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Research Article

Role of Oxidative Stress & Glycation of Hemoglobin in Relation with Pathophysiology of Neuropathic Pain

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Abstract: Oxidative stress might be caused by an imbalance between the production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. Glutathione (GSH) deficiency is related to a number of diseases, including neurodegenerative diseases and peripheral neuropathies. In chemical terms, oxidative stress is considered as a large decrease in the reducing capacity of cellular redox couples including glutathione. The purpose of the present study was to evaluate the role of oxidative stress in patients with neuropathic pain no precise studies carried out demonstrating effect of GSH in neuropathy caused by oxidative stress. Hence this study was done to find correlation of GSH with the progression of peripheral neuropathies. However, there have been may indicate better treatment options. For the determination of GSH, Hb and other indices the blood samples of patients suffering with neuropathic pain were collected from Karachi, Pakistan. The results showed significant difference in GSH levels between normal individuals and in patients with neuropathic pain. These differences were also found significant among the neuropathic pain patients with the history of diabetes and other peripheral neuropathies. In conclusion, the reduced levels of GSH in patients with neuropathic pain may indicate the involvement of oxidative stress which might be more in patients suffering from diabetic neuropathy.
Keywords: Neuropathic pain, Oxidative stress, GSH, Neuropathy, Diabetes..

INTRODUCTION

Oxidative stress is a consequence of imbalance between the production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates to repair the damage. It can be pathophysiological consequence of overload of oxidants, with respect to the antioxidant defense system developed by cells to counteract oxidation [1]. All metabolic and life sustaining process at cellular level need to maintain a reducing environment within their cells, this reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state like diabetes can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids [2-4] and DNA Oxidative stress is imposed on cells as a result of one of three factors i.e. increase in oxidant generation, decrease in antioxidant protection, failure to repair oxidative damage [4]. Cell damage is induced by reactive oxygen species (ROS). ROS are either free radicals, reactive anions containing oxygen atoms, or molecules containing oxygen atoms that can either produce free radicals or are chemically activated by them, patterns are hydroxyl radical, superoxide, hydrogen peroxide, and peroxynitrite [5]. The main source of ROS in vivo is aerobic respiration, although ROS are also produced by peroxisomal b-oxidation of fatty acids, microsomal cytochrome P450 metabolism of xenobiotic compounds, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism, and tissue specific enzymes. Under normal conditions, ROS are cleared from the cell by the action of superoxide dismutase (SOD), catalase, or glutathione (GSH) peroxidase. The main damage to cells results from the ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA. Cheeseman in 1993 said that oxidative stress and ROS suspected to be involved in several pathological processes such as inflammation, cancer, and neurodegenerative diseases [6, 7]. (GSH) is a tripeptide reduced(GSH) form with its role in redox reaction and alteration of free radicals and neutralizing toxic effects of harmful compound. Diabetes mellitus which is associated with increased lipid peroxidation caused by

oxidative stress has been also linked with diabetic nephropathy and oxidative stress was reported [5]. It is suggested that oxidative stress affects the progress of diabetic complications and could restructure these complications. Our work has focused on its presence in peripheral nerves [*8]*. Antioxidant enzymes are reduced in peripheral nerves and are further reduced in diabetic nerves. Oxidative stress appears to be primarily due to the processes of nerve ischemia and hyperglycemia auto-oxidation. The indexes of oxidative stress include an increase in nerve, dorsal root, and sympathetic ganglia lipid hydroperoxides [1, 9]. The most unswerving and responsive indicator is a decrease in reduced glutathione. Experimental studies also showed that diabetic neuropathy results in myelinopathy of dorsal root ganglion via lipid peroxidation [7, 10] and mitochondrial dysfunction that not only increase reduced oxygen species but in turn damage respiratory and metabolic utility of cell, resulting in a nerve torn and ultimately neuropathy [10]. The diabetic peripheral neuropathy (DPN) is marked by altered electrophysiology, declined blood flow and related metabolic deviations in peripheral nerve functions assumed to be linked with chronic hyperglycemia, tissue hypoxia, and oxidative stress [**9]. The major consequence of** oxidative stress results from a cell or tissue failing to detoxify the free radicals that are produced during metabolic activity. This study was an effort to explore not only the concept that diabetes altered metabolic pathways but their relation with ROS and oxidative stress. Evidences support the idea that both chronic and acute hyperglycemia cause oxidative stress in the peripheral nervous system that can promote the development of diabetic neuropathy [*8]*. The smashed proteins due to oxidative trauma have lessened biological activity leading to loss of regular functions that ultimately leads to cell death [9]. Many investigational studies been proposed that oxidative stress may lead to changes in basic liver metabolism and blood indices that may be associated with disturbances of the antioxidant barrier of the organism. The aim of our study was to evaluate this whole mechanism in relation with damaged vascular and nervous tissues. The low GSH level may directly be associated with an increase of production of reactive oxygen species or the changes in blood indices by obvious glycation due to chronic hyperglycemic conditions [11].

