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EFFECT OF SUBACUTE ORCHIDECTOMY ON HEAMATOLOGICAL PARAMETERS AND LIPID PROFILE IN WISTAR RATS

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ABSTRACT

The effect of hypogonadism on some cardiovascular risk factors remains controversial and occasionally conflicting. This study was designed to determine the effect of orchidectomy induced hypogonadism on some hematological predictors of cardiovascular health status and body defense system. Fifty Wistar rats (25 male and 25 females) were used for this study. Male wistar rats were grouped into five groups (n=5), Group 1 consisted of the bilaterally orchidectomized rats, Group 2 were unilaterally castrated, Group 3 were sham operated, while group 4 and 5 were the experimental and passive controls respectively. The study lasted for six weeks. Conventional methods were applied for sample collection. Laboratory analysis of concentrations of testosterone, FSH, LH, lipid profile, full blood count, ESR, PCV and Hb concentration were conducted. Data was subjected to statistical analysis using one way analysis of variance (ANOVA) to compare means ($P \leq 0.05$). Bilateral orchidectomy was found to significantly decrease testosterone concentration (65.62%), triglycerides concentration (17.52%), red blood cell count (53.24%), total white blood cell count (54.64%) and percentage lymphocytes (26%) in the differentials. Significant increase in concentrations of LH (82.81%), FSH (97.12%), MCV (70.76%), MCH (76.23), total cholesterol (12.45%),

LDL (25.34%) and percentage neutrophils relative to the differentials (69.4%) were also observed in the group. No significant change in PCV, Hb, MCHC, thrombocyte count, ESR, HDL. In conclusion, bilateral orchidectomy lead to characteristic macrocytic normochromic aneamia, increased susceptibility to infection and dyslipideamia associated with hypercholesteroleamia and elevated LDL concentration which may increase atherosclerotic plaques formation. Though the procedure may be used in a number of procedures, the subacute complications that follows the procedure appears to be highly detrimental to healthy leaving.

KEY WORDS : FSH, MCV, PCV, Hb, MCHC.

INTRODUCTION

Aging is associated with a number of challenges such as increased physical dependency, risk of cardiovascular disorders (CVD), greater incidence of infection and hypogonadism [1]; [2]. The social and individual cost of hypogonadism may best be understood by exploring its effect on the entire body system. The role of hypogonadism in cellular growth led to a breakthrough in the management of prostate cancer. Bilateral orchidectomy is the gold standard for androgen ablation in men with metastatic prostate cancer [3]. Testosterone was reported to possess vasodilatory properties by enhancing release of NO and improving vascular smooth muscle sensitivity to NO [4], this effect is highly diminished in cases of atherosclerosis, a vascular condition that is highly affected by the lipid profile of an individual. Various studies have related testosterone to testosterone level to a particular lipid profile, yet most of the findings presented conflicting data and occasionally inconclusive results [5]; [6]. Hypogonadism affects wide range of systems that may either depend directly or indirectly on nature of formed elements of the blood and other hematological indices, example include wound healing [7], atherosclerosis [8], erectile dysfunction, depression, aneamia, reduced muscle mass and bone density [9]. Studying the comprehensive effect of testosterone depletion in animal model may provide a comprehensive insight on the way and manner the procedure alter blood composition and constituents in relation to serum testosterone and gonadotropin levels. Low serum testosterone is prospectively associated with dyslipideamia which predispose aged men to high incidence of CVDs [10]. The increasing use of orchidectomy in some clinical practice (prostate cancer, epididymal neoplasia, testicular carcinoma, torsion and orchitis etc), cryptorchidism, sex reassignment surgeries and also its re-introduction in the punishment of repeated sex offenders [11]; [12] has

made it necessary to study the effect of the procedure on some underlying atherosclerotic and cardiovascular dependent hematological parameters. This study was aimed at studying the mid-term effect of orchidectomy on some hematological determinants of risk of atherosclerosis in male adult wistar Rats. Specifically the study analyzed the effect of mid-term bilateral and unilateral orchidectomy on the following parameters: Lipid profile (serum cholesterol, triglycerides, low density lipoproteins and high density lipoproteins) and hematological indices (red blood count, total leukocyte count, total platelet count, differential leukocyte count, mean corpuscular volume, mean corpuscular heamoglobin, mean corpuscular heamoglobin concentration and erythrocyte sedimentation rate) in relation to some sex hormones level obtained (follicle stimulating hormone, luteinizing hormone and testosterone).

