Annals of Health & Health Sciences
Vol. 1, No. 2 July-December 2014, 139-142
DOI: 10.5958/2322-0422.2014.00530.X

Short Communication

A Study of the Effect of Isolated Folic Acid Deficiency on Lens in Adolescent Rats

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ABSTRACT

Background and Objectives: India accounts for 20% of the world's blind population and 90% of the reversible blind people are from developing nations. Micronutrients like folic acid have been linked to cataract and 33% of the Indian population suffers from folate deficiency. This study was done to find evidence of cataractogenesis in the younger age group of folate-deficient people. **Methods:** After taking animal ethical clearance, 12 Wistar albino rats weighing 120–150 grams and corresponding to 18–20 years of human age, were fed folate-deficient diet for 45 days and sacrificed on the 46th day. The enucleated eyeballs were subjected to histopathological examination, under light microscopy, for any lens abnormality. **Results:** The study stated that the lens capsule epithelium and the arrangement and regularity of the lens fibers were normal. There was no evidence of thickening, degeneration, swelling or disruption of the lens fibers. **Conclusion:** It was inferred that the lens cells could store folic acid as intracellular folylpolyglutamate or bound to internalized receptor proteins. Folic acid is essential in the synthesis of an important antioxidant Glutathione from glutamic acid, cysteine and glycine. Glutathione in turn protects the cells from oxidative damage incurred during normal metabolic and optical activities in the eye lens.

Keywords: Folic acid deficiency, Cataract, Free radicals, Glutathione redox system, Antioxidant, Formiminoglutamic acid

INTRODUCTION

India is one of the participants of the vision 2020 program of World Health Organization (WHO). With a population of one billion, it accounts for 20 percent of the 37 million blind population across the globe. Cataract accounts for 47.8% of all reversible blindness and of these, 90% of cataract blindness is seen in developing countries[1,2,3].

A number of indicators of poor nutrition have been found to be associated with increased risk of cataract. Lack of micro nutrients in the diet, one of which is folic acid, can be a cause for cataractogenesis. Folate deficiency, which has traditionally been linked to poverty, afflicts ~ 33 percent of the Indian population[4]. Folic acid is a naturally occurring water soluble Vitamin B9. This vitamin is found in foods such as dark green leafy vegetables, citrus fruits and liver[5]. Along with folate, Vitamins B6 and B12 are critical in the production of glutathione, an antioxidant and protective enzyme which helps prevent or limit oxidative damage to the cells. Conversion of homocysteine to cysteine leads to the synthesis of glutathione from glutamic acid and glycine[5,6].

In the body, folate functions as a coenzyme of co-substrate in the synthesis of nucleic acids (DNA and RNA) and amino acids and is essential in the cell for the synthesis of Thymidine triphosphate (dTTP) from deoxyuridine monophosphate (dUMP). Under folate-deficient conditions uracil is incorporated into DNA instead of thymidine, leading to chromosome breakage and micronucleus formation[7,8]. Deficiency of folate and other methyl donors may also result in telomere instability by causing inadequate maintenance of methylation in the sub-telomeric sequences, which leads to telomere dysfunction[9,10].

Free radicals are generated by the normal activity of mitochondria, peroxisomes, lipoxygenases, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and cytochrome p450 as well as by normal metabolic processes and by absorption of light. Glutathione plays a considerable role as an antioxidant here. The human eye is constantly exposed to light both natural and artificial. Light transmission through the optical system is vital for vision and the body's circardian rhythm[11]. Exposure to ultraviolet radiation can cumulatively have an effect on the crystalline lens and manifest as cataract. There have been many articles which have linked folic acid deficiency with cataract formation in the above 40 years age group[5].

Our study aims to understand the relationship between folic acid deficiency and its effect on lens in rats whose age correlates to about 18–20 human years[12].

MATERIALS AND METHODS

During a previous research work on the in vitro effect of methanol on folate-deficient rat hepatocytes[13], twelve Wistar strain albino rats of either sex, in their post weaning period, (weighing 120–150 gm) were studied in 2001. Ethical clearance was obtained. The rats were maintained under standard laboratory conditions and were fed a folic acid deficient diet[14] and water ad libitum. The rats were divided into two groups of six rats each.

Group A rats were fed a folate-deficient diet for 45 days and at the end of this period their folate-deficient status was assessed and confirmed by formiminoglutamic acid (FIGLU) excretion in urine[15]. Group B were similarly fed a folate-deficient diet, but intragastric administration of Vitamin E (400 mg/kg BW) was done for this group of rats for the last 7 days of the 45 day folate-deficient diet schedule. FIGLU test confirmed their folate-deficient status, too. On the 46th day, the animals were sacrificed. Their hepatocytes were extracted and subjected to methanol insult after which the reduced glutathione (GSH), glutathione reductase (GSSG-R), Catalase and superoxide dismutase (SOD) levels were estimated. These levels were found to be markedly reduced in Group A as compared to Group B[13].

