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## ORIGINAL ARTICLE

# Oxidative Stress Induced Lipid Peroxidation And DNA Adduct Formation In The Pathogenesis Of Multiple Myeloma And Lymphoma

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**Objective:** To assess the oxidative stress status by quantification of byproducts generated during lipid peroxidation and DNA breakdown products generated during DNA damage in the blood serum of multiple myeloma and lymphoma patients.

**Material & Methods:** Case control study comprised of 40 patients of multiple myeloma and 20 patients of lymphoma along with 20 age and sex-matched healthy subjects as controls. Levels of Malondialdehyde and 8-hydroxy-2-deoxy-Guanosine were measured to study the oxidative stress status in the study subjects.

**Results:** The level of markers of DNA damage and lipid peroxidation were found to be raised significantly in the study subjects in comparison to healthy controls. The results indicate oxidative stress and DNA damage activity increase progressively with the progression of disease.

**Conclusion:** Oxidative stress causes DNA damage and Lipid peroxidation which results in the formation of DNA adducts leading to mutations thereby indicate the role of oxidative stress in the pathogenesis of multiple myeloma and lymphoma.

*Key words: Oxidative Stress, Malondialdehyde, 8-hydroxy-2-deoxy-Guanosine, Multiple myeloma, Lymphoma.*

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Multiple myeloma is a cancer formed by malignant plasma cells. Normal plasma cells are found in the bone marrow and are an important

part of the immune system. The exact cause of multiple myeloma is not known. Hodgkin's lymphoma is a cancer of lymph tissue found in the

lymph nodes, spleen, liver, bone marrow, and other sites. Non-Hodgkin's lymphoma is cancer of the lymphoid tissue, which includes the lymph nodes, spleen, and other organs of the immune system. Although the specific etiological factors of multiple myeloma and lymphoma are not yet known, considerable evidence indicate that both genetic and environment may play a role in their evolution.

Oxidative stress, mediated by reactive oxygen species, may result in direct DNA damage as well as in lipid peroxidation, protein modification, membrane disruption, and mitochondrial damage. Some human studies have provided evidence of the potential role of oxidative stress in cancer etiology (Halliwell and Gutteridge, 1999, Marnett, 2000). ROS initiate autocatalytic lipid peroxidation, which generates a large variety of potential genotoxic breakdown products, including alkoxyl radicals (LO.), peroxy radicals (LOO.), and aldehydes, such as malondialdehyde (MDA). MDA is an important lipid oxidative damage product because it has both mutagenic and carcinogenic activity. It can cause cross linking in lipids, proteins and nucleic acids (Freeman, 1982, Flohe et al., 1985). Oxidative stress also damage DNA resulting in the formation of bulky adducts. 8-hydroxy-2-deoxy Guanosine is one of the major product of oxidative damage that is abundant, mutagenic and can be detected reliably (Kasai et al., 1984, Kasai and Nishimura, 1984). This stable pre-mutagenic oxidative modification has also been recognized as a potential marker connoting target organ damage from reactive oxygen species (ROS) and its level is well correlated with the incidence of cancer (Poulsen et al., 1998). An attempt has been made to investigate the level of oxidative damage by quantification of levels of Malondialdehyde and 8-hydroxy-2-deoxy Guanosine in study subjects and controls.

Association between markers of oxidative stress and DNA damage has been studied in relation to the disease to understand the pathogenesis of disease.

## **MATERIALS AND METHODS**

A case control study was conducted in the Department of Biophysics, Institute of Medical Sciences, Banaras Hindu University. The study comprised of 40 patients of multiple myeloma and 20 cases of lymphoma (Hodgkin's and non-Hodgkin's lymphoma) selected from the Department of Medicine, University Hospital, Banaras Hindu University. The controls consisted of 20 healthy volunteers with a socio-economic status similar to that of the patients. Ethical approval and permission for the study was taken from the Ethical Committee of Institute of Medical Sciences, Banaras Hindu University (India). Informed consent was taken from all the study subjects purely for research purpose. With all aseptic precautions blood samples of all study subjects were collected. After centrifuging the blood at low speed, serum was separated and stored at - 20°C for biochemical investigations. All chemicals and reagents used in the study were of analytical grade from Sigma-Aldrich Co, USA.

### ***Estimation of Malondialdehyde (MDA) – A lipid damage marker***

Assay of oxidative damage in the serum of the patients as well as healthy control samples was assessed by measurement of products of lipid peroxidation in serum by the thiobarbituric acid (TBA) method (Burge and Aust, 1978). MDA, which is a stable end product of fatty acid peroxidation, reacts with TBA at acidic conditions to form a complex that has maximum absorbance at 532 nm.