METHODOLOGY

A cross sectional study was carried out at the department of physiology, university of Karachi and civil hospital Karachi from 2010 to 2014. With the help of statistician and epidemiologist, sample size was calculated through creative research systems website with level of significance 5%, margin of error 5%, confidence interval 95%. Only diagnosed cases of peripheral & diabetic neuropathy were included. All the samples were collected personally by non probability sampling technique after Subjects were recruited from civil hospital Karachi & Al-Madad Organization. The subjects were asked to sign the consent form after full explanation of the study. The samples have been collected from two different types of patients i.e diabetic neuropathy & peripheral neuropathy. Base line history from all subject were collected through a questionnaire, which included question mainly on current health of the individual & complete history. First set of samples was collected from patients suffering with peripheral neuropathy. The total 116 subjects, both males and females between 40 to 70 years of age were selected from the Department of Neurology Civil Hospital Karachi. Second set of samples was collected by the 172 patient of diabetic neuropathy from AL-MADAD Foundation, under the supervision of Diabetoligist. Samples were collected from all the subjects for the study of GSH levels, Hb, RBC, Hematocrit, MCV, MCH, MCHC, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, HbA1c & Platelets. For the determination of GSH a lysate of RBCs was prepared by adding 200µl of whole blood and adding 20ml of $D.H_2O$ along with thec determination of Hemoglobin concentration by using Drabkin's reagent. The Hemolysate of 20ml was then added to 30ml of a solution of Glacial metaphosphoric acid 1.67/dl+disodium EDTA 0.2g/dl+Nacl30g/dl, at standing for several minutes mix it then filtered with addition of 20ml of filtrate to 8ml of 0.3M Na_2HPO_4 solution and read at 412nm wave length. Another reading was taken by adding 1ml of DTNB reagent (20mg DTNB/dl) again at 412nm wave lengthand the difference between two readings was used to evaluate GSH concentration in nanomol/g of Hb by Blood GSH conc. = (OD2-OD1)×E×101/ Hb (g/dl) (E=0.5421).

RESULTS

Scatter diagram (Fig. 1) displayed the pain scores on Y- axis & diabetic duration on Y-axis, respectively, it can be seen that, pain score reduces with increasing age of diabetes. The results of pain scores were compared with the diabetic history. We used correlation test, p-value <0.05 considered as statistically significant. The values of blood GSH in patients with neuropathic pain shows a significant decline when compared with normal subjects. Moreover we also observe a significant decline among patients suffering from diabetic neuropathy and other peripheral neuropathies. *T*he mean value of GSH Levels of Peripheral Neuropathy, it was found 0.289 on average with an standard deviation of 0.069, while in the scores of GSH Levels of Diabetic neuropathy the mean was 0.162 with an standard deviation of 0.082, the significant p-value <0.01 was obtained using the independent sample t-test shows the significant differences in the GSH Levels of Diabetic neuropathy and scores of GSH Levels Peripheral Neuropathy. Horizontal bar diagram in (Fig: 2) displayed the mean of GSH between peripheral neuropathies & diabetic neuropathy Patients,

respectively, it can be seen that, GSH have lesser mean values in diabetic neuropathy Patients as compared to peripheral neuropathies Patients.

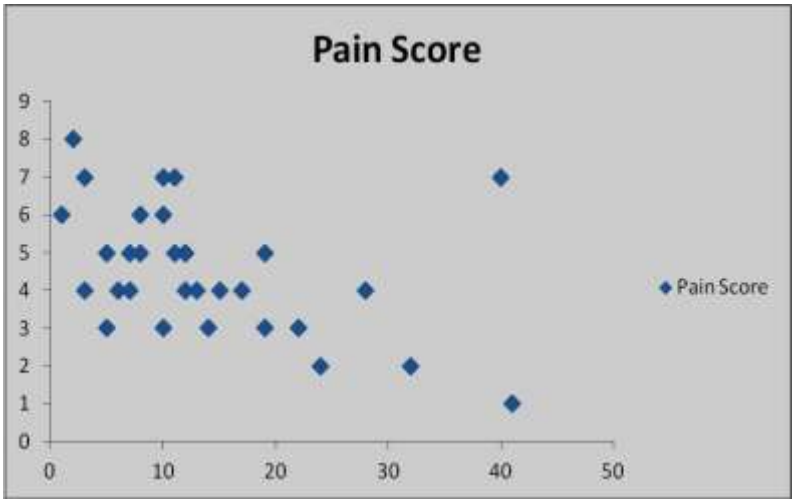

Fig. 1: Correlation of Pain score & Diabetic History

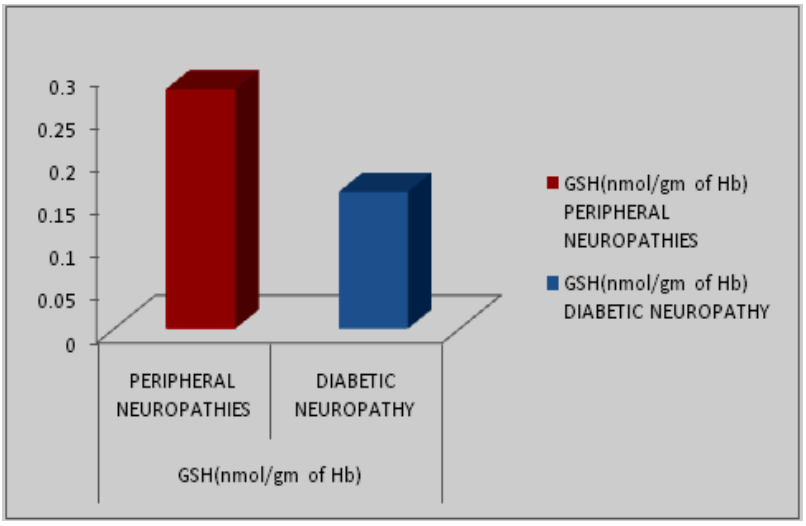

Fig. 2: Comparison of Diabetic Neuropathy & Peripheral Neuropathy

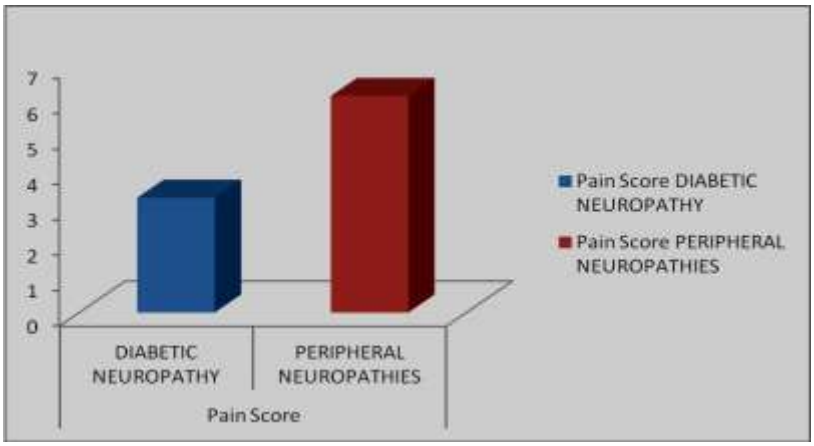

Fig. 3: Comparision of pain score between Diabetic Neuropathy & Peripheral Neuropathy

Horizontal bar diagram in (Fig. 3) displayed the mean of pain score between Diabetic Neuropathy & Peripheral Neuropathy, respectively, it can be seen that, pain score have lesser mean values in Diabetic Neuropathy as compared to Peripheral Neuropathy. We give the Correlation between Hb, RBC, Hematocrit, MCV, MCH, MCHC, Total WBC, Neutrophils,

Lymphocytes, Monocytes, Eosinophils, Platelets Count significant.

Table 1: Correlation between Hb, RBC, Hematocrit, MCV, MCH, MCHC, Total WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Platelets Count and GSH

	Hb	RBC	Hemat-ocrit	MCV	MCH	MCHC	WBC fa	Neutro-phils	Lympho-cytes	Mono-cytes	Eosino-phils	Pletelet fa	GH
Hb	1.00												
RBC	0.38	1.00											
Hematocrit	.927*	.631**	1.00										
MCV	0.22	-.782**	-0.03	1.00									
MCH	0.23	-.805**	-0.08	.981**	1.00								
MCHC	0.19	-.652**	-0.20	.640**	.777**	1.00							
Wbcfa	0.21	0.08	0.16	0.01	0.03	0.11	1.00						
Neutrophils	-0.10	-0.19	-0.19	0.09	0.11	0.18	0.35	1.00					
Lymphocyt	0.11	0.07	0.15	0.03	0.02	-0.06	-0.29	-964**	1.00				
Monocytes	-0.11	.519*	0.14	-558*	-626**	-660**	-0.30	-0.16	-0.07	1.00			
Eosinophils	0.14	0.17	0.15	-0.16	-0.15	-0.04	-0.15	-0.36	0.16	0.34	1.00		
Platelet fa	0.02	-0.01	-0.06	-0.08	-0.02	0.17	0.624*	0.18	-0.22	-0.12	0.32	1.00	
GSH	.946**	0.41	.884**	0.14	0.15	0.15	0.24	-0.03	0.01	-0.06	0.21	0.17	1

