Original Article

Histological Study of Preventive Role of Cyanacobalamin (Vitamin B-12) On Heat Induced Testicular Injury

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ABSTRACT

Objective: The purpose of this study was to investigate the possible role of Cyanacobalamin (Vitamin B-12) in reducing the hazardous effects of heat on seminiferous tubules of testes in albino rats.

Study Design: Experimental Study

Place and Duration of Study: This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, for 6 weeks from October 2010 to November 2010.

Materials and Methods: Thirty adult albino rats of 200-250 grams of weight and 90-120 days of age were taken for this study. They were divided into three groups A (control), B (heat treated), and C (heat plus Cyanocobalamin treated). They were further subdivided into A1&A2, B1&B2 and C1&C2, based on duration of treatment of 4 weeks and 6 weeks respectfully. At the end of study histological examination of seminiferous tubules of testes were seen by applying Periodic Acid Schiff Iron Hematoxylin stain.

Results: There was marked damaging effects of heat (42^0C) on seminiferous tubules of testes with disorganized germinal epithelium and vacuolation. This damage to spermatogenic cell series was well protected with concomitant treatment with Cyanacobalamin (vitamin B-12). There was restoration of germinal epithelium and marked decrease in vacuolation.

Conclusion: This study proved protective role of Cyanacobalmin (Vitamin B-12) in heat induced damage in testes of albino rats.

Key Words: Heat, Cyanacobalamin (Vitamin B-12), Testes

INTRODUCTION

Heat is the sensation of an increase in temperature or the energy which produces the sensation of heat[1]. Heat injury is an acute life-threatening situation when core temperature rises above 41°C[2]. Both excess heat and excess cold are important causes of injury. Prolonged exposure to elevated ambient temperatures can result in heat cramps, heat exhaustion, and heat stroke[3,4].

Various types of testicular injuries, including hormonal perturbations, heat exposure[5], Sertoli cell toxicants such as Tacrolimus, an immunosuppressant,[6] and germ cell toxicants like x-radiation,[7] immobilization stress,[8] alcohol,[9] Cadmium,[10] fungicides such as Mancozeb,[11] all result in germ cell apoptosis. Heat is produced by cellular metabolism, and lost through the skin by both vasodilatation and sweating and through the lungs in expired air. In health, the body core temperature is maintained at 37°C by the hypothalamic thermoregulatory center.[2] Studies in cell lines and animal models suggest that heat directly causes tissue injury.[12,13] Increasing testicular temperature above normal levels results in altered spermatogenesis in mammals due to effect of heat,[14] which brings about oxidative stress on the seminiferous tubules.

The effects of Cyanacobalamin (Vitamin B-12) deficiency is most pronounced in rapidly dividing cells, such as the erythropoietic tissue of bone marrow and the mucosal cells of the intestine.[15] Vitamin B-12 is important in cellular replication, especially for synthesis of RNA and DNA and deficiency states have been associated with decreased sperm count and motility.[16,17,18,19]. Vitamin B-12 (1000mcg/day) was administered to men with a sperm counts less than 20 million/ml. By the end of the study, 27 percent of the men had a sperm count over 100 million/ml.[20]

Several studies have been done on testicular tissue injuries by various agents such as heat, drugs, and radiations along with protective role of anti-oxidants like vitamin A, vitamin E and Selenium, but the role of vitamin B12 has not been explored in histological studies, so this study was planned.

MATERIALS AND METHODS

This experimental study was conducted in the department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, for 6 weeks from October 2010 to November 2010.

Thirty (30) young male albino rats about 90-120 days of age and 200-220 gm of weight were obtained from animal house of BMSI, JPMC, Karachi.

Animals were divided into three groups A, B, and C. Each group was further subdivided into two subgroups, A1, A2; B1, B2; and C1, C2; based on the duration of treatment i.e. 4, and 6 weeks respectively. Each subgroup comprised of five animals.

The animals were kept under observation for one week, prior to the commencement of study for assessment of their health status. The animals were kept in plastic cages and were maintained on 12 hours light and 12 hours dark cycle. The standard laboratory chow and tap water were available ad libitum.

- Group-A (A1& A2) animals served as control.
- Group-B (B1&B2) animals had received heat at 42ºC for six hours daily with electric room heater and maintained with room thermometer.
- Group-C (C1&C2) animals received heat as given in group B and injection Cyanacobalamin, at dose of 500 mcg/kg body weight[21] intraperitoneally. All the animals were observed daily for their physical activity and weighed weekly. They were sacrificed under deep ether anesthesia at the end of their respective period of treatment. A mid line incision was made up to scrotum and extended upwards to the thoracic region.

The testes were fixed in Bouin's fluid for 24 hours. After 24 hours each testis was cut longitudinally into two equal halves and again post fixed in fresh Bouin's fluid for next 24 hours. The tissues were kept in capsules and then washed in running water for 3 to 4 hours to remove excess fixative. After fixation, tissues were processed in ascending strengths of alcohol, infiltrated and embedded in paraffin. 4µm thick longitudinal sections were cut on rotatory microtome. Sections of tissues were floated on hot water bath at 42ºC and transferred on albumenized glass slides. The slides were placed on hot plate at 37ºC for 24 hours for fixation of tissues and stained with Periodic Acid Schiff Iron Hematoxylin Technique. The morphological examination was done under light microscope.