### **Estimation of 8-OHdG - Marker of oxidative DNA damage**

Serum samples of all the study subjects were used for the measurement of 8-OHdG levels using competitive in vitro enzyme-linked immunosorbent assay (ELISA) kit obtained from Cayman Chemical Company U.S.A (Shigenaga and Ames, 1991). 8-OHdG measurements were performed using micro ELISA plate pre-coated with anti-mouse IgG. 50µl sample, 50µl 8-OHdG AChE (Acetylcholinesterase) tracer and 50µl 8-OHdG monoclonal antibody were added to each well and incubated at 40°C for 18 hours. After the wells were washed five times, 200 µl Ellman's reagent was added to each well. The wells were incubated at room temperature in the dark for 100 minutes. The absorbance was read at wavelength of 420 nm. ELISA assay displays IC50 (50% B/B0) and IC80 (80% B/B0) values of approximately 100 and 30 pg/ml respectively.

### **RESULTS**

Our case control study consisted of 40 patients of multiple myeloma and 20 cases of lymphoma (Hodgkin's and non-Hodgkin's lymphoma) who reported for treatment in the Department of

Medicine, University Hospital, Banaras Hindu University, Varanasi. Study also included 20 healthy volunteers who acted as age matched controls. The characteristics of the study group are given in the table 1.

The levels of Malondialdehyde in myeloma patients and lymphoma were found to be elevated in comparison to controls indicating occurrence of the oxidative damage. The difference between these values was statistically significant as shown in Table 2. 8-hydroxy-2-deoxy-Guanosine level was measured to assess DNA damage due to the oxidative stress in multiple myeloma and lymphoma along with normal controls. As shown in Table 2, the mean level of 8-OH-dG was very significantly increased in patients when compared to normal healthy controls.

### **Interrelationships among oxidative stress markers**

Regression analysis conducted on data pooled from all the groups revealed significant association between all examined indices. A significantly positive relationship was found between 8-OHdG and MDA in both Multiple myeloma and lymphoma (Figure 1 and Figure 2).

**Table 1:** Characteristics of patients

	Multiple Myeloma	Lymphoma
Number of patients	40	20
Age		
<50	20	16
>50	20	4
Duration of Disease		
<6 months	26	13
>6 months	14	7
Stage	Durie Salmon stage	Ann Arbor classification
I	5	4
II	9	8
III	26	5
IV	-	3

**Table 2:** Levels of Malondialdehyde (mmol/L) and 8-OHdG (pg/ml) in patients with Multiple myeloma, Lymphoma and Controls

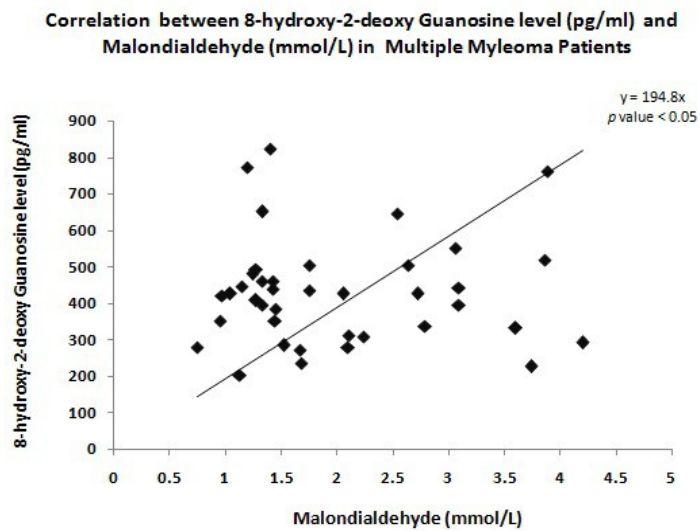
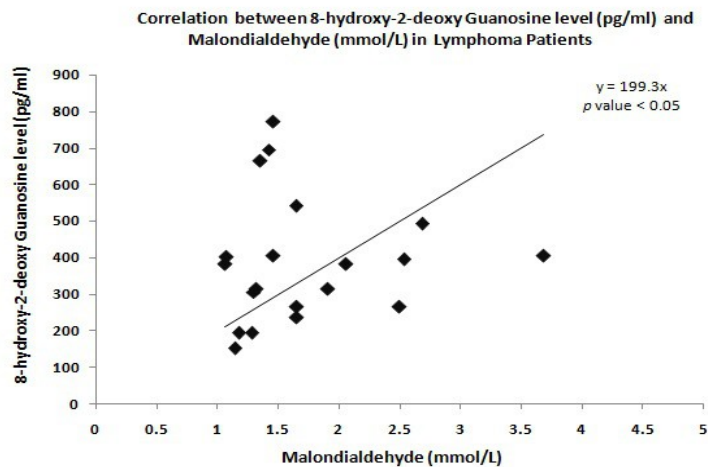
Groups	Malondialdehyde (mmol/L)	8-OH dG (pg/ml)
Multiple myeloma	1.98 ± 0.95*	501.75 ± 480.39**
Lymphoma	1.74 ± 0.68#	388.91 ± 170.91##
Controls	1.12 ± 0.38	177.69 ± 86.07

\*Multiple myeloma vs. Controls T value= 3.558; *p* value 0.001

#Lymphoma vs. Controls T value = 3.387; *p* value 0.002

\*\* Multiple myeloma vs. Controls T value= 2.828; *p* value 0.001

##Lymphoma vs. Controls T value= 4.727; *p* value 0.001

**Figure 1:** The scatter graph shows correlation between MDA level (mmol/L) and 8-hydroxy-2-deoxy Guanosine level (pg/ml) in patients with multiple myeloma. The graph shows with the increase in the MDA level there is an increase in the level of 8-hydroxy-2-deoxyguanosine and the correlation is statistically significant.**Figure 2:** The scatter graph shows correlation between MDA level (mmol/L) and 8-hydroxy-2-deoxy Guanosine level (pg/ml) in patients with lymphoma. The graph shows with the increase in the MDA level there is an increase in the level of 8-hydroxy-2-deoxyguanosine and the correlation is statistically significant.

**DISCUSSION**

ROS are free radicals and other molecules with unpaired electrons (such as  $O_2^-$  and  $H_2O_2$ ) that are highly reactive and can react with biologic macromolecules, peroxidise lipids to form mutagenic MDA, modify the structure and function of proteins, and cause oxidative damage to DNA (Márquez et al., 2007, Ottaviano et al., 2008 and Marnett et al., 2003). Although these modifications can be efficiently removed by repair mechanisms, a persistent increase in ROS could lead to accumulation of mutations resulting in tumor induction (Basu et al., 1989, Hwang and Bowen, 2007 and Ishikawa et al., 2008). Under normal conditions, intracellular ROS are maintained at a low level by various enzyme systems which maintain the in vivo redox homeostasis. Excessive production of ROS or inadequacy in a normal cell's antioxidant defense system (or both) can cause the cell to experience oxidative stress. Tumor cells usually have an imbalanced redox status resulting in the damage to DNA, proteins and lipids. Higher levels of DNA damage and deficient DNA repair may predispose individuals to cancer (Ishikawa et al., 2008). Under these circumstances, the cells may develop cytogenetic alterations, such as deletions, amplifications and/or mutations in critical oncogenes and tumor suppressor genes, leading to cellular transformation and neoplasm (Kumar et al., 2008 and Valko et al., 2006). Thus, an elevated exposure to genotoxic agents may contribute to hematological malignancies. Also the increased ROS may play a broader role in cellular processes associated with initiation and development of many cancers.

The present study was designed to assess the role of free radicals (in terms of byproducts generated during lipid peroxidation) and DNA break

down products generated during DNA damage. MDA was assessed to demonstrate the free radical activity and 8-hydroxy 2- deoxy Guanosine was assessed to demonstrate the DNA damage activity. In our study, the mean 8-hydroxy-2-deoxy Guanosine levels and MDA levels in Multiple myeloma and lymphoma cases were higher than healthy controls. The difference between these values were statistically significant ( $p < 0.001$ ). The correlation between MDA level and 8-hydroxy-2-deoxy Guanosine levels in studied subjects shows with the increase in the MDA level there is an increase in the level of 8-hydroxy-2-deoxy guanosine and the correlation is statistically significant ( $p < 0.001$ ).

Thus, we infer that increased level of oxidatively modified DNA and lipids observed in our study subjects creates a picture of increased oxidative stress in the system. Oxidative stress by definition is the difference between pro-oxidants and antioxidants (redox imbalance). Also a positive association is found between the malondialdehyde and 8-OHdG levels, and malignancy risk. Both oxidative stress and DNA damage increases significantly in cases of multiple myeloma and lymphoma and the rise in the level of one is simultaneous to the rise in other suggest an association between the two which is also evident from the correlation studies. We hypothesize that an altered pro-oxidant-antioxidant balance may lead to an increased oxidative damage and consequently play an important role in multiple myeloma and lymphoma.

Potential role for ROS in the regulation of cellular process controlling malignant transformation holds a lot of promise in understanding etiology and progression of multiple myeloma and lymphoma.

## CONCLUSION

DNA damage has largely been emphasized as the major factor of pathogenesis in the progression of the disease. The identification of genetic factors that predispose individuals to disease may be useful in the development of diagnostic tests to help identify those at-risk for this cancer, leading to early diagnosis and treatment, as well as the identification of new drugs.

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