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Evaluation of Testosterone, Dihydrotestosterone and Gonadotropins among Long Distance Drivers in Benin City, Nigeria

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Abstract

Background: Reseach reports over the years have pointed to the vulnerability of the testes to heat and other physical agents of cell damage. A possible source of intense heat is the drivers' seat of heavy duty trucks and trailers. For the drivers of these vehicles, testicular heat is likely to occur, and initial injury/damage within the testes is likely accompanied by an increase in the serum concentration of FSH and LH from the anterior pituitary gland. This research was aimed at evaluating the serum concentration of Testosterone, Dihydrotestosterone (DHT), Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in drivers of heavy duty trucks and trailers (long distance drivers), as compared to values obtained from age-matched control subjects.

Method: This study was carried out on drivers of heavy duty trucks. A total of sixty (60) subjects and sixty controls were recruited for this study. Venous blood was collected and serum extracted for the evaluating the parameters. ELISA method - an enzyme immunoassay test which follows a typical two-step capture or "sandwich" type assay, was used for the laboratory estimation of these hormones. Data obtained was analyzed using SPSS.

Result: There was significant difference in the FSH concentration (at $p<0.001$) and LH concentration ($p<0.05$) in long distance drivers as against control subjects. DHT and Testosterone however did not differ significantly between the study and control groups. Both ANOVA and Correlation studies showed that these elevations in FSH and LH increase with intensity of heat, and duration of contact with the seat. Among the parameters, DHT and Testosterone correlated positively ($r = 0.697$, $p<0.001$); FSH and LH also correlated positively ($r = 0.607$, $p<0.001$); while DHT correlated negatively with FSH concentration ($r = -0.460$, $p<0.001$). Age also significantly correlated positively with FSH contration ($p<0.01$).

Conclusion: In conclusion, the aberrations observed in the concentration of parameters measured in this study indicate that there is significant hypergonadotropism due to prolonged exposure to testicular heat among these subjects.

Keywords: Follicular Stimulating Hormone,Luteinizing Hormone, Testosterone, Dihydrotestosterone

1. Introduction

There are published research works which point to the vulnerable nature of the male gonads due to exposure of the scrotum and the testes to relatively high temperature. Other physical properties e.g. electromagnetic as well as ultrasound waves, may exacerbate defects in spermatogenesis secondary to heat. Other studies have also concluded that this adverse effect of heat is largely irreversible [1]. Consequently, the result of a study published by Kandeel and Swerdloff in 1988 on the use of heat in male contraception further confirms the possible detrimental effect of high temperature on spermatogenesis [2].

The testes are the male gonads in mammals and primates. As with the female homologue – the ovaries, the testes produce both spermatozoa and testosterone. Synthesis and secretion of testosterone is regulated by the action of luteinizing hormone, produced by the anterior pituitary gland while spermatogenesis is regulated by both testosterone and follicular stimulating hormone. Spermatogenesis takes place in the pair of testes, which are similar in size and sacked in the scrotum [3].

The testes are engulfed in a layer of tough membranous material known as tunica albuginea. Each testis is composed of a network of tiny tubules - seminiferous tubules, lined with a layer of germ cells which starts to differentiate at onset of puberty and persists till old age [3]. The testicular tissues are delicate structures which can be damaged by environmental insults; hence there was a 21st century study which reported a slight but steady decrease in human testicular size and weight [4].

Spermatogenesis is optimum at 2℃ less than 37℃, hence the need for the scrotal sac [5]. As a result of this temperature requirement, testicular temperature is kept at 35℃. Previous researches have hinted that higher temperatures affect spermatogenesis. As a result, the body has developed certain adaptations to keep tesicular function optimum [5][6]. The scrotum responds to temperature variation by adjusting the cremasteric muscle. At cold temperatures, there is a contraction, resulting in shortening of the cord, which subsequently raise the testicles closer toward the body. At hotter temperatures when cooling is required, it relaxes to keep the content of the scrotum at 35℃. Apart from temperature changes, contraction is also elicited by stress conditions (e.g fight) as an involuntary protective mechanism. This adaptation of the scrotum is effectively utilized during temperature changes in the body [7].

