinsella (1987). Initiation of lipid peroxidation in biological systems: Critical Reviews in Food Science and Nutrition. 25: 317-364.

[9] Lee C, E Kim and MR Yun (2010). 4-Hydroxynonenal enhances MMP-9 production in murine macrophages via 5-lipoxygenase-mediated activation of ERK

and p38 MAPK. Toxicology and Applied Pharmacology. 242: 191-198.

[10] Lipinski and E Pretorius (2012). "Hydroxyl radical-modified fib- rinogen as a marker of thrombosis. The role of iron," Hematology. 17: 241-247.

[11] Esterbauer P, G Eckl, and A Ortner (1990). Possible mutagens derived from lipids and lipid precursors: Mutation Research. 238: 223-233.

[12] Pryor WA (1989) .On the detection of lipid hydroperoxides in biological samples: Free Radical Biology and Medicine: 7: 177-178.

[13] Verslegers K, I Lemmens, JK Van Hove and L Moons (2013). Matrix metalloproteinase-2 and -9 as promising benefactors in development, plasticity and repair of the nervous system.Progress in Neurobiology. 105: 60-78.

[14] Holick M (2007). Vitamin D deficiency. N Engl J Med 357: 266–281.

[15] Hart G, J Furniss, D Laurie and S Durham (2006) Measurement of vitamin D status: background, clinical use, and methodologies. Clin Lab 52: 335–343.

[16] McGrath J, K Saari, H Hakko, J Jokelainen, P Jones, M Jarvelin (2004). Vitamin D supplementation during the first year of life and risk of schizophrenia: a Finnish birth cohort study. Schizophr Res 67: 237-245.

[17] Ohkawa H, N Ohishi and K Yagi (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry, 95: 351-358.

[18] Kakkar R, SV Mantha, J Radhi, K Prasad and J Kalra (1997). Antioxidant defense system in diabetic kidney: a time course study. Life sciences, 60: 667-679.

[19] Aebi H (1974). Catalase. In Methods of Enzymatic Analysis (Second Edition), 2: 673-684.

[20] Moshage H (1997). Nitric Oxide Determinations: Much Ado About NO•-Thing?.

[21] Esterbauer KH, K Cheeseman and MU Dianzani (1982).Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe2+ in rat liver microsomes: Free Radical Biology and Medicine. 12: 41-142.

[22] Esterbauer R, R Schaur, and H Zollner (1991).Chemistry and Biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radical Biology and Medicine. 11:81-128.

[23] Roberts II, JP Fessel, and SS Davies (2005).The biochemistry of the isoprostane, neuroprostane, and isofuran

pathways of lipid peroxidation: Brain Pathology.15: 143-148.

[24] Brambilla L, P Sciaba and L Faggin (1986).Cytotoxicity, DNA fragmentation and sister-chromatid exchange in Chinese hamster ovary cells exposed to the lipid peroxidation product 4-hydroxynonenal and homologous aldehydes: Mutation Research. 171: 169-176.

[25] Zarkovic (2003). 4-Hydroxynonenal as a bioactive marker of pathophysiological processes: Molecular Aspects of Medicine. 24: 281-291.

[26] Rivera S, M Khrestchatisk, R Kaezmarck, L Rosenberg and DM Jaworski (2010). Metzincin proteases and their inhibitors: foes or friends in nervous system physiology? J Neurosci. 30:15337-15357.

[27] Castellani K, D Honda and X Zhu (2004) "Contribution of redox-active iron and copper to oxidative damage in Alzheimer disease. Ageing Research Reviews. 3:319-326.

[28] Steinbrenner H and H Sies (2009). Protection against reactive oxygen species by selenoproteins: Biochimica et Biophysica Acta: General Subjects. 1790: 1478-1485.

[29] Moldovan and NI Moldovan (2004).Oxygen free radicals and redox biology of organelles: Histochemistry and Cell Biology. 122: 395-412.

[30] Zulfiqar HF, Javed A, Afroze B, Ali Q, Akbar K, Nadeem T, Rana MA, Nazar ZA, Nasir IA, Husnain T (2017). HIV diagnosis and treatment through advanced technologies. Frontiers in Public Health. 7; 5: 32.

[31] Nadeem T, Khan MA, Ijaz B, Ahmed N, ur Rahman Z, Latif MS, Ali Q, Rana MA (2018). Glycosylation of recombinant anticancer therapeutics in different expression systems with emerging technologies. Cancer Research. 1; 78(11): 2787-98.

[32] Imran A, Qamar HY, Ali Q, Naeem H, Mariam RI, Saima AM, Kanwal N, Fawad AL, Sabar MF, Nasir IA (2017). Role of molecular biology in cancer treatment: A review article. Iranian Journal of Public Health. 46(11):1475.