RESULTS

The morphological examination of Periodic Acid Schiff-Iron Hematoxylin stained 4µm thick sections of testes in control subgroups; A1& A2 revealed seminiferous tubules cut in various planes of section under the light microscope. The tubules were compact, regularly arranged with intact basement membrane. The germinal epithelium was regularly arranged in various stages of spermatogenesis and the lumen contained spermatozoa. Vacuoles were not visualized .The Leydig cells were seen in groups in interstitial spaces which surround the seminiferous tubules and the dispersed chromatin material was seen within the cells (Figure-1).

On morphological examination of testicular tissue in subgroup B-1(4 weeks heat treated) animals, the seminiferous tubules were slightly distant but regularly arranged with intact basement membrane. Germinal epithelium was disorganized and germ cells were less in number. Spermatogenic material was compact in most of the basal layer.

Vacuoles were visualized. Leydig cells were dispersed and reduced in number and chromatin material in some cells was compact and showed some pyknosis (Figure-2).

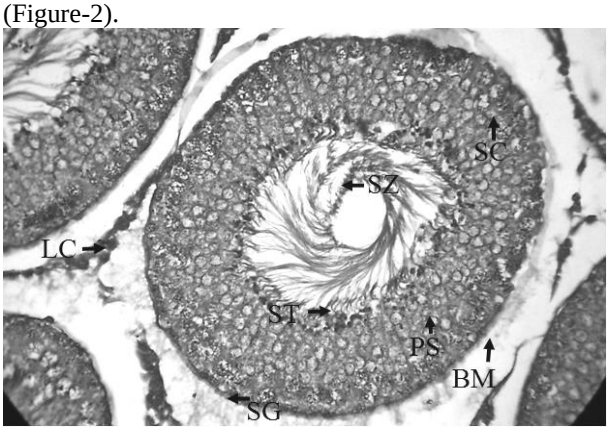

Figure No.1: PAS-Iron haematoxylin stained, 4 µm thick sections of testes of control albino rat, showing a seminiferous tubule with intact basement membrane (BM), spermatogonia (SG), primary spermatocytes (PS), spermatids (ST), sertoli cell (SC), lumen contained spermatozoa (SZ) and Leydig cells (LC) in the interstitial space. (Photomicrograph x40)

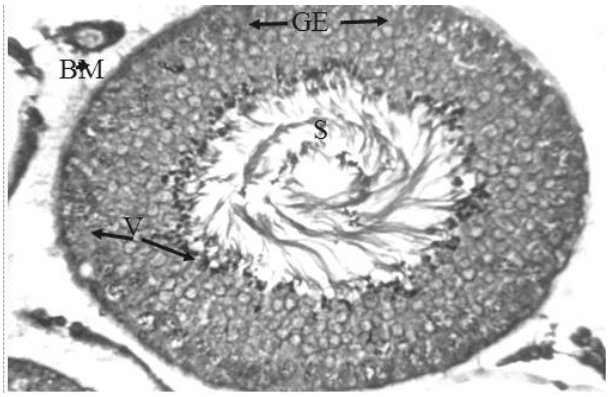

Figure No.2: PAS-Iron haematoxylin stained, 4 µm thick sections of seminiferous tubule, after 4 weeks heat treated showing disorganized germinal epithelium (GE) with more vacuolation (V), slough (S) but intact basement membrane (BM). (Photomicrograph x40)

The morphological examination of testes in subgroup B-2 (6 weeks heat treated) showed that the tubules were more widely separated as compared with B-I animals. There was shrinkage of seminiferous tubules. The germinal epithelium was scanty. Interstitial spaces were increased. Necrosis was seen at some sites. Compact chromatin in some tubules with pyknosis was seen. Slough was present in the lumen of most of the tubules. The Leydig cells were dispersed, less in number and reduced in size and nuclei showed pyknotic changes (Figure-3).

The morphological examination of seminiferous tubules in subgroup C-1 (4 weeks heat plus vitamin B12) showed the restoration of spermatogenic cell series similar to control. The basement membrane was intact

and vacuolation was remarkably decreased. Leydig cells were restored (Figure-1).

The morphological examination of seminiferous tubules in subgroup C-2 (6 weeks heat plus vitamin B12) revealed seminiferous tubules with slight widening of interstitial spaces, but basement membrane was intact. Lumen contained spermatozoa with some slough (Figure-4)

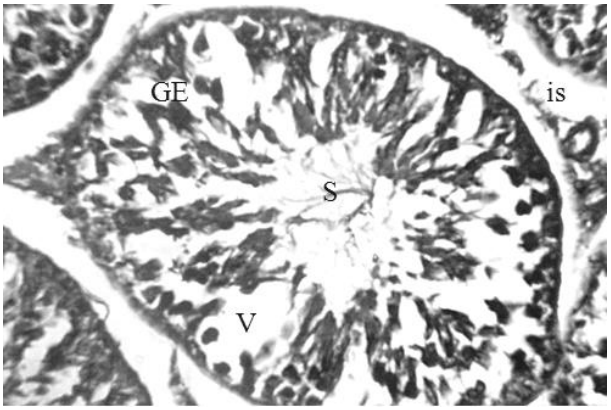

Figure No.3: PAS-Iron haematoxylin stained, 4 µm thick sections of seminiferous tubules, after 6 weeks heat treated showing scanty germinal epithelium (GE), widened interstitial space (IS), more vacuolation (V) and slough (S) in the lumen. (Photomicrograph x40)