Studies have shown that when the scrotum is subjected to long-term heating, the spermatogenic functions are adversely affected. Vigodner et al., in 2003, reported that spermatozoa from cryptochid testis in hamsters showed marked reduction in survival rate. According to the study, the few that eventually survived showed evidence of DNA damage. According to Setchell, 2006 in an experiment conducted on mice, increased heat (achieved by induced cryptochidism or microwave irradiation) resulted in production of spermatocytes with defective function. The cells failed to fertilize mature oocytes. Also in the same study, reactive oxygen species (ROS) were implicated in the degree of damage by heat. It was reported for instance that the damages were more severe in mice whose genes for superoxide dismutase (SOD) were knocked off.

Automobile engines generate an enormous amount of heat daily. These engines are situated beneath or close to the driver's seat. For short distance drivers or non-commercial drivers, exposure to heat may be insignificant. However, this is not the case with long distance drivers (especially trailer drivers). Sitting very close to automobile engines for a long period of time on a daily basis may cause significant damage on the gonads. This study aims to evaluate the possible effect of testicular heat on gonadal hormone synthesis on this population of long distance drivers.

1.1 Justification of Study

The aetiology of male gonadal defect is multifactorial, ranging from anatomic defects, endocrinopathies, immunologic reactions, gene mutation, radiation, chemotherapy, and exposure to other environmental toxicants. However, a potentially potent agent of reduced spermatogenesis is exposure to heat. Heat causes protein coagulation and aggregation both in vivo and in vitro. All cells are made of proteins (intracellular and membrane proteins).

Evaluation of the effect of heat on the gonads is particularly not an easy task as this may involve direct examination of the gonadal tissues (fine needle aspiration cytology or direct biopsy) to check for the presence of defective proteins or denatured protein products. Another method involves the direct examination of ejaculated spermatozoa to check for abnormality in morphology. It may also involve the estimation of heat shock proteins generated secondary to heat exposure. However, the previous reports which suggested that these effects of heat may be reversible particularly undermine the usefulness of histological and immunological examinations listed above. Also, these defects in sperm morphology or denaturation of proteins may be due to other factors (e.g. chemical agents, ionizing radiation, environmental toxicants, etc). The aforementioned methods (histology and immunochemical) do not take into cognisance the possibility of non-heat causes for the defects.

The most important function of the male gonads is its role in spermatogenesis. Therefore, if spermatogenesis is not affected by heat and other functions are affected (as may be seen if the only tissues affected are not involved in spermatogenesis), then there is minimal societal burden. But on the other hand, if spermatogenesis or all testicular functions are affected, then it becomes a public health issue that must be addressed.

This study therefore evaluated the serum levels of FSH, LH, Testosterone and DHT among long distance drivers exposed to long duration of testicular heating. Defective gonadal function results in defective androgens secretion by the gonads. Therefore, in this study the androgens secretion function of the testes as well as pituitary response was analyzed among long distance drivers in Benin City, Nigeria.

1.2 Aim of study

To determine the serum concentration of testosterone, dihydotestosterone, follicular stimulating hormone and luteinizing hormone among long distance drivers in Benin City Nigeria.

1.3 Ethical approval

Ethical approval was sought from the Ethics Review Committee of Edo State Ministry of Health, Benin City, prior to commencement of blood samples collection from voluntary males drivers in Benin City.

In addition, written informed consent was obtained from individual participants and utmost confidentiality maintained throughout the study.

1.4 Hypothesis

H_0:There is no difference in the gonadal androgens concentrations of long distance drivers and control subjects

H_1:There is a significant difference in the gonadal androgens concentrations of long distance drivers and that of control subjects

2. Materials and Methods

2.1 Study design

This study was a case-control study conducted on both male long distance drivers and non-drivers, between the ages of 20-55 years. This is the age range of drivers that volunteered for the study.

