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ASSESSMENT OF TESTOSTERONE AND OESTROGEN LEVELS OF YOUNG ADULTS MALE SMOKERS OF MARIJUANA (*CANNABIS SATIVUS*) IN PORT HARCOURT, NIGERIA

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ABSTRACT

The use of marijuana (*Cannabis sativa*) in Nigeria is considered particularly high and could have damaging consequences. Thus, this study was designed to assess testosterone and oestrogen levels of young adult males involved with marijuana use in Port Harcourt, Nigeria. The study population includes; 90 males comprising 50 marijuana (smokers) and 40 (non smokers), their age ranging between 17-41 years and the duration of marijuana use was between 0.6 to 15years. Measurement of the weight, height and waist circumference was done using standard procedures. BMI and WHR were derived appropriately and serum testosterone and estrogen levels were determined by a microplate enzyme immunoassay kit. The study shows that the mean ±SD of BMI for the smokers and non smokers was 22.60 ± 3.41kg/m^2 and 21.37 ± 1.96 kg/m^2, WHR was 0.86 ± 0.05 and 0.90 ± 0.11, testosterone was 6.94 ± 2.64 ng/mL and 7.37 ± 2.25 ng/mL and estrogen was 12.45 ±15.21 ng/mL and 9.40 ± 5.32 ng/mL respectively. While significant difference ($p<0.05$) was seen between the means of BMI, others did not show significant differences ($p>0.05$). The average smoking period was 4.97 ± 3.57 years and T/E ratio was 0.56. No significant correlations was seen between testosterone and estrogen and the other parameters in both smokers and non smokers but testosterone was positively correlated ($r=0.27$) with estrogen in the smokers ($p<0.05$). The study shows that even in the population studied, the levels of testosterone and estrogen was reduced in the smokers when compared with the non smokers and the effect of duration of expoure to smoking was also inconsistent. The implication of the finding is that marijuana

could have damaging effects on the fertility profile of smokers in Port Harcourt. Thus efforts short be doubled to educate youths on the danger of this practice on their procreativity potential.

KEYWORDS: Male, obesity, infertility, testosterone, estrogen, body mass index, waist to hip ratio.

INTRODUCTION

It has been reported that exposures to certain drugs and toxins may play a role in male infertility and recreational use of illicit drugs is an important factor for consideration when assessing the etiology of male infertility in couples diagnosed with infertility. Some of the illicit drugs that have been found to adversely impact male fertility includes marijuana, opioid narcotics, methamphetamines, cocaine, and anabolic-androgenic steroids (AAS).[1] Marijuana has been reported to rank highest (20.10%) among all illicit drugs used by young males within the ages of 26-34 years according to the survey made by the National Survey on Drug Use and Health (NSDUH) in 2009 in the USA.[2] When marijuana derived from the dried leaves and flowers from the marijuana plant (*Cannabis sativa*) is smoked, the psychoactive cannabinoid compound called delta-9-tetrahydrocannabinol (THC) is released.[3] The cannabinoids can be synthesized endogenously and are known to modulate several pathophysiologic processes, including neuropathic pain, mood disorders, movement disorders such as Parkinson disease and Huntington disease, disease processes such as cancer, atherosclerosis, and obesity, as well as reproductive functions.[4-5]

The negative impact of THC on male reproductive physiology has been well documented and it has been reported that testosterone levels in the plasma of chronic marijuana smokers were significantly lower when compared with age-matched controls who had never used marijuana, and the reduction was dose dependent.[6] Some reports of the cardiovascular effects of marijuana exposure stated that administration to healthy marijuana users led to an increase in both pulse rate and blood pressure.[7] Despite these concerns, the idea that marijuana use is safe is deep seated in the public especially amoung young adults and even among some health-care professionals. In Nigeria, as at the time of this study, there are scanty documented reports on the effect of marijuana use on the ferility profile of young adults despite widespread knowledge of its use. Thus, this study was designed to assess testosterone and oestrogen levels of young adult males involved with marijuana use in Port Harcourt, Nigeria in order to allay concerns about the possibility that marijuana use might

impair male sexual function or even lead to impotence among the teaming population of young adults involved in its use.

MATERIALS AND METHODS

Study population

The study was conducted among young male adults residing in various waterfronts in parts of Port Harcourt, Nigeria. They were randomly selected. Both the study subjects (marijuana users) and the control subjects willingly gave their consent to participate in the study. The study population includes; 90 males comprising 50 marijuana users (smokers) as study subjects and 40 apparently healthy males (non smokers) as control subjects, their age ranging between 17-41 years and the duration of marijuana use among the study subjects was between 0.6 to 15years.

Ethical approval

The ethical approval for the study was granted by the Local Ethics Committee of the Rivers State Health Management Board, Port Harcourt and the Research Ethics Committee, Faculty of Science, Rivers State University, Nkpolu- Oroworukwo, Port Harcourt, Nigeria.

Blood sample collection

5ml of venous blood was collected under aseptic condition from the ante cubital vein into sterile plain containers from the selected subjects. Then serum was separated by making a centrifugation for all samples by digital centrifuge 1500 rpm for 2 min according to the method of Sood.[8]

Inclusion and exlusion criteria

Only subjects who accepted to have used marijuana or are currently smoking marijuana were included as study subjects. It is also pertinent to state that most of the users also smoked cigarettes. Normal healthy subjects as control group incuded people who have never smoked marijuana or even cigarettes. However, subjects who showed signs of psychiatric illness and also reported being treated for liver disease were excluded from the study.