**: Correlation is significant at the 0.01 level (2-tailed), *: Correlation is significant at the 0.05 level (2-tailed)

The data in Table: 1 shows significant correlation between HB concentration, GSH and Hematocritat level 0.01 (two tailed). Also the data indicates that RBC concentration have significant correlation with Hematocrit, MCV, MCH, MCHC and Monocytes, significant correlation between GSH and Hematocrit, significant correlation between MCV & MCH, MCHC, significant correlation between MCH& MCHC, monocyte; significant correlation between MCHC & monocyte, Furthermore significant correlation between Neutrophils &Lymphocytes at level 0.01 (two tailed).

DISCUSSION

Studies indicate that GSH play essential role in providing defense against ROS [12]. However many related chief defensive mechanism beside this can save radicals-mediated tissue damage. Our study revealed the reduced glutathione level among neuropathic pain patient's declines in comparison with the normal healthy individuals. This not only indicate the association of reduce GSH levels with increase amount of ROS but also free radicals as a result of damaged vascular and nervous tissues. Our findings point out towards the imbalance of glutathione antioxidant system that endorse the classic views about oxidative stress and progress of disease. The other major finding with respect to Diabetes and progressed diabetic neuropathy with and without expressed pain is somewhere in the middle unexplored though our results supported the literature about the antioxidants and their power of healing against ROS. Our study revealed that increase production of ROS leads to massive tissue damage that in vicious cycle keep on increasing ROS and it disables the decreasing GSH to cope with this elevated harmful substances. This poor condition of eliminating reactive oxygen species and free radicals from tissues is one of the factors which may further result in the progression of neuropathy [13]. Other than this the causes of increased oxidative stress vary with the age, gender, duration, environment etc. as there are variety of other sources hat can enhance oxidative stress other than Diabetes however is almost impossible to outline all processes and unknown mechanisms that can lead to oxidative stress but preliminary stage of pathophysiological pathway is much important to observe with the help of nature of products formed during oxidation especially of macromolecules accumulating in tissues so that the relationship between oxidative stress and the progress of problems in diabetes can be deal with. The determination of glycated by products is significant study point to observe the level of oxidative stress in relation to hyperglycemia in diabetes and other vascular complication occur as chronic persistant of free radical formation. These glycated products as glycoxidation products of blood and relation of these with other bold indices and GSH, [14] was significant in this study that not only provided an hint of the actual

pathophysiological status of oxidative stress in diabetic neuropathic pain patients but also was a rather than increasing oxidative harm to nerves and vessels [*8*,15]. This was not only helpful in diagnosing the patients properly with respect to their pain intensity but also was effective in recommendation of therapies. In due course with the help of our findings we wanted to do such investigation about expected pathophysiological and biochemical mechanisms that can further lead to the advancement of effectual strategies for restrictive damage from glycation and oxidation of macromolecules [16]. alterations of collagens, elastins, myelin sheath proteins and structural changes in tissues of vascular wall, basement membranes are associated with the development of diabetic Neuropathy (Table 1). The enzyme deficiency may result in mild to moderately severe hemolytic anemia upon exposure to various endogenous chemicals as well as environmental changes though hereditary deficiency of the enzyme can also be a rare a rare case as diabetes mellitus is as micro vascular and metabolic altered snag that results in multifactorial symptoms and ailments . Chronic hyperglycemia might result in abnormal red blood cells, oxidative stress, and lympathetic denervation of the kidney related to autonomic neuropathy. These factors promote a hypoxic environment in the renal interstitium, which leads to impaired production of erythropoietin by the peritubular fibroblasts. Improperly reduced erythropoietin level is an important cause of early anemia in patients with diabetes mellitus [11]. It can be concluded that there is strong significant impact of oxidative stress (low glutathione) on glutathione peroxidase, glutathione reductase level could reduce hemoglobin concentration in diabetic neuropathic patients which is suggested to be a pathophysiological contributor of anemia in diabetic patients with nephropathy [16].

CONCLUSION

The present study suggested the role of oxidative stress in the pathogenesis of diabetic and vascular complication that is indicative for not only many pathways of hyperglycemia also invite further imminent In molecular mechanisms of the metabolic basis and pathophysiological mechanisms lead to painful diabetic neuropathy

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