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Int J Hum Genet, 10(4): 247-250 (2010)
PRINT: ISSN 0972-3757 ONLINE: 2456-6360
DOI: 10.31901/24566330.2010/10.04.07

Changes in Anti-Oxidant Enzyme Profile during Haematological Malignancy

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KEYWORDS Blood Cancer. Glutathione Peroxidase. Superoxide Dismutase

ABSTRACT Reactive oxygen species can initiate lipid peroxidation and DNA damage leading to mutagenesis, carcinogenesis and cell death, if the antioxidant system is impaired. The present work aimed to study the changes in the quantitative *in vitro* activities of the antioxidant enzymes – glutathione peroxidase and superoxide dismutase in the whole blood of our study group. Our study group included both blood cancer patients and age, sex matched healthy controls. We found significant decreases in the activities of both glutathione peroxidase and superoxide dismutase in patients as compared with healthy controls. Our previous work has indicated elevated arsenic concentration in the biosamples of patients. Thus, haematological malignancy is found to be associated with changes in the antioxidant defense system which is correlated with arsenic toxicity.

INTRODUCTION

Haematological malignancy is a type of cancer which affects the way the body makes blood and provides immunity from other diseases, the major forms being leukemia, lymphoma and multiple myeloma. Another closely associated haemato-logical disorder is myelodysplastic syndrome which results in ineffective production of blood cells and varying risks of transformation to acute leukemia as stated by Besa (1992).

West Bengal is one of the worst arsenic affected areas of the world. Das et al. (1995) estimated that over 8 lakh people in West Bengal are chronically exposed to arsenic through drinking water.

It was found by Zima et al. (1996) that reactive oxygen species (ROS) and other free radicals are the mediators of phenotypic and genotypic changes that lead from mutation to neoplasia. Anti-oxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPx) protect against the harmful effects of ROS. Genetic variation in the genes coding for these enzymes (SOD2 and GPX1, respectively) alters ROS production and therefore may provide a mechanism for the incidences of cancer was confirmed by Lightfoot et al. (2006).

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Previous studies have reported lowered activities of GPx and SOD during haematological malignancies (Bakan et al. 2003; Bewick et al. 1987). Earlier studies have also reported that arsenic induces oxidative stress by lowering activities of antioxidant enzymes – GPx and SOD (Nandi et al. 2005; Ganyc et al. 2007; Das et al. 2005). The present work aimed to study the anti-oxidant enzyme profile changes among the increasing incidence of haematological malignancy cases in West Bengal, which is one of the worst arsenic affected areas of the world.

METHODOLOGY

Study Group: Our study group comprised of 100 individuals which included both untreated blood cancer patients (n=70) and age, sex matched healthy controls (n=30). The control population was from arsenic nonaffected area. Haematological malignancy cases studied included leukemia, lymphoma, multiple myeloma and myelodysplastic syndrome. The studies involving human subjects were reviewed and approved by the Ethical Committee of the Institute. Informed consent was obtained from the patients.

Estimation of Glutathione Peroxidase Activity: This method is based on Paglia and Valentine (1967). Glutathione Peroxidase catalyzes the oxidation of Glutathione (GSH) by Cumene Hydroperoxide. In the presence of Glutathione Reductase and NADPH the oxidized Glutathione (GSSG) is immediately converted to

the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340nm is measured.

Quantitative *in vitro* determination of glutathione peroxidase activities in whole blood were estimated with 0.05ml heparinized whole blood using Ransel glutathione peroxidase assay kit (Randox, United Kingdom). The samples were assayed by UV-Visible spectrophotometer (Spectronic, USA) at a wavelength of 340nm. Appropriate negative and positive controls were maintained with each batch of estimation.

Estimation of Superoxide Dismutase Activity: This method based on Woolliams et al. (1983) employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of I.N.T. under the conditions of the assay.

Quantitative *in vitro* determination of superoxide dismutase activities in whole blood were estimated with 0.5ml heparinized whole blood using Ransod superoxide dismutase assay kit (Randox, United Kingdom). The samples were assayed by UV-Visible spectrophotometer (Spectronic, USA) at a wavelength of 505nm. Appropriate negative and positive controls were maintained with each batch of estimation.

Statistical Analysis: In all cases, the data was analyzed statistically following the Student's *t* test.

RESULTS

Decreased Anti-Oxidant Enzyme Activity in Cancer Patients: Quantitative *in vitro* glutathione peroxidase activity was estimated in both patients and healthy controls. It was found that glutathione peroxidase activity in healthy individuals was 31.05 ± 1.1 U/g Hb and that in patients were 18.14 ± 0.21 U/g Hb. Thus, the glutathione peroxidase activity of patients showed about 2 folds decrease as compared to healthy individuals ($p \leq 0.001$). Quantitative *in vitro* superoxide dismutase activity was estimated in both patients and controls. It was found that superoxide dismutase activity in healthy individuals was 1608.5 ± 13.47 U/g Hb and that in patients were 958.5 ±11.11 U/g Hb. Thus, the superoxide dismutase activity of patients showed about 40% decrease as compared to healthy individuals ($p \leq 0.001$) (Table 1).