MATERIALS AND METHOD

This study was conducted in accordance to the recommendations of international standard for the protection of animals [13]. The study lasted for a total of six weeks. Twenty five adult (12-13 weeks old) male albino rats of wistar strain; *Rattus norvegicus* (200g-250g) were used for this study. The animals were obtained from animal house of the department of human physiology, Bayero University, Kano-Nigeria. Animals in each group (except group 5 animals) were caged (38cm×46cm×24cm), transferred to the laboratory, allowed to acclimatize for a week, at maintained room temperature (30-33oC), obeying the circadian cycle (12 hours light and 12 hrs dark), fed low cholesterol diet and water given *ad libitum*. Animals in group five (Passive control) were allowed to remain in the animal house (without isolation), allowed free access to food, water and female counterparts.

Animal Groupings

The male rats were randomly divided into the following groups

Group I: Bilaterally Orchidectomized

Group II: Unilaterally Orchidectomized

Group III: Sham Operated

Group IV: Laboratory Control

Group V: Passive Control

ORCHIDECTOMY

All surgical procedures were carried out at the Physiology research laboratory, Faculty of Medicine, Bayero University Kano. The procedure was carried out in adherence to the

technical rules of asepsis under general aneasthesia. A premedication of atropine (Atropine, Laborate Pharmaceuticals, Panipat, India. 132103-NF, A4-1953) was administered intraperitoneally (0.02 mg/kg). Five minutes afterward, a combination of Ketamine hydrochloride (Ketajet ®, Sterfil Laboratories, India) at a dose of 20 mg/kg body weight and diazepam (Valium, Roche LTD, Basel, Switzerlands) at a dose of 2 mg/kg body were administered intraperitoneally [14]. The rats were immobilized, while the operation was performed by depilating the fur in the scrotal area and subsequent disinfection of the skin with methylated spirit, 1cm median anterior scrotal incision was made, the tunica vaginalis was opened and testes were externalized. The ductus deferens, main arteries and veins were isolated and ligated using 3-0 ethicon thread (Johnson & Johnson). Subsequently, the duct and blood vessels were severed allowing the testicle and epididymis to be removed below the level of ligation. Group 1 animals had both the two testes removed, Group II had only the left testis removed from each member of the group, no testis was removed in group III animals, testis was only exposed without isolation or removal. The incision was closed by suturing the scrotal skin with catgut chrome 3-0 (chromic catgut Polysuture ®) then swabbed with povidone iodine solution. The rats were kept under warmth till they regain consciousness. They were then housed in separate cages (1 rat /cage) and allowed free access to food and water *ad libitum* [15].

HORMONAL ASSAYS

After the follow-up period of six weeks, the rats were anaesthetized in the morning as previously described. 5 ml of blood was collected in plain containers through retro-orbital plexus technique under aseptic condition [16], after which the samples were allowed to clot at room temperature for 15 minutes and then centrifuged at 3000 r.p.m. for 5 minutes using bench top centrifuge (MSE Minor, England). The serum layer was aspirated and transferred by the use of pasteur pipette into smaller sterile blank tubes for hormonal assays as conducted by Sadri and Ahmadi [17]. Tubes were coated with aluminum foil to protect from light, placed in ice to prevent the denaturing effect of higher temperatures. The samples were immediately forwarded to hematology lab of Medicus Clinic and Diagnostic Center, Nassarawa, Kano, for the following;

Determination of serum testosterone concentration

Isolated sera of all the animals were assayed for the testosterone values using testosterone enzyme Iimmunoassay test kit (TEIA Test Kit, Catalog No: TEST-96) with sensitivity and

assay range of 5 – 1800 ng/dL. The principle of the test was based on competitive binding between testosterone in the test specimen and testosterone- conjugate for a constant amount of rabbit anti-testosterone.