2.2 Study location

Study was conducted in Benin City, a city in the South-South geopolitical zone of Nigeria.

2.3 Study population

This consists of adult males who are fully engaged in long distance driving as a source of livelihood in Benin City. This study include both healthy non-driver male subjects (Control Group) who had no chronic clinical illness and had their baby within one year of unprotected sexual intercourse, as well as male long distance drivers (Test Group) who are exposed to prolonged periods of testicular heat.

All individuals who participated in this study were well enlightened on the nature of the study and informed consent was received.

2.4 Sample size determination(cochran's formula)

The 'Case-Control Study Design' was adopted for this study and sample size is derived as follows:

$$S = \frac{N}{(1 + (N-1/x)}$$

$$\text{Where } N = \frac{Z^2pq}{e^2}$$

Where: z=z-score
p=estimated % of subjects the alternate hypothesis should apply to
q=1-p (i.e 0.5)
e=confidence limit (0.05)
x=estimated population being studied

Calculation:
z=1.96;p=0.5;q=(1 – 0.5);e=0.05

$$N = \frac{1.96^2 \times 0.5 \times (1 - 0.5)}{0.05^2}$$

$$= 384$$

$$S = \frac{N}{(1 + (N-1/x)} \quad \text{i.e} \quad \frac{385}{1 + (383/70)}$$

Minimum sample size required = 60 [8]

2.5 Inclusion criteria

Long distance drivers who are exposed to prolonged periods of driving heavy duty vehicles.

2.6 Exclusion criteria

Non long distance drivers who are not exposed to significant amount of testicular heat nor have any infertility issues.

2.7 Sampling Methodology

2.7.1 Sampling duration

Venous blood samples was obtained from both Test and Control subjects between the periods of April 24, 2018 to May 5, 2018.

2.7.2 Sample collection

Blood samples was collected in a sterile plain container by venipuncture. Serum were collected sequel to centrifugation, and frozen at -20^{0}C.

2.7.3 Sample preparation

Frozen serum samples were thawed and brought to room temperature prior to laboratory analysis.

3. Laboratory Analysis And Techniques

3.1 Testosterone and 5'DHT assay

3.1.1 Principle

The principle of Testosterone and DHT assay in this study was competitive binding ELISA (Enzyme Linked Immunosorbent Assay). Serum containing the antigen of interest (in this case, testosterone or DHT) is mixed with a fixed amount of enzyme labelled antigen. They compete for the binding sites of the antibodies coated onto the wells. A wash step removes excess unbound antigen or antibody prior to the addition of an enzyme substrate conjugated with a chromogen (TMB). The reaction is stopped and optical density measured with the use of a microplate reader. Hormone concentration is inversely proportional to intensity of the colour developed. Concentration and optical density of a set of standards (calibrators) is used to plot a calibration curve, and this is used to calculate the concentration [9].

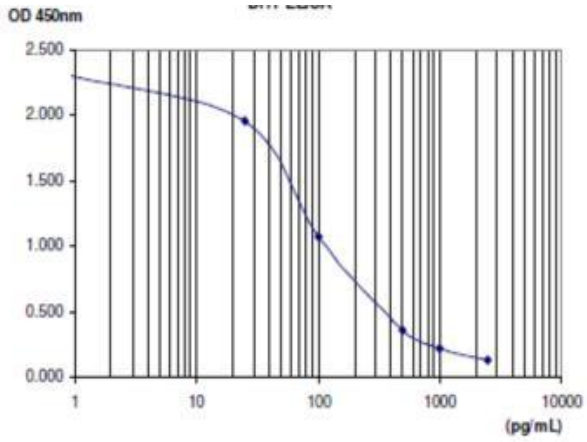
3.1.2 Procedure

50 µ L each of calibrators, control samples and samples were dispensed into formatted wells of the microtiter plate. Using a 100 µ L pipette, freshly prepared working enzyme conjugate was dispensed into each of the wells, and the mixture swirled for 30 seconds. Wells were then incubated for 1hr at room temperature. The plates were then washed 5 times with 300 µ L of diluted Wash Buffer. An 8-channel Micropipettor was used to add 100 µ L of TMB Substrate Solution. The wells were then incubated at room temperature for 15 mins. 50 µ L of stopped solution was then dispensed into each well to stop the reaction and subsequently convert the blue colour to yellow. The plates were gently

swirled to ensure proper mixing and colour conversion prior to reading at 450nm.