Table 1: Anti-oxidant enzyme activities in the study group

Types	*Glutathione Peroxidase activity (U/g Hb)Mean±S.E*	*Superoxide Dismutase activity (U/g Hb)Mean±S.E*
Healthy	31.05 ± 1.1	1608.5 ± 13.47
Patient	18.14 ± 0.21*	958.5 ± 11.11*

*Statistically significant at pd"0.001 (Fisher's *t* test)
S.E. = Standard Error

***Distribution of Anti-Oxidant Enzyme Activities among Different Patient Groups*:** Glutathione peroxidase activities were studied in the different groups of patients. It was observed that multiple myeloma patients showed the lowest glutathione peroxidase activity values among all groups of patients. Superoxide dismutase activities were studied in the different groups of patients. It was observed that multiple myeloma patients showed the lowest superoxide dismutase activity value among all groups of patients (Table 2).

Table 2: Anti-oxidant enzyme activities among different patient groups

Types of patients	*Glutathione Peroxidase activity (U/g Hb) Mean±S.E*	*Superoxide Dismutase activity (U/g Hb) Mean±S.E*
Acute myeloid leukemia	18.5±0.19	968.7±10.6
Acute lymphoid leukemia	18.9±0.24	955.7± 9.5
Chronic myeloid leukemia	19.1±0.17	979.1±12.6
Chronic lymphoid leukemia	17.4±0.21	956.6±10.4
Hodgkin's lymphoma	17.6±0.22	948.4±11.8
Non- Hodgkin's lymphoma	18.5±0.18	967.4±11.5
Multiple myeloma	16.9±0.27	940.2±10.6
Myelodysplastic syndrome	18.2±0.23	952.2±11.9

S.E. = Standard Error

DISCUSSION

Devi et al. (2000) reported that reactive oxygen species can initiate lipid peroxidation and DNA damage leading to mutagenesis, carcinogenesis and cell death, if the antioxidant system is impaired.

Glutathione peroxidase (GPx) enzymes are a group of selenoproteins that are necessary to regulate intracellular concentration of hydroperoxides and thought to play a role in antioxidant defense as stated by Holben and Smith (1999). GPx is a selenium containing tetrameric glycoprotein. Changes in GPx activities

have been noted in different type of haematological malignancies. Lowered GPx activities have been observed in chronic lymphocytic lymphoma patients by Bakan et al. (2003), in leukemia by Zhou et al. (2007) and in non-Hodgkin's lymphoma by Lightfoot et al. (2006). In our study we have observed a significantly lowered GPx activity in patients as compared with controls. This lowered glutathione peroxidase activity in patients is an indicator of lowered selenium status which in turn is directly correlated with increased cancer risk and progression.

Intracellular antioxidant enzymes like superoxide dismutase (SOD) are responsible for the removal of ROS such as superoxide free radicals. SOD is an essential primary antioxidant enzyme that converts superoxide radical to hydrogen peroxide and molecular oxygen within the mitochondrial matrix. SOD activities have been studied in haematological malignancy cases. Lowered SOD activities have been noted in malignant lymphoma by Bewick et al. (1987), in chronic lymphocytic lymphoma by Bakan et al. (2003) and in acute myeloid leukemia by Saito et al. (1984). Our study revealed significantly lowered SOD activity in patients. These results suggest an abnormality in the regulation of the expression of the SOD gene in the pluripotent stem cells. This in turn leads to impairement of the antioxidant defense system as stated by Gonzales et al. (1984).

We have studied the anti-oxidant enzyme activity in haematological malignancy cases and found it to be around 2 folds lowered than in controls. On studying this anti-oxidant enzyme activity in detail in the various groups of haematological malignancy cases we have found it to be decreased in all the groups which is in conformity with the activity in the patient group as a whole. However, the level of decrease varied with the type of haematological malignancy, being the lowest in multiple myeloma patients.

Pi et al. (2002) reported that chronic exposure of arsenic through drinking water among Chinese residents results in induction of oxidative stress. Our previous studies have revealed that the arsenic concentrations in the biosamples (hair, nail) in patients were around 3 to 4 folds higher than in controls, having values above 1000µg/kg i.e. in the toxic range. This high arsenic concentration correlated with about 2 folds decrease in the anti-oxidant enzyme activity in patients as compared with controls.

Thus, it can be said that the genotoxic mechanism of arsenic involves decreased activities of oxygen-radical-scavenging enzymes such as GPx, SOD.

Our study area involves only haematological malignancy case reports. Our study has indicated that the mechanism of carcinogenic activity of arsenic is associated with the impairment of the anti-oxidant defense system of the body. As epidemiologic studies have provided substantial evidence for the association of arsenic with cancers of the skin (non-melanoma), lung, and bladder (Chiou et al. 1995; Tsuda et al. 1995), so we may presume that the anti-oxidant activity in arsenic associated malignancies other than haematological malignancies may also be lowered.

CONCLUSION

Thus, our study indicates that arsenic toxicity is positively correlated with increased carcinogenicity and negatively correlated with the cellular antioxidant defense which may be one of the factors for the increasing incidence of haematological malignancy in West Bengal.

RECOMMENDATION

Osur findings indicate the importance in formulating a public health policy regarding treatment of arsenic contaminated water which may in turn help in reducing the increasing incidences of haematological malignancy cases in West Bengal.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support for the present work provided by Council of Scientific and Industrial Research, New Delhi -110012, India. The authors are also grateful to the Secretary, Ramakrishna Mission Seva Pratisthan, Kolkata, India for kind co-operation and support.

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