During the same time, the rats were enucleated and their eyeballs were preserved in formalin. The eyeballs were embedded in paraffin wax blocks; sections were cut and mounted on slides. They were stained with hematoxylin-eosin and studied under light microscopy by a competent histopathologist for any abnormalities in the lens.

RESULT

The report stated that the lens capsule epithelium and the arrangement and regularity of the lens fibers were normal. There was no evidence of thickening, degeneration, swelling or disruption of the lens fibers. Hence, the report concluded that there was nothing abnormal detected in any of the eye lens specimens.

Table 1: Effect of Folic acid Deficiency on Hepatic Enzymes and Lens

Parameters	Group A (n = 6) Folate-Deficient Diet	Group B (n= 6) Folate-Deficient Diet + Vitamin E
FIGLU Test	+	+
Hepatocytes (GSH,GSSG-R, SOD)	Significantly reduced	Not significantly reduced
Lens: Histopathology	No abnormality	No abnormality

DISCUSSION

In our study no cataractous changes were observed in the lenses of the adolescent rats which corresponded to an approximate 20 years in human age[12].

Highly reactive species can be generated in the eye through photochemical pathways[16] causing oxidative stress which is a common initiator of cataractogenesis of the lens. It is associated with progressive changes in the physical and chemical properties of its structural proteins, mainly crystallins. These changes include crystallin crosslinking, aggregation, loss of solubility, conformational alterations, fragmentation and enzyme inactivation in the lens fibers[5,17].

Normal young lenses maintain an optimal activity of antioxidant enzymes and high concentrations of ascorbate and glutathione and hence minimize the alterations wrought by excessive oxidation. If this balance of pro- and antioxidants is disturbed, cataractogenesis occurs[18]. A high concentration of GSH, a major intracellular antioxidant, has been found to protect the lens from oxidative damage due to toxic chemicals[19]. Thus, depletion of GSH seriously affects GSH-dependent enzymes such as glutathione

peroxidase (GPx), glutathione reductase (GR) and glutathione transferase (GST) as well as the glutathione redox system, rendering the cells susceptible to a toxic challenge[20]. GR maintains the intracellular level of GSH by preserving the integrity of cell membranes and by stabilizing the sulfhydryl groups of proteins.

In our study, as the rats were folic acid deficient, their glutathione levels would have decreased leading to oxidative stress in the lens and formation of cataract. But no such changes were observed in any specimen. This could be because the rats may not have been totally folate-deficient as the lens and lenticular epithelial cells are capable of storing folic acid as folylpolyglutamate within the cytoplasm[6,21] or bound to an internalized receptor protein[22,23]. It could also be that very little folic acid is required in the lens to produce Glutathione. The lens has many antioxidant systems apart from the glutathione redox system[24]. So, even if the rats were totally folate-deficient, their other antioxidant systems like SOD, catalase, etc. probably were capable of quenching the free radicals produced.

Approximately 40 micronutrients (the vitamins, essential minerals, and other compounds required in small amounts for normal metabolism) are required in the human diet[25]. Micronutrient deficiency can mimic radiation (or chemicals) in damaging DNA by causing single- and double-strand breaks, or oxidative lesions, or both. Folic acid is one such micronutrient whose deficiency mimics radiation[26]. Damage to DNA, lipids and proteins induced by such free radicals may be controlled by antioxidants (such as Vitamins C and E) as well as enzymatic mechanisms such as superoxide dismutase, catalase and glutathione peroxidise[26].

As the study rats were deficient solely in folic acid and not in other vitamins and micronutrients, only the production of glutathione would have been impaired. The other antioxidant systems and enzymatic mechanisms would have ably tackled the oxidative stress.

CONCLUSION

The age of the rats studied was equivalent to about 20 human years. As the study was conducted on a younger group of rats, the folate-deficient status was probably not significant enough to show any effect on the lens. A comparatively less folate level was required in the lens to combat oxidative damage. Folic acid was probably stored within the cells during the pre-weaning stages when a normal diet was fed to the rats. As only folic acid was withheld, other antioxidants like Vitamin A, Vitamin C and Vitamin E, perhaps played a role by stepping up to combat the oxidative stress caused in the glutathione-deprived lens cells.

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How to cite this article: Datta NJ, Geetha S. A Study of the Effect of Isolated Folic Acid Deficiency on Lens in Adolescent Rats. Ann Health & Health Sci 2014; 1(2): 139-142.

NO|370|DMC|KLR|Phy|2014

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		ISSUE NO.	01
		REV NO	00
		DATE	1-11-2005

145
17/9/2014.

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