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ORIGINAL ARTICLE

Alteration in MDA, GSH level and hematological changes due to thiamine deficiency in *Mus musculus*

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ABSTRACT

It is known that thiamine deficiency may lead to Alzheimer's diseases in humans. The present study has thus been conducted to understand the role of thiamine deficiency with respect to alteration in the peripheral blood of Swiss albino mice. For this purpose, adult Swiss albino mice (6–8 week old) were divided into three groups. The first group was control; the second (group II) and the third group (group III) were made thiamine deficient for 08 and 10 days respectively. Thiamine deficiency was induced in mice by injecting pyrithiamine (5 µg/10 g bwt) and feeding a thiamine deficient diet. The erythrocytes, leukocytes count, hemoglobin, hematocrit value, mass cell volume, mean corpuscular hemoglobin in blood of mice were determined by hematoanalyzer. Malondialdehyde (MDA) and reduced glutathione (GSH) level was also determined in serum of treated and non-treated groups. A significant reduction in leukocyte and erythrocyte count was observed in both the thiamine deficient groups as compared to control. Levels of hemoglobin and hematocrit value were also declined in the thiamine deficient groups. Enhancement in mass cell volume (MCV) level and decline in mean corpuscular hemoglobin (MCH) levels were observed in both thiamine deficient groups with respect to control. Inter-group comparison of all parameters also showed a significant value at $p<0.01$. In comparison with the control group, elevation in MDA and decline in GSH level was observed in both thiamine deficient groups which were statistically significant. These data indicate that thiamine deficiency leads to significant alterations in the hematological parameters as well as in MDA and GSH level.

KEY WORDS: blood; hematological alterations; thiamine deficiency; Alzheimer's diseases

Introduction

Nutrition is one of the basic needs for proper functioning of various organ systems. Early malnutrition may have harmful effects not only on the immune function but also on the development of the nervous system, gastrointestinal tract, and it can cause metabolic syndrome (He *et al.,* 2009). Thiamine (vitamin B1) is a water soluble vitamin and cannot be synthesized. Thiamine is essential in the body for energy metabolism, specifically 0.33 mg of thiamine is required for generation of 4400 kJ of energy (Smithline *et al.,* 2012). The primary active form of thiamine is thiamine diphosphate (ThDP). It is a cofactor of several multienzyme complexes related to glucose metabolism, *i.e.* pyruvate dehydrogenase complex (PDHC), α-ketoglutarate dehydrogenase complex, and transketolase. Thiamine deficiency (TD) provides a pertinent experimental system to understand the neurodegenerative disorder in which mitochondrial dysfunction attributes to the failure of tricarboxylic citric acid (TCA) cycle enzymes (Sheu *et al.,* 1998; Sharma *et al.,* 2013).

Further, blood is a protective, regulatory, and homeostatic connective tissue and consists of blood cells and plasma (Nasyrova *et al.,* 2006; Eze *et al.,* 2010). It is an essential medium which circulates around the body within the cardiovascular system and acts as a transportation system for many substances, such as O_2, CO_2, drugs, hormones and xenobiotics. In blood, transportation of oxygen is achieved through the presence of hemoglobin (Ashton, 2013). Blood profiles in living beings determine the internal environment and recognizing the causes of impairment in homeostasis as corroborated by marked fluctuations in physiological indices in different internal and external environmental conditions (Koubkova, 2002; Sattar & Mirza, 2009). Blood values are used for

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determining the level of stress as well as the well-being of the animal. Hematological examination is a manifestation of an animal's responses to its external and internal environments (Koubkova *et al.,* 2002; Carlosa *et al.,* 2015), nutritional deficiencies and stress (Agarwal *et al.,* 2016). The hematological parameters are biological tools to assess alterations in the health and physiological status which could not be detected during physical examinations (Kronfeld & Medway, 1969). Thiamine deficiency might affect hematopoiesis and various other organ systems. Further, erythrocytes are the most important blood cells in the human body. The main function of red blood cells (RBCs) is to carry oxygen to the cells during respiration (Johnston & Morris, 1996; Chineke *et al.,* 2006). Decrease in the level of RBC's causes anemia (Tejashwini & Padma, 2015), White blood cells are an important part of the body's immune system. They protect against certain bacteria, viruses, cancer cells, infectious diseases. Low white blood cell counts (WBC) may indicate that a person is in risk of infection, whereas high WBC counts generates antibodies in phagocytosis and high degree of resistance to diseases (Soetan *et al.,* 2013) and might indicate an existing infection and tissue damage (Tejashwini & Padma, 2015).