3.1.3 Calculation

Testosterone/DHT concentration was obtained from the calibration curve. Serial dilutions of the testosterone/DHT standards were made and prepared as described above. Then a calibration curve was obtained from the dilutions as depicted below. The optical densities of each sample (wells) were traced against concentration axis on the calibration curve.



[SOURCE: Eagle biosciences inc. package insert]

3.2 Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH) assay

3.2.1 Principle

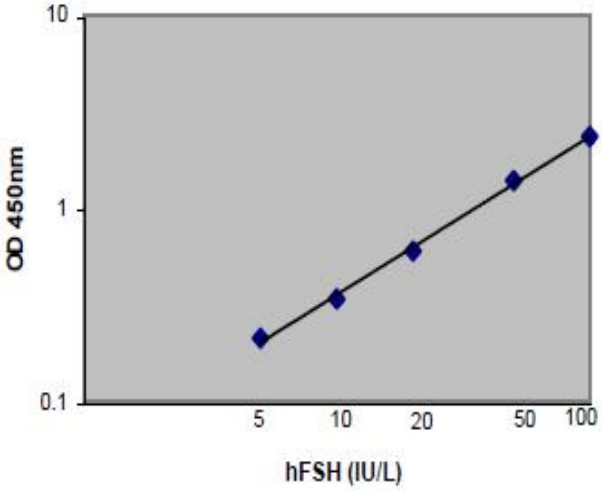
The principle of this ELISA test followed a typical two-step capture or "sandwich" type assay, which made use of two highly specific monoclonal antibodies: A monoclonal antibody specific for FSH/LH was immobilized onto the microwell plate and another monoclonal antibody specific for a different region of FSH/LH was conjugated to horse radish peroxidase (HRP). FSH/LH from the sample and standards were allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate was added. The enzymatic reaction was terminated by addition of the stop solution. The absorbance was measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of FSH/LH in the sample [10][11][12].

3.2.2 Procedure

Working reagent of anti-FSH-HRP and anti-LH-HRP conjugate were freshly prepared. Wash buffer concentrate was also appropriately diluted prior to analysis. 25 µl each of calibrators, controls and sample were dispensed into correspondingly labelled wells. Then 100 µl of biotinized assay buffer was dispensed into each well using a multichannel pipette. The well was then incubated in a plate shaker at 200rpm for 30 minutes at 250C, and then washed 3 times with 300 µl of wash buffer. 100 µl of working conjugate solution was then dispensed into each well and incubated in a plate shaker at 200rpm for 30 minutes at 250C. A similar wash procedure was done as described above. Then 100 µl of TMB substrate was dispensed into each well and the contents were incubated for 20 mins. 50 µl of stop solution was added to the wells to stop the reaction and change the final colour to yellow. The plates were gently swirled to ensure proper mixing and colour conversion prior to reading at 450nm.

3.2.3 Calculations

The optical densities of the sample were read and traced on the calibration curve to obtain the concentration. The calibration curve for FSH/LH assay is depicted below.



[15][4][9]

3.3 Statistical Analysis

Data collected was analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0 (IBM Inc., USA). The Students t-test and analysis of variance (ANOVA) was used to compare means, while correlation study was applied to determine the degree of correlation between parameters. A confidence limit of 95% ($p < 0.05$) was considered significant.