Determination of serum LH and FSH concentration

LH and FSH Microplate Immunoenzymometric Assay (Accubind Elisa microwells, Monobind Inc. Lake Forest, CA 92630, USA, Product code: 625-300 and Product code: 425-300) respectively were used for this assessment [18].

LIPID PROFILE

Blood collection and serum isolation was same as earlier described.Serum total cholesterol and high-density lipoprotein cholesterol were determined using Randox Laboratory kit reagents (Randox CHOL) of analytical grade. While serum triacylglyceride level was estimated using Randox Laboratory test kit for triglycerides (Randox TRIGS) of analyticalgrade [19]. Serum Low density lipoprotein (LDL) cholesterol was calculated using Friedewald's equation [LDL = TC - HDL - TG/5.0 (mg/dL)] [20]. Total Lipid was presented as the sum total of all the components of the lipid profile as measured.

HEMATOLOGICAL ANALYSIS

Blood samples were collected in EDTA containers by retro-orbital plexus technique. Hemoglobin concentration (Hb), packed cell volume (PCV), total red blood count (TRBC), total white blood count (TWBC), differential white blood count (DC), platelets count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were analyzed using automated heamatology analyser (Sysmex K2X1: SYSMEX corporation, Japan) [21]. ESR was estimated by Westergren method.

STATISTICAL ANALYSIS

Values were recorded as mean ± standard error mean (SEM). Statistical analysis was conducted using the SPSS software (version 20). In order to compare mean values, the collected data was subjected to one-way ANOVA analysis, followed by Bonferonni's post hoc test for multiple comparisons. The significance level was set as $P< 0.05$.

RESULTS

Table 1 shows the changes in serum concentrations of testosterone, LH and FSH. Significant

reduction of testosterone levels in orchidectomized animals (P<0.05) compared to nonorchidectomized ones was revealed, while significant increase (P<0.05) in serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) level was observed in the bilaterally orchidectomized group as compared to the control groups, a marked increase ingonadotropins was also observed in unilateral groups but no significance (P>0.05) was recorded in the serum level of LH, nevertheless FSH was significantly increased as compared to the control.

Table 1: Serum concentrations of Testosterone (ng/dl), LH (mIU/ml) and FSH (mIU/ml) of male wistar rats (n=5)

	I	II	III	IV	V
T (x10^3 ng/dl)	0.36±0.031	0.79±0.010*	1.07±0.044*	1.06±0.043*	1.01±0.024*
LH (mIU/ml)	10.00±1.673	9.60±0.748	5.00±0.447*	6.80±1.393	4.60±0.927*
FSH(mIU/ml)	9.60±2.462	9.40±0.748	4.80±0.663*	6.00±1.049	3.80±0.663*

* Mean ±SEM. (P ≤ 0.05).

Table 2 shows the extent to which orchidectomy affected lipid parameters in wistar rats. Serum total cholesterol (TC), low density lipoprotein (LDL) and total lipids (TL) were significantly (P<0.05) increased while triglycerides (TG) was significantly decreased in bilaterally orchidectomized animals. No significant change in serum high density lipoprotein (HDL) concentrations between groups.

Table 2: Lipid Profile; Serum concentrations of High density Lipoproteins (HDL; mg/dl), Low Density Lipoproteins (LDL; mg/dl), Total Cholesterol (TC; mg/dl), Triglycerides (TG; mg/dl) and Total Lipids (TL; mg/dl) of rats in each group (n=5).

	I	II	III	IV	V
HDL (mg/dl)	46.60±1.030	45.80±0.860	48.80±1.020	47.60±1.631	48.40±1.077
LDL (mg/dl)	124.00±5.050	98.20±2.596*	95.40±3.669*	102.00±2.121*	99.40±2.676*
TC (mg/dl)	186.00±3.962	170.60±2.337*	165.40±4.155*	167.00±2.490*	164.00±3.114*
TG mg/dl	76.60±1.435	85.40±1.691	92.40±2.421*	92.80±2.577*	93.40±4.297*
TL (mg/dl)	433.00±9.394	395.00±3.578*	402.00±2.000*	409.40±3.868	405.20±8.027*

* Mean ±SEM. (P ≤ 0.05).

Observations on heamatological indices were presented in table 3. Significant increase (P<0.05) in mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) and percentage neutrophil (Neu), together with significant decrease in red blood cell count (RBCC), percentage lymphocyte (Lym) and total white blood cell count (TWBC) was recorded in bilaterally orchidectomized groups. No significant change in packed cell volume

(PCV), heamoglobin concentration (Hb), mean corpuscular heamoglobin concentration (MCHC), percentage monocyte (Mon), percentage eosinophil (Eos), erythrocyte sedimentation rate (ESR) and platelet count (PC).

Table 3: Effect of orchidectomy on RBC count (cells/mm3), packed cell volume (%), Hb (ng/dl), MCV (fl), MCH (pg), MCHC (g/dl), neutrophil (%), eosinophil (%), basophil (%), lymphocyte (%), WBC count (cells/mm3), ESR (mm/hr) and platelets count (cell/mm3) (n=5).

	I	II	III	IV	V
RBCC(x10^{6}cells/mm^3)	4.140±0.1833	6.520±0.4398*	9.240±0.2561*	8.680±0.3787*	8.640±0.2694*
PCV (%)	32.80±1.855	33.00±2.168	43.80±5.248	38.20±3.470	41.40±1.208
Hb (ng/dl)	10.98±0.554	10.90±0.702	14.56±1.805	12.32±1.111	13.16±0.696
MCV (fl)	79.46±4.329	52.41±7.151*	47.53±5.843*	44.04±3.743*	48.03±1.594*
MCH (pg)	26.61±1.331	17.33±2.389*	15.80±2.009*	14.21±1.203*	15.30±0.958*
MCHC (g/dl)	33.54±0.591	33.05±0.265	33.13±0,239	32.31±0.589	31.75±1.138
Neu (%)	69.40±1.778	64.40±1.749	55.40±2.829*	57.20±4.289*	58.20±1.985
Lym (%)	26.00±2.025	31.60±1.778	40.80±2.888*	40.20±4.224*	38.40±2.112*
Mon (%)	2.60±0.678	2.00±0.316	1.40±0.245	1.40±0.245	2.20±0.510
Eos (%)	2.00±0.316	2.00±0.000	2.40±0.510	1.50±0.289	1.20±0.250
TWBC(x10^{3}cells/mm^3)	3.88±0.229	6.50±0.467	7.92±0.839*	8.94±0.857*	8.80±0.401*
ESR (mm/hr)	4.40±1.122	3.60±0.510	6.00±2.302	3.00±0.447	2.40±0.510
PC (x10^{4}cells/mm^3)	19.50±1.962	18.82±0.542	20.24±0.999	21.54±1.096	21.80±.860

* Mean ±SEM. ($P \leq 0.05$).

DISCUSSION

The most obvious consequence of orchidectomy was massive decrease in circulating testosterone, and one would expect the metabolic effects of castration to be those of testosterone deficiency. Significant decrease in serum testosterone level occurred in the orchidectomized groups as compared to other groups. This is in agreement with Coyotupa *et al.* [22]. Orchidectomy does not completely abolish the secretion of androgens because they may be compensated for by other sources other than the testes, such the adrenal gland. This explains the minute amount of the hormone detected in the bilaterally orchidectomized group. Though significantly lower than in the control, the serum concentration of testosterone among unilaterally castrated rats was significantly higher than that of the bilaterally orchidectomized groups. This may best be explained by the possible compensatory role of the contralateral testes. Both bilateral and unilateral treatments caused significant increase in serum FSH. Significant increase in LH was more conspicuous in the bilateral than the unilateral group which had insignificant increase in LH concentration. Accordingly, the presence of one normal testis after unilateral castration might have been sufficient to preserve

the existing feedback control system already operating between the pituitary and the gonads. Interestingly, this finding implies that a single normal testis has the capacity to produce sufficient seminiferous tubular agent, as well as testosterone, for proper communication with the hypophysis. Although testosterone is an important physiological regulator of pituitary gonadotropin secretion in the normal adult male rat [23], the results reported here indicate that some factors other than peripheral serum testosterone may be influential in the regulation of pituitary gonadotrophins in serum. Particularly important was the finding that although bilateral orchidectomy caused a massive fall in serum testosterone level when compared to that of unilateral groups, the difference in rise of gonadotropin level between groups was not proportionate. Bilateral orchidectomy significantly ($P < 0.05$) increased total cholesterol (TC), Low density lipoprotein (LDL) and total cholesterol (TL). In agreement with this, it was reported that increase in total cholesterol and low density lipoprotein cholesterol followed orchidectomy in prostate cancer patients [24]; [25]. This may be attributed to the decrease in activity of hepatic lipase (HL) and lipoprotein lipase (LPL) which depends on gonadal hormones [26] for its function. Contrary to the outcome of our study, John and Lars-Eric [27] reported no significant change in serum cholesterol level among castrated animals. The increase in serum concentration of cholesterol in the bilaterally orchidectomized group may also be due to increase in the concentration of acetyl CoA arising probably from increase β-oxidation of fatty acids, since acetyl CoA is a key substrate in the biosynthesis of cholesterol [28]. Such increase in serum concentration of cholesterol may be harmful as it may aggravate erectile dysfunction [29], increase incidence of atherosclerosis and hypertension [30]. Low density lipoprotein is the primary transporter of plasma cholesterol. The significant increase in LDL may be assumed to have occurred in response to increased serum total cholesterol. It is termed bad cholesterol because it builds up slowly in the walls of arteries, thus forming plaque that increases the risk of atherosclerosis, high blood pressure and subsequently leads to stroke [31]. LDL increase in response to low testosterone concentration is likely to be through down regulation of the peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ system [32]. The increase in the number of LDL particles in the present study could also result from reduction LDL uptake by the LDL receptor [33]. Serum triglyceride concentration was significantly ($P<0.05$) decreased in bilaterally orchidectomized groups, this result is in agreement with Veloso *et al.* [34], in which reduced blood levels of triglycerides and VLDL cholesterol in young orchidectomized rats were reported. These changes may be related to the age of castration and the feeding habit. Contrary to this finding, an increase in serum TG levels of castrated rabbits was reported by Zhao *et al.*, [35].

Nevertheless, data from the Physicians Health Study demonstrated that LDL particle size has an inverse relation with triglyceride levels [36]. Thus, it can be suggested that the fall in triglycerides level may be related to the size and radius of LDL particles in this study, which may be assumed to be large.

No significant change was recorded in the serum concentration of high density lipoprotein, this is in agreement with the findings of Alexandersen and Christiansen, [37], which reported no change in HDL and increased LDL in hypogonadal men. According to Veloso *et al.,* [34], Hypogonadism secondary to total bilateral epididymectomy and orchiectomy do not modify the serum values HDL fractions. According to Isidori *et al.,* [5] androgenic therapy elicited no change in HDL fractions, this imply that HDL metabolism is not solely dependent on testosterone. Many more studies confirmed that no change in serum HDL recorded following testosterone replacement therapy [38] [39]. Most studies that reported increase in serum TG upon castration also reported decrease in HDL [24] [25] [40], this imply that metabolism of HDL and TG are closely associated, this claim may be supported by the action of hepatic triglyceride lipase on the metabolism of TG and HDL which is highly dependent on level of estrogen, testosterone and rate of aromatization of testosterone to estrogen [41]. Similar to the findings of Kelani and Durotoye [42], Orakwe *et al*., [43] and Hassan [44], Significant decrease in red blood cell count (RBCC) was recorded. Lowered testosterone level in the orchidectomized group may have caused decrease erythropoietin production by decreasing activation rate of androgen receptors in erythroid cells of the bone marrow. Contrary to our expectation, no significant change was observed in heamoglobin concentration and packed cell volume. Zhao *et al.* [35] observed no significant change in Hb and PCV of castrated New Zealand white rabbits at baseline and at six weeks after castration, but they later recorded significant decrease in Hb and PCV as at eighteen weeks after castration. Significant ($P<0.05$) increase in mean corpuscular volume (MCV) and mean corpuscular heamoglobin as observed in the bilaterally orchidectomized indicate that the red blood cells of the bilaterally orchidectomized rats are macrocytic normochromic erythrocytes (large sized RBCs with normal heamoglobin concentration). Because of its large size, it has increased hemoglobin content and ability to cover almost the same volume as a normocytic cell will do. This might have being the reason why the Hb and PCV recorded insignificant decrease in the study group. Castration has been shown to elicit physiological stress, inflammatory reactions, painassociated behavior, suppression of immune function, and a reduction in performance to varying degrees [45]. According to Elshaikh *et al.* [46], positive correlation exists between

CD4/CD8 T cells and testosterone level. A study they conducted on HIV negative patients with prostate carcinoma revealed decrease in CD4, CD8 and total white blood cell count as testosterone declined to castrate level upon 3D radiation treatment. No significant change was observed in erythrocyte sedimentation rate.

(ESR) among groups.

The values were all within normal range (2-15 mm/hr). ESR is a non-specific measure of inflammation. It is elevated in cases of inflammation (of any cause). Usually, ESR decreases in cases of aneamia, in this study, the significant reduction in erythrocyte count in bilaterally orchidectomized groups did not cause proportionate decrease in ESR, probably because the change observed in the heamoglobin (Hb), hematocrit, mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) and mean corpuscular heamoglobin concentration (MCHC) presented a macrocytic normochromic type of aneamia characterized by RBCs with large membrane surface area, which may increase the number of negative charges on the RBC membrane (high zeta potential). This might have created balance between the low RBC count (a pro sedimentation factor) and high zeta potential (anti-sedimentation factor) of the few erythrocytes [47]. Significant increase in percentage of neutrophil and decrease in lymphocyte percentage was observed in orchidectomized groups, though the number of both the neutrophil and lymphocyte are at reduced level. Castration causes decrease in gammainterferon which exerts a suppressive effect on lymphocyte function. It increases neutrophil numbers and the neutrophil : lymphocyte ratio [48]. Androgens exert potent regulatory influence over the immune system, although the full nature of these effects and mechanism underlying hormone–induced changes in host immunity are poorly understood. Several observations indicate that sex hormones serve as important regulators of lymphopoisis. Thymic involution that occurs during puberty is associated with the onset of sex hormone production and can be delayed by castration prior to puberty [49]. Castration of mice after puberty reverses thymic involution and leads to thymic hypertrophy, a process that can be reversed by replacement of androgen or estrogen. The production of B lymphocyte is regulated by physiologic level of androgens [49]. Non significant change in eosinophil and monocyte agrees with works of Kelani and Durotoye [42], Orakwe *et al.*, [43] and Hassan [44]. In conclusion, bilateral orchidectomy induced hypogonadism is associated with severe aneamia of hypochromic type, pancytopeania, hypercholesteroleamia, high LDL which pose risk of atherosclerotic plaques formation and increased risk of thrombotic disorders. Base on the

findings of this study, It is thereforerecommended that before carrying out orchidectomy:Subject's cardiovascular health status in relation to dyslipidaemia, anaemia and many other risk factors should be carefully assessed.The immune status of subjects should be taken into consideration prior to the procedure in order to avoid further immuno-compromise due to leukocytopenia.

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