Additionally, hemoglobin (Hb) is the oxygen-carrying protein in blood, providing an indication of the capacity of the blood to oxygenate the tissue for oxidation of ingested food to release energy for the other body functions as well as to transport carbon dioxide out of the body (Ugwuene, 2011; Omiyale *et al.,* 2012; Isaac *et al.,* 2013; Soetan *et al.,* 2013). Further, thiobarbituric acid reactive substance (TBARS) is the end product of lipid peroxidation (LPO), which is an important event induced by oxidative stress related to the pathogenesis of several diseases (Halliwell & Gutteridge, 1969; Reznick & Packer, 1993). Increased reactive oxygen species (ROS) production in thiamine deficiency can trigger cell membrane damage, including LPO and alterations in the functional integrity of ion channels and transporters (Jhala & Hazell, 2011). GSH acts as a direct scavenger and is a major intracellular redox tampon system (Blokhina *et al.,* 2003). It is a tripeptide containing cysteine that has a reactive sulfhydryl group with reductive potency, and thus it plays a critical role in detoxification of ROS (Urso & Clarkson, 2003; Jozefczak *et al.,* 2012). It has facile electron donating capacity linked to its sulfhydryl group (Kidd, 1997). GSH removes the free radicals overproduced and decreases its cellular concentration offering a defense against oxidative stress. Glutathione is one of the major outcomes of free radical-mediated injury leading to the production of a range of quite stable end products that are capable to initiate LPO (Ferreiro *et al.,* 2012). Previous studies suggest that thiamine deficiency reduces the concentration of thiamine in the spleen (Fitzsimons *et al.,* 2005; Ketola *et al.,* 2008). In this perspective, the present study was conducted to identify the effect of thiamine deficiency in hematological variables, as well as the MDA and GSH level in Swiss albino mice.

Materials and methods

Chemicals

Pyrithiamine hydrobromide was procured from Sigma (MO, USA). 5, 5'-dithiobis-2-nitrobenzoic acid, (DTNB), ethylene diamine tetraacetic acid (EDTA), tris hydrochloride, trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA) were purchased from Sisco research laboratories (Mumbai, India). All other chemicals were used of analytical grade.

Animal care and monitoring

Swiss albino Male mice (6–8 week old) were procured from C.C.S. Haryana Agricultural University, Hisar. They were fed pelleted diet (Hindustan Uniliver Limited) and water *ad libitum*. After 7 days of adaptation, the mice were used for experimental purpose. Maintenance and treatment of animals was done in accordance with the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), New Delhi.

Experimental design

The animals were divided into three groups with minimum of six animals in each group and treated as follows:

Group I	Control
Group II	Thiamine deficient for 08 days (TD 08)
Group III	Thiamine deficient for 10 days (TD 10)

Induction of thiamine deficiency (TD)

Mice were made thiamine deficient by injecting the pyrithiamine hydrobromide (5 µg/10 g of body weight) intraperitoneally daily for 8 and 10 days and fed with thiamine deficient pelleted diet (MP Biomedical, Mumbai, India). Control animals were fed a normal diet.

Determination of hematological parameters

After the 8 and 10 days exposure to thiamine deficient diet and pyrithiamine hydrobromide, blood was collected from retro-orbital sinus of mice. 20 µl of blood of different experimental groups were kept in EDTA vial and 500 µl sample diluent was added. The samples were loaded on the hematoanlayzer (PocH-100i). Hematological parameters such as RBC, WBC count, hemoglobin (Hb), hematocrit value (HCT), mass cell volume(MCV) and mean corpuscular hemoglobin(MCH) were determined in control and treated groups.

