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8-HYDROXY-2-DEOXY GUANOSINE IS A NOVEL NEW BIOCHEMICAL MARKER FOR PATIENTS WITH MULTIPLE SCLEROSIS AND CORRELATION WITH PARAOXANASE-1 AND MDA

Ibtisam K. Mohaisn[1*], Lamia S. Ashoor[2] and Bushra H. Ali[3]

[1]Department of Basic Medical Sciences, Collage of Density, University of Missan, Iraq.
[2]Department of Basic Science, College of Agriculture, University of Baghdad, Iraq.
[3]Department of Chemistry, College of Education for Pure Sciences, University of Baghdad, Ibn – ALhaitham, Iraq.
*e-mail : ibtisam_kareem@yahoo.com

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ABSTRACT : Multiple sclerosis (MS) is a chronic, inflammatory demyelinating disease of central nervous system with complex etiopathogenesis that affects young adults. The aim of this study focuses on determination 8-H-2-dG, MDA and PON1 in multiple sclerosis disease and finds the relationship between newly marker 8-H-2-dG with MDA and PON1. Our results showed that patient with MS had significantly higher ($p < 0.05$) frequency of 8-H-2-dG and MDA compared to healthy control group (0.359±0.0035, 0.597±0.009) and (2.646±0.089, 5.829±0.09), but highly significant decrease ($p < 0.001$) in G1 (823.321±53.669) compared to healthy control group (1060.84±65.446) for PON1. Our findings suggest that increase oxidative damage may represent a common pathophysiological mechanism and in other hand that 8-H-2-dG, MDA and PON1 may be used as a novel biochemical marker in MS disease.

Key words : Multiple sclerosis, oxidative damage, 8-H-2-dG, MDA, PON1.

INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory demyelinating disease of central nervous system with complex etiopathogenesis that impacts young adults (Lee et al., 2015), and MS impacts younger and middle aged character and leads to a range of disabilities that can alter their daily routines (Yara *et al*, 2010). Although, the exact cause of MS is still undetermined, the disease is mediated by adaptive immunity through the infiltration of T cells into the central nervous system (Bjelobaba *et al*, 2017). MS causes the Focal neurological symptomsand biochemical changes in the molecular level and the variation of neural cells such as loss or alteration of sensation, motor function, visible signs such as blurred vision or transient blindness, disturbance of conjugate eye movements, bladder and bowel dysfunction and cognitive impairment (Induruwa *et al*, 2012 and Jafarzadeh *et al*, 2014). Autoimmune diseases (ADs) are chronic conditions initiated by way of the loss of immunological tolerance to self-antigens (Todorovic-Dilas *et al*, 2011).

It is a heterogeneous group of disorders in which more than one modification in the immune system can be specific to a particular tissue or organ or might also be systemic, non-specific, involving multiple tissues or organs (Ray *et al*, 2012). One possible cause behind this is a lack of understanding of pathogenic mechanisms driving progressive multiple sclerosis. Due to the indolent nature of symptom progression, current disease criteria used to signify the course of disease (Lublin *et al*, 2014) indicate diagnosis is generally retrospective and based totally on history of gradual worsening. Clearly, diagnosis is primary based on clinical judgment, as there is no fully reliable diagnostic test (Ontaneda *et al*, 2015).

In latest years, the elements involved in the etiology of the disease have also included oxidative stress (OS), which is described as an imbalance between the generation of reactive oxygen species (ROS) and the mechanisms that are responsible for their elimination, andthe imbalance between OS agents and antioxidants leads to OS activating the inflammatory process (Phaniendra *et al*, 2015). In the absence of enough antioxidant defenses, ROS can reason oxidative damage to macromolecules resulting in oxidation of lipids, proteins and deoxyribonucleic acid (DNA) (Griffiths, 2002). Some reseach report that ROS play a main role in myelin phagocytosis (Ghabaee *et al*, 2010 and Tasset *et al*, 2012). The inflammatory response gives rise to the manufacturing of both ROS and Reactive

Nitrogen Species RNS through monocyte interactions with brain endothelium; ROS manufacturing induces cytoskeletal rearrangements, loss of blood-brain blood BBB integrity, tight-junction alteration and the extravasation of leukocytes into the central nervous system (Van *et al*, 2011; Witherick *et al*, 2011).