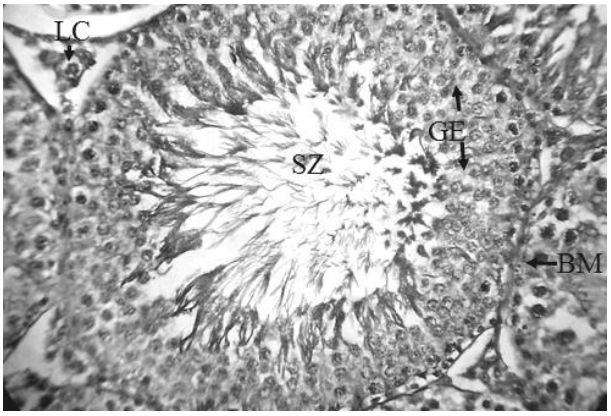

Figure No.4: PAS-Iron haematoxylin stained, 4 µm thick sections of seminiferous tubules, after 6 weeks heat with cyanacobalamin treated albino rats showing germinal epithelium (GE), with widening of interstitial spaces, intact basement membrane (BM), spermatozoa (SZ) in the lumen, and restored Leydig cells (LC). (Photomicrograph x40)

DISCUSSION

In the present study in heat treated group, the testes showed atrophy of seminiferous tubules, degeneration of germinal epithelium, specifically of spermatocytes and early spermatids producing vacuolation and slough. Kumar et al[3] described that persistent or excessive injury causes cells to pass the threshold into irreversible injury. This is associated with extensive damage to all cellular membranes, swelling of lysosomes, and vacuolization of mitochondria with reduced capacity to generate ATP. Yin et al[12] observed the effects of heat on testicular germ cells in adult mice with experimental cryptorchidism. They described the presence of vacuolation in the tubules representing sites of germ cells undergone apoptosis. Ren et al[22] showed degenerative changes (vacuoles) in experimental cryptorchidism in adult male rats. Impaired detoxification of reactive oxygen species and concomitant oxidative stress may be implicated in the biochemical mechanism responsible for testicular dysfunction in cryptorchidism.

Lue[23] reported that short exposure of the testis to heat causes degeneration of germ cells and apoptosis and germ cell death in the adult rat. A single exposure (43ºC for 15 minutes) of the rat testis to heat resulted in selective, but reversible, damage to the seminiferous epithelium through increased germ cell apoptosis[24,14] and mentioned that transient scrotal hyperthermia and Levonorgestrel enhanced testosterone-induced spermatogenesis suppression in men through increased germ cell apoptosis. The increased suppression of spermatogenesis is due to accelerated apoptosis mediated by the mitochondrial pathway of signaling. Apoptosis occurred mainly in the round spermatids and spermatocytes.

The animals in group C showed restoration of germinal epithelium, along with decrease in vacuolation. This could be the role of vitamin B12 in restoring serum testosterone levels and in reducing the oxidative stress. As B12 is reducing the stress, ACTH levels were decreased to near normal levels, resulting in might be near normal levels of LH which restored normal morphology of germinal epithelium. Courten and Ploen[25] in their study on adult cryptorchid testis observed that intratesticular infusion of Lactate showed improvement in various stages of spermatogenesis. Matsukiet al[26] in their study had noted that Minocycline decreased the effects of heat by decreasing the rate of apoptosis in mice testis. Yang et al[10] observed restoration of germinal epithelium by α-Tocopherol in Cadmium-induced testicular damage, by reduction of free radical damage and oxidative stress. The Leydig cells appeared smaller in size and their nuclei were darkly stained and some of them also showed pyknotic changes in heat treated animals as compared to control animals. The probable reasons for these changes may be hormonal disturbances and oxidative injury. As described by Norman[27], stress causes disruption of HP axis hormones. Lipid peroxidation due to oxidative stress of the cell membrane of Leydig cell causes reduction in the steroidogenesis[28]. The results of the present study are in agreement to Kanter and Aktas,[29] who also observed scrotal hyperthermia- induced damage in Leydig cells.

The Leydig cell arrangement was restored in group C animals. Their number increased and their nuclei were lighter stained as compared to heat treated group B

animals. The probable reason was due to the effects of vitamin B12 in decreasing stress so the ACTH levels are decreased and testosterone levels are increased to near normal. This increased level of testosterone helps is restoring the normal architecture of Leydig cells.

CONCLUSION

It is concluded that high ambient temperature severely damages the testicular tissue in albino rats, which becomes highly damaging with increased time period and can be protected by Cyanocobalamin.

It is suggested that, the results could be considered promising enough in humans who are working at high ambient temperature.

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