Estimation of malondialdehyde (MDA) and GSH level

Serum separation

After exposure duration, whole blood samples were drawn from retro orbital sinuses of control and treated mice. Blood samples were collected and allowed to clot for half an hour and centrifuged at 3500 rpm for 10 min at 4 °C. Serum was isolated and used for determination of oxidative stress markers: thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH).

Estimation of MDA level

MDA assay has been widely used to measure LPO. MDA is one of the end products derived from the breakdown

of polyunsaturated fatty acids and related esters. MDA (LPO product) reacts with TBA and forms MDA-TBA adduct which gives a characteristically pink color in high temperature with acidic environment. The LPO product was read at 512 nm spectrophotometrically (Ohkawa *et al.*, 1979).

Assessment of reduced glutathione (GSH) level

GSH level served as an index for determining the extent of oxidative stress. GSH was determined by Ellman's method. First, the serum sample is made protein free and then reduced glutathione reacts with DTNB and forms 5-mercapto-2-nitorbenzoic acid giving a light yellow color. Spectrophotometrically reduced glutathione level was measured at 412 nm (Ellman's 1959).

Data analysis and statistics

The data were represented as Mean±SEM. The statistical analysis was done by using one-way analysis of variance (ANOVA) (Statistical Package of Social Science editor 16). Intergroup comparisons were made by *post-hoc* comparison analysis and significant level was measured at 99%.

Results

The data on erythrocyte count, Hb level and MCH level indicated a significant decline in all three parameters in both 8- and 10-day thiamine deficient mice in comparison to control (Figure 1). The hematocrit value also showed a similar pattern of changes (Figure 2) as erythrocyte count and Hb level. The decline in MCH level was more pronounced in 10-day thiamine deficient mice with respect to 8 days and this difference was statistically significant (Figure 2). Whereas MCV was increased significantly in 8 and 10 days thiamine deficient groups as compared to non-thiamine deficient group (Figure 2).

The leukocyte count declined significantly ($p<0.01$) in thiamine deficient groups for 8 and 10 days with respect to control group. The decline was more pronounced in 10-day thiamine deficient mice with respect to 8 days, which was statistically significant (Figure 3).

GSH level was considerably decreased ($p<0.01$) in both the thiamine deficient groups as compared to control group (Figure 4). Maximum reduction of GSH level was observed in 10-day thiamine deficient group as compared to the 8-day thiamine deficient group and non-treated group which was statistically non-significant.

The level of MDA was significantly ($p<0.01$) higher in the serum of both the treated groups as compared to control group. The elevation of MDA level was more pronounced ($p<0.01$) in the mice of 10-day thiamine deficient group with respect to 8-day deficient mice (Figure 5).

Discussion

Thiamine is an essential water-soluble vitamin and its availability is a prerequisite for normal cellular

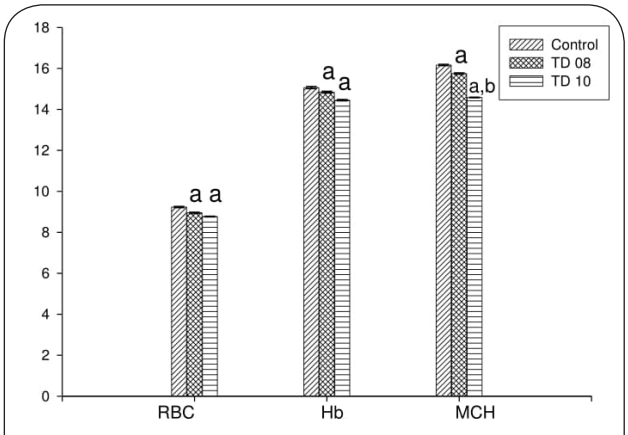

Figure 1. Erythrocyte count (10^6/µl). Hemoglobin (g/dl) and mean corpuscular hemoglobin level (pg) of peripheral blood of thiamine deficient and non-thiamine deficient mice. a- $p<0.01$ with respect to control; b- p<0.01 with respect to 8 days thiamine deficient group.