Aim of study

The aim of this study focuses on determination 8-H-2-dG, MDA and PON1 in multiple sclerosis disease and finds the relationship between newly marker 8-H-2-dG with MDA and PON1.

MATERIALS AND METHODS

Subjects

This study was performed on 25 female patients with age (25-35) years who diagnosed by physicians as a multiple sclerosis in Misan governorate. The patients compared with 25 apparently healthful in the identic rangel of age.

In this study sample was collected five mL of venous bloods, placed in to plain tubes until coagulation was performed. Serum was separated from blood cells by centrifugation 4000 r.p.m.

Assay method

Determination of serum of 8-H-2-dG

This assay that can be used for quantification of 8-H-2-dG in urine, cell culture, plasma and other sample matrices. The ELISA utilize an 8-H-2-dG coated plate and HRP- conjugated antibody or detection which allows for any assay range of 0.94-60 ng/mL, with sensitivity of 0.59 ng/mL.

Determination of MDA

The concentration of MDA,which is the consequence of lipid peroxidation and a marker of oxidative stress, was measured using thiobarbiturc acid.

Determination of PON1

The quantitive sandwich enzyme immunoassay (ELISA) technique was employed for determination of PON1.

Statistical analysis

Mean values and standard deviations (SD) were used for continuous variables. Student t-test was used to compare the significance of the difference in the mean values of control and patient groups ($p<0.001$) was considered high significance.

RESULTS

Table 1 showed the levels of BMI, 8-H-2-dG, MDA and PON1 concentration sera of G1 for patient and healthy control, respectively. The results showed high significant in levels for BMI,8-H-2-dG and MDA (Figs. 1, 2 & 3) increase. As well as highly significant decrease ($p<0.001$) in G1 (823.321±53.669) compared to control group (1060.84±65.446) for PON1 (Fig4).

In addition, Table 2 showed poorly significant positive correlation was observed between MDA and 8-H-2-dG for control and G1 (Fig. 1), but showed negative significant for PON1and 8-H-2-dGfor control group and negative poorly significant for G1 (Fig. 2).

DISCUSSION

MS is chronic disorder that influences mainly young adults. Currently, that is believedas MS is a biphasic disease (Fiorini *et al*, 2013). Initially, inflammatory processes dominate, and the process is associated with polymorphonuclear leukocyte (PMN) passage into the brain tissue (Tasset *et al*, 2012) then stimulates the adhesion of monocytes to the vascular endothelium (Usatyuk and Natarajan, 2012). The extravasation of leukocytes into the CNS generates cytokine caused synaptic hyper excitability (Rossi *et al*, 2014) and ultimately leads to chronic neuroinflammation (Mossakowski *et al*, 2015).

The recruitment and extravasation of immune cells throughout the activated vascular endothelium of the blood brains to consider to critical step in MS pathogenesis (Frohman *et al*, 2006). MS also assocated with significant amount of cerebral vascular endothelial dysfunction (Alexander, 2010) and with cerebral hypoperfusion

Table 1 : The serum level of 8-H-2-dG, MDA and PON1 of MS patients and control group.

Parameter	Mean ± SEM C	Mean ± SEMG1	C vs G1
BMI (K/m^2)	24.952± 0.796	25.9± 1.122	H.S.
8-H-2-dG (Pg/ml)	0.359±0.0035	0.597±0.009	H.S.
MDA(μmol/l)	2.646±0.089	5.829±0.09	H.S.
PON-1 (mL U /mL)	1060.84 ±65.446	823.321 ± 53.669	H.S.

Table 2 : Correlation relation analysis between biochemical parameters between control and studied group.

Parameter	r &P	C	G1
MDA& 8-H-2dG	r	0.142	0.130
	P	P>0.05	P>0.05
PON-1 & 8-H-2dG	r	- 0.445	- 0.126
	P	P>0.05	P>0.05

P values < 0.05 considered significant (S)
P values< 0.001 considered high significant (HS)P values>0.05 considered non- significant (NS) r values mean correlation coefficient.

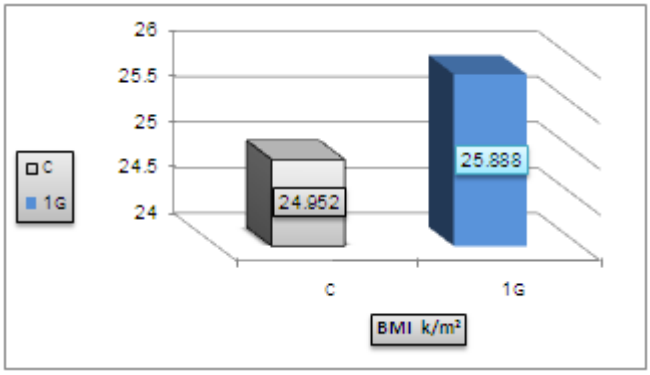

Fig. 1 : Levels of BMI in healthy control group (C) and patient group (G1) with MS disease, column represent the mean values ±SD.

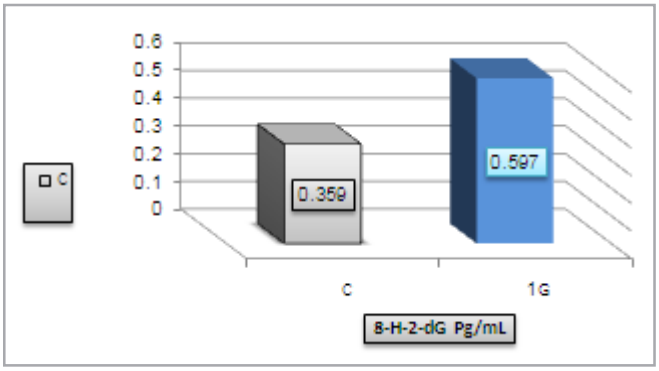

Fig. 2 : Levels of 8-H-2-dG in healthy control group (C) and patient group (G1) with MS disease, column represent the mean values ±SD.

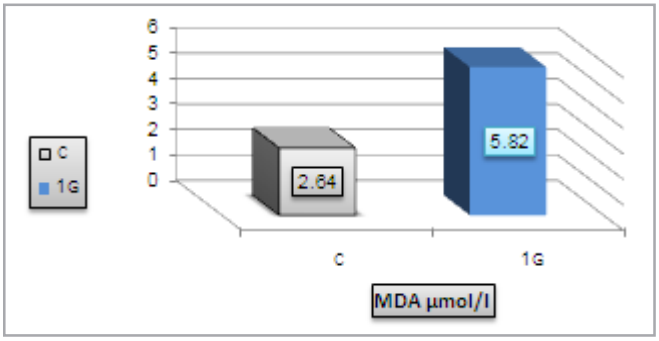

Fig. 3 : Levels of MDA in healthy control group (C) and patient group (G1) with MS disease, column represent the mean values ±SD.

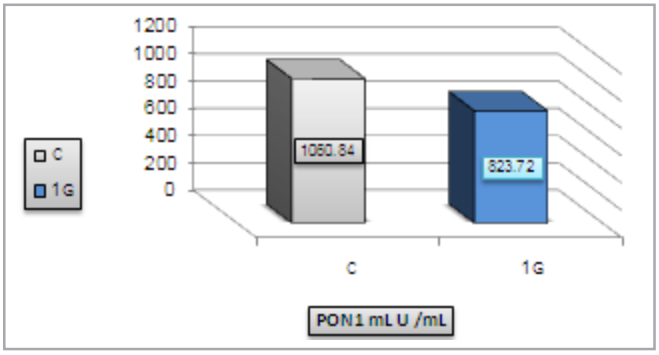

Fig. 4 : Levels of PON1 in healthy control group (C) and patient group (G1) with MS disease, column represent the mean values ±SD.

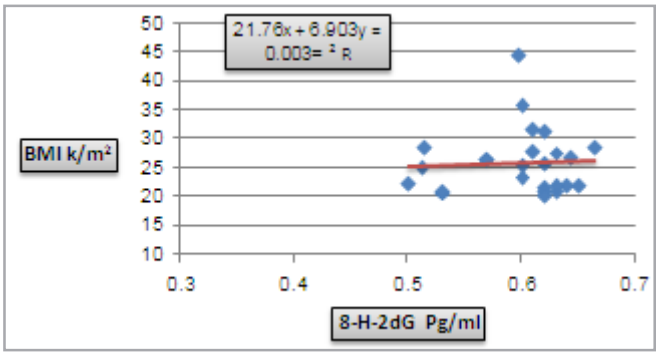

Fig. 5 : Correlation between 8-H-2-dG and BMI.