4. Results

4.1 Hypothesis Testing (Test of Significance)

Table 4.1:Comparison of measured parameters in cases and controls

	MEAN ± SEM (Cases)	MEAN ± SEM (Controls)	SIGNIFICANCE (p-values)
FSH	8.358 ± 0.3669	5.597 ± 0.1452	**0.000 ****
LH	6.277 ± 0.2690	5.235 ± 0.1260	**0.001 ***
TESTOSTERONE	7.710 ± 0.4310	8.730 ± 0.3588	0.071
DHT	1.158 ± 0.0782	1.257 ± 0.0753	0.367

P < 0.05

4.2 Analysis of Variance (ANOVA)

Table 4.2.1:ANOVA table showing the effect of duration of driving and age on FSH levels in the study group

FACTORS	MEAN VALUES			F-VALUE	SIGNIFICANCE	INTERPRETATION
	MINIMAL	**MODERATE**	**SEVERE**			
	≤5YRS	**5 – 10YRS**	**>10YRS**			
DURATION	5.240[a]	7.030[a]	9.358[b]	*13.829*	**0.000**	**Significant**
	<35 yrs	36 – 46 yrs	>47 yrs			
AGE	5.103[a]	7.991 [b]	9.450 [b]	*3.971*	**0.024**	**Significant**

p<0.05

Table 4.2.2:ANOVA table showing the addictive effect of period of driving and daily exposure (average working period/day) on FSH level

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1919.870	1	1919.870	259.309	**0.000**
PERIOD OF DRIVING	22.158	3	7.386	0.998	0.401
DAILY EXPOSURE	63.186	2	31.593	4.267	**0.019**

P<0.05

4.3 Correlation and Regression

The table below as well as the scatter plots summarises the results obtained from the correlation and regression analysis between parameters as well as between factors and parameters.

FSH correlated positively with age at $p<0.05$. Other parameters however didn't show significant correlation.

There is also a very strong positive correlation between DHT and testosterone (at $p<0.001$). DHT also strongly correlated negatively with FSH level.

Also, FSH and LH correlated positively as $p<0.001$.

"Intensity of heat" correlated positively with FSH, Testosterone and DHT level, but not with LH.

LH correlated however with "exposure to Smoke" ($p<0.05$)

"Average period of rest per day" also correlated negatively with DHT level

Table 4.3:Summary of correlation between parameters and factors

		AGE	DURATION	FSH	LH	T	DHT
AGE	Pearson Correlation	1	.232	.396**	.157	.124	.072
	Sig. (2-tailed)		.074	**.002**	.230	.345	.584
	N	60	60	60	60	60	60
DURATION	Pearson Correlation	.232	1	.567**	.188	-.333**	-.439**
	Sig. (2-tailed)	.074		**.000**	.149	**.009**	**.000**
	N	60	60	60	60	60	60
FSH	Pearson Correlation	.396**	.607**	1	.607**	-.190	-.465**
	Sig. (2-tailed)	**.002**	**.000**		**.000**	.145	**.000**
	N	60	60	60	60	60	60
LH	Pearson Correlation	.157	.188	.607**	1	.078	-.148
	Sig. (2-tailed)	.230	.149	**.000**		.554	.259
	N	60	60	60	60	60	60
TESTO	Pearson Correlation	.124	-.333**	-.190	.078	1	.697**
	Sig. (2-tailed)	.345	**.009**	.145	.554		**.000**
	N	60	60	60	60	60	60
DHT	Pearson Correlation	.072	-.439**	-.465**	-.148	.697**	1
	Sig. (2-tailed)	.584	**.000**	**.000**	.259	**.000**	
	N	60	60	60	60	60	60

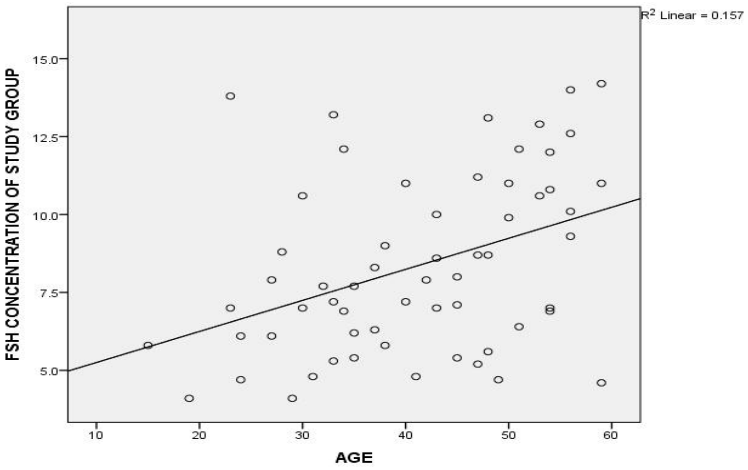


Figure 4.1:Correlation of FSH level with Age

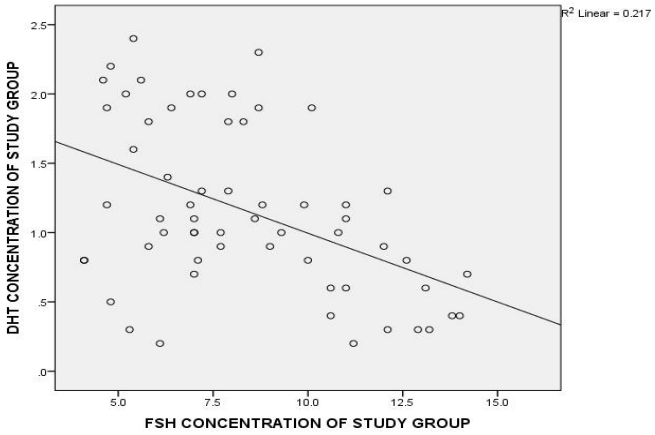


Figure 4.2:Negative correlation between DHT and FSH level

5. Discussion,Conclusion and Recommendation

5.1 Discussion

There was a significant increase in FSH levels in the study population as against that of control group ($p<0.001$). Similar reports according to Jegou et al., 1984 [13], stated that FSH level in rats was elevated after prolonged exposure to heat. This was followed by significant weight loss in the testes and reduction in fluid production. The rise in FSH level appeared to have resolved the effect of excess heat as fluid production improved later in the study. Similar observation was also made by Galil et al., in 1987 [14]. In their study, they reported that FSH level increased as intensity of heat increased. Report by Namiki et al., 1987 [15] however disagreed with this finding. Apparently, this may be because Namiki and colleagues focused only on short term effect of heat and the temperature of 370 is arguably mild, compared to that in this study (as high as 590C).

One way analysis of variance (ANOVA) which grouped the study subjects into 3 categories according to duration of driving in years (exposure to heat), showed a significant difference in FSH levels among the categories ($p<0.001$). Multiple comparisons study using Least Significance Difference (LSD) showed that the most exposed individuals to heat (i.e. group iii) is the reason for the significant difference in FSH levels between the categories. The subjects least exposed to heat had a significantly lower FSH value as reported in chapter 4. Roth and colleagues reported a similar observation in cattle. According to the report, the cattle exposed to a considerable amount of heat had elevated FSH and larger follicles [16].

The correlation table (table 4.3.) also showed a strong positive correlation between 'Exposure to Heat' and 'FSH' concentration. This shows that FSH levels rose consistently with intensity of heat / exposure to heat. This means that the ;period of

contact with heat' and 'temperature of the driver's seat' may be the most important factors responsible for the FSH rise.

Luteinizing hormone (LH) level was also significantly elevated in the study subjects, as compared to control group ($p<0.05$). This is in agreement with earlier report published by Galil and Setchel in 1987 [14]. Correlation analysis showed a strong positive correlation of LH with FSH among these subjects ($p<0.001$). These findings are similar to the findings of a 2014 report by Odiba and colleagues. They reported a significant correlation between FSH and LH concentration in infertile women of unknown cause [17].