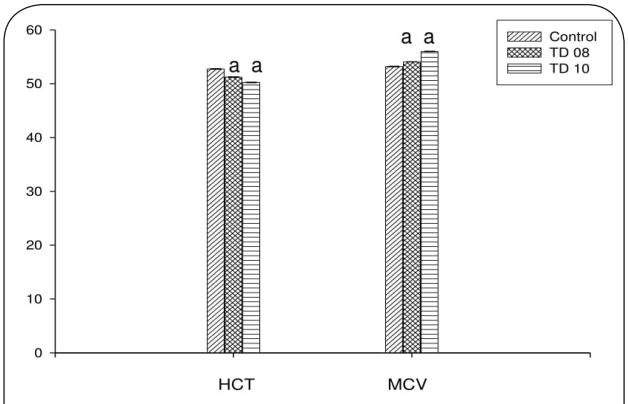

Figure 2. Hematocrit value (%) and mass cell volume (fl) of peripheral blood of thiamine deficient and non-thiamine deficient mice. a- $p<0.01$ with respect to control.

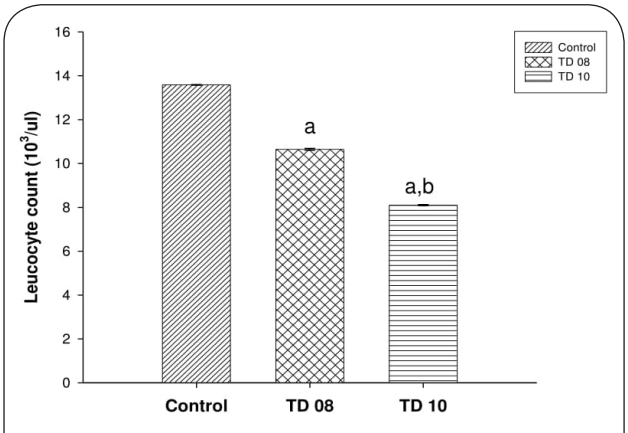

Figure 3. Leucocyte count of peripher blood of thiamine deficient and non-thiamine deficient mice. a- $p<0.01$ with respect to control; b- p<0.01 with respect to 8 days deficient group.

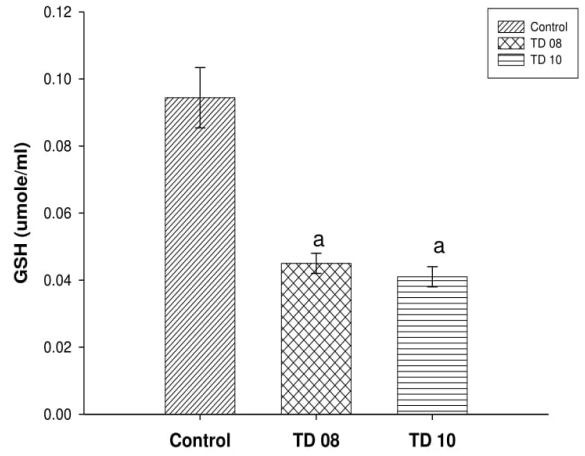

Figure 4. GSH level of serum of thiamine deficient and non-thiamine deficient mice. a- $p<0.01$ with respect to control.

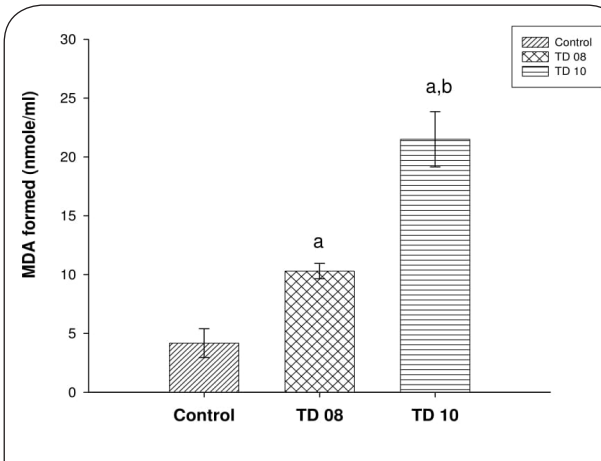

Figure 5. MDA level of serum of thiamine deficient and non-thiamine deficient mice. a- $p<0.01$ with respect to control; b- $p<0.01$ with respect to 8 days deficient group.

metabolism of the brain (Liu *et al.,* 2016) and needed for various other physiological functions of the body. It serves as a specific cofactor of certain enzymes involved in energy metabolism of cells and its deficiency may affect enzymes of the TCA cycle (Sharma *et al.,* 2013; Sharma & Bist, 2014). Thiamine deficiency may also be associated with brain degenerative conditions such as Parkinson's and Alzheimer's disease (Hazell & Butterworth, 2009; Hazell, 2009; Karuppagounder *et al.,* 2009; Hirsch &Parott, 2012). Blood is a loose connective tissue which is first to be affected by a stress introduced into the body and therefore proposes an insightful and consistent indicator, which could be effectively used to assess the magnitude of oxidative stress (Lakshmanan *et al.,* 2013). The results showed alterations in the erythrocyte and leukocyte count, Hb, HCT, MCH, MCV in peripheral blood of thiamine deficient groups as compared to control. The decline in erythrocyte count may be due to inhibition of pyrimidine 5-nucleotidase that results in an accumulation of nucleotides in the erythrocyte. This enzyme inhibition and nucleotide accumulation affect erythrocyte membrane stability and survival by alteration of cellular energetics. Falahtakar *et al.* (2014) reported another reason for the reduction in RBC and Hb level as due to altered hematopoiesis. It directly affects the blood forming organs which results in the excessive destruction in RBC synthesis (Badraoui *et al.,* 2011). The decline in RBC count in peripheral blood generates free radicals and causes oxidative stress (Stohs, 1990; Fibach *et al.,* 2008), supporting the present study by observing the elevation in MDA and decline in GSH level in the serum of thiamine deficient groups.

Further, decrease in leukocyte count was observed in the thiamine deficient group and Hb, MCH hematocrit value was also decreased in exposed animals with thiamine deficiency. It may reflect anemia which is often mainly due to destruction of erythrocytes. Nevertheless, the decline in erythrocyte count is ~3% (TD 08 days) and ~5 %(TD 10 days) as compared to reduction in leukocyte count, which was 21.6% (TD 08 days) and 40.3% (TD 10 days) with respect to control group, indicating more pronounced changes in leukocyte count then erythrocyte count.

The data of the present study show the elevation in MDA level and reduction in GSH level in thiamine deficient mice which may act as key markers of oxidative stress. Several previous reports showed an increase in MDA level due to thiamine deficiency in different organs, such as heart (Shangari *et al.,* 2003), liver and brain of thiamine deficient mice (Sharma *et al.,* 2013; Sharma & Bist, 2014), exhibiting the conditions of stress. As the thiamine deficiency increases MDA level which represents the direct relation with dose and duration. GSH level in serum was found to be reduced in thiamine deficient exposed group as compared to control. These results are in agreement with earlier findings of Sharma *et al.* (2013; 2014) regarding the reduction in GSH level in brain mitochondria as well as liver tissue in thiamine deficient mice. Earlier, Shangari *et al.* (2003) also reported a reduction in cellular GSH level in rat hepatocytes under thiamine-deficient conditions. Glutathione is involved in various cellular functions, ranging from the control of physical and chemical properties of cellular proteins and peptides to the detoxification of free radicals. Reduction in GSH level and increase in MDA level promotes stress in serum of thiamine deficient group, which acts as a key marker of oxidative stress.

Conclusion

The current study concluded that thiamine deficiency alters the changes in hematological parameters and induces oxidative stress in Swiss albino mice, which may lead to neurodegeneration.

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