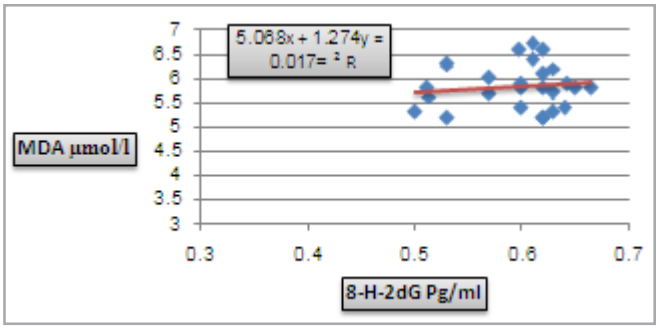

Fig. 6 : Correlation between 8-H-2-dG and MDA.

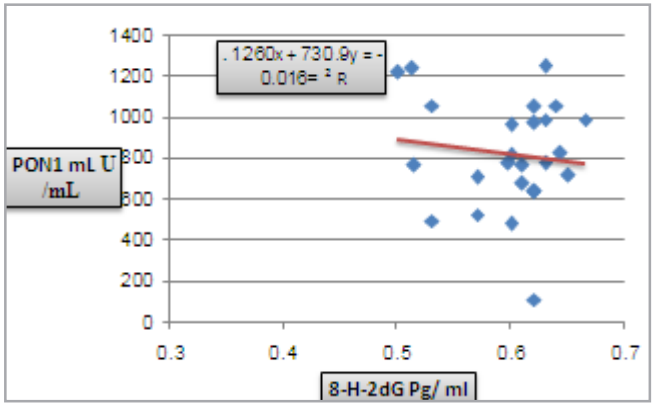

Fig. 7 : Correlation between 8-H-2-dG and PON1.

(Keyser, 2008).

In this context, the generation of reactive oxygen species ROS which is important into both normal physiology and in the pathogenesis of many disease (Alessandro *et al*, 2015). Oxidative damage results from biochemical interactions between reactive oxygen ROSthen target biomolecules. ROS can damage nucleic acids, lipids and proteins; this damage figures prominently within the etiology and progression of numerous cancer as our biomarker of oxidative damage (Michael and Gregory, 2006). The biomarker 8-H-2-dG established a commonly measured and sensitive marker of DNA damage (Athanasios, 2009) and recent research have revealed the role of 8-H-2-dG through avarious analytical techniques among blood cells or in urine have established it as a very important biomarker disease (Athanasios, 2009).

In this study, the levels of 8-H-2-dG were found high significant in patients with MS of G1 than the healthy control group.

Also, this study include MDA (malondialdehyde) as biomarker for MS, level of MDA were found high significant in patients group G1 compared to healthy control group.

This result is agreed with Al Neaimy (2014) that found the level of MDA in patient MS is increased and the lipid peroxidation a marker of oxidative stress is in patients of MS, also this study agreed with study of Bianca *et al* (2011), which found lipid profile such as LDL,TG and total cholesterol levels are associated with increased disability progression in MS, and also agreed with study of Saam *et al* (2016), which found MDA considerably augmented in MS patients who did not receive any medicine compared to the control group.

In this study, PON1 concentration was lower in MS patients of G1 compared to healthy control group. This result is in accordance with result of previous studies that compared the concentration of PON1 and individual antioxidant enzyme in MS patients. This study agreed with Almeshhadani (2015), Arinna *et al* (2016) and also agreed with study of Ibtisam *et al* (2014) about PCOS autoimmune diseases (ADs).

CONCLUSION

Based on results of this study, we conclude that 8-H-2-dG, MDA and PON1 have important role in diagnosis of MS disease. Most likely, this biomarker affects the incidence of MS by reduced for complete dysfunction of CNS. A future investigation evaluating the mechanism of MS disease protective antioxidant intraction with inflammation and large clinical trial might be needed.

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