These findings (ANOVA and Correlation analysis) which shows a consistent positive correlation between FSH and LH suggests that both testosterone production and spermatogenesis may be concomitantly affected by excess heat. These effects are however neutralized by the corrective effect of FSH and LH which are elevated.

There was no significant difference in Testosterone level of the study population and control subjects ($p>0.05$). This suggests that the elevation of LH synthesized by the anterior pituitary gland may just be enough to correct the possible damage caused by the excessive exposure to heat. Testosterone also correlated positively with DHT ($p<0.001$), to show that the same insult affecting testosterone also affects DHT. Also, among the study population, testosterone however correlated negatively with 'intensity of heat' ($p<0.05$). This suggests that there may be some degree of damage in testosterone synthesis or structure.

There was also no significant difference in DHT concentration between study population and control subjects. There are 3 possible explanations for this: (1) this is most likely due to the fact that testosterone was also not significantly reduced in these subjects. (2) Exposure to heat may not have any effect on the structure or function (activity) of 5 α reductase in the testes. (3) Circulating levels of 5 α reductase (synthesized in the skin, prostate gland and hair) can augment for any defect in the structure and function of the local (testicular) 5 α reductase.

However, there was a significant negative correlation between DHT concentration and FSH concentration ($r = -0.465^{**}$, $p<0.001$). This finding explains that though there was no significant difference in DHT between control and study population, there is a consistent drop in DHT as FSH is increasing. This drop in DHT is however not significant, possibly due to the compensatory role of FSH and LH.

Multifactorial analysis of variance which included the two factors -'duration of driving (in years)' and 'average daily exposure' demonstrated an additive or synergistic effect on FSH level (table 4.2.2). In this analysis, 'average daily exposure' was not significant ($p<0.05$), while 'period of driving (in years)' was significant. However, combination of the two factors (intercept) was very significant ($p<0.001$). This therefore explains that when these two factors are combined (as seen in drivers who work for a long period of time in a day and also have been driving for a long time), there may be heat-induced hypergonadotropism.

There was a significant correlation of FSH concentration with age ($p<0.05$). Also, analysis of variance (ANOVA table 4.2.1) which stratified age into categories also showed a significant difference in their means. Multiple comparisons using Tukey and LSD showed that group 2 and 3 (subjects above 30 yrs) is significantly higher than group 1 (less than 30 yrs). This finding is in tandem with earlier reports by Bribiescas, 2005; Ramasamy et al., 2009; Araujo and Wittert, 2011 [18][19][20]. Their various reports hinted that FSH consistently rises with age, as testicular function diminishes with age of the individual.

5.2 Conclusion

This study postulates that there is hypergonadotropism (since FSH and LH are elevated) in these subjects more than in control subjects. Test of significance however suggests that FSH level is more significantly elevated than LH, which is also buttressed by the lack of correlation between LH and any of the factors considered in this study. This suggests that the follicles and sertoli cells of the testes (which are targets of FSH action) may be more vulnerable to heat than the interstitial leydig cells which is the target for LH.

Correlation studies and ANOVA also suggests 'duration of contact with the hot seat' as well as 'Age of the driver', also influence FSH increase. The significant correlation of FSH with Age suggests that the self repair/regenerative potential of

the cells of the testes may be diminishing with age, thereby requiring the action of FSH from the anterior pituitary gland to activate repair processes (within the ageing testicular cells).

In summary, there is hypergonadotropism in long distance drivers of heavy duty vehicles, while testosterone and DHT levels are not significantly different.

5.3 Recommendations

1.Workers occupationally exposed to testicular heat should as much as possible, reduce contact with the source of the heat. In this case, drivers should reduce the duration of sitting on the driver's seat once it becomes hot.

2.A more comprehensive research which will include other occupationally exposed individuals to testicular heat as well as exposure to other environmental toxicants like hydrocarbons and heavy metals is proposed.

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