Measurement of weight, height and waist circumference

The height was measured (in metres) to the nearest 0.1 m with a calibrated meter rule placed horizontally against the wall. The subjects were barefooted and wore light clothing. The subjects stood 0n a flat surface, with weight distributed evenly on both feet, heels together

and the head positioned so that the line of vision is perpendicular to the body. The hands hung freely by the sides and the head, back, buttocks and the heels were in contact with the vertical board. The moveable headboard was brought onto the topmost point of the head with sufficient pressure to compress the hair. The weight of the subjects was measured (in kilograms) with participants wearing light clothing and no shoes, and recorded to the nearest 0.1 kg as recommended by the World Health Organization[9] (WHO, 1995). The Hana Bathroom Scale (Pese-Personne Br 9011) weight balance made in China was used to measure the weight of the participants. Body mass index (BMI) was calculated as BMI= body mass in kg/height in m^2. Based on the waist measuring at the narrowest place and hip measuring at the widest place, Waist-to-Hip-Ratio (WHR) was calculated according to the following formula: WHR = waist (cm)/hip (cm).[9]

Determination of serum testosterone and estrogen concentations

The Accubind ELISA Microwells which is a Testosterone Test System and Estrogen Test System was used for the quantitative determination of total testosterone and estrogen concentrations in the serum samples by a Microplate Enzyme Immunoassay. The kit was manufactured by Monobind Inc. Lake Forest, CA 92630, USA.

RESULTS

The present study was designed to investigate the effect of marijuana consumption on the levels of testosterone and estrogen in young male adults involved in its use. The study (smokers) and control (non smokers) subjects were age-matched. The results of testosterone, estrogen, body mass index (BMI), waist-to hip ratio (WHR), duration of exposure (years of intake of marijuana) and testosterone/eostrogen ratio is shown in table 1.

Table 1: Mean±SD of biochemical and anthropometric parameters.

Parameters	Study subjects (n=50)	Control subjects (n=40)	p-value
Age (years)	23.62 ±4.10	23.80 ± 4.87	0.85, NS
BMI (Kg/m^2)	22.60 ± 3.41	21.37 ± 1.96	0.04, p<0.05
WHR	0.86 ± 0.05	0.90 ± 0.11	0.40, NS
Duration of exposure (yrs)	4.97 ± 3.57	-	
Testosterone (ng/ml)	6.94 ± 2.64	7.37 ± 2.25	0.4089, NS
Oestrogen (ng/ml)	12.45 ±15.21	9.40 ± 5.32	0.243, NS
Testosterone/Estrogen ratio	0.56	0.78	

Note: *Normal range for serum testosterone = 2.5 – 10 ng/mL

Normal range for estrogen: 4 – 94 ng/mL
Normal range for WHR in men: >9.0

Significant difference in mean was observed in the BMI ($p<0.05$) between the smokers and non smokers although the levels of the parameters were within acceptable limits. No significant difference in means ($p>0.05$) was seen in the other variables. The average years that the subjects were exposed to marijuana was 4.97 ±3.57 years.

The effect of duration of exposure (years that the subjects smoked) to marijuana and the levels of testosterone and estrogen is shown in table 2. The table shows that smoking of marijuana among the smokers which ranged from between 0.6 to 15 years did not have significant ($p>0.05$) effect on the levels of testosterone and estrogen between the age classes. However, while steady inconsistent increase in the means was observed in the levels of testosterone between the age classes as the years of exposure increases, the reverse was the case with estrogen levels. Thus, while the testosterone levels which was 7.57 ± 2.97 ng/mL for 0-3years at baseline declined to 6.18 ± 2.27 ng/mL at 4-7 years, it rose to 8.13 ± 2.26 ng/mL for 12 and above years and estrogen levels declined from 12.59 ± 14.83 ng/mL for 0-3years to 7.13 ± 1.70 ng/mL for 12 and above years.The levels of the two hormones did not rise above or decrease below the level for normal population.

Table 2: Effect of duration of exposure to marijuana on testosterone and estrogen levels.

Duration of exposure to marijuana (years)	Testosterone (ng/mL) Mean ± SD	Estrogen (ng/mL) Mean ± SD
0 -3 (n=23)	7.57 ± 2.97	12.59 ± 14.83
4 – 7 (n=17)	6.18 ± 2.27	11.93 ±17.47
8 – 11 (n=6)	6.42 ± 2.17	11.28 ± 13.79
12 and above (n=4)	8.13 ± 2.26	7.13 ± 1.70
p-value	p>0.05, F=1.273	p>0.05, F=0.1482

Note: *Normal range for serum testosterone = 2.5 – 10 ng/mL

Normal range for estrogen: 4 – 94 ng/mL

As seen in tables 3 and 4, no significant correlations was seen between testosterone and estrogen and age, BMI and WHR non smokers but testosterone was positively correlated with estrogen in the marijuana smokers. However, the percentage of the subjects with mean values of measured parameters outside the range stipulated for normal populations were considerably similar between the two populations studied. While 100% of both the smokers and non smokers had estrogen levels within the levels stipulated for normal populations, the levels of testosterone in the subjects who had consummed marijuana showed that while 94.00% had testosterone level within the normal range, 6.00% of the subjects had levels above normal range. The results of BMI values shows that while 88% of the study subjects

had normal BMI values, 4% were obese and 8% deficient BMI values. In the control subjects, 95.50% of subjects had normal BMI values while 7.50% were deficient in BMI (table 5).

Table 3: Correlation coefficients of testosterone and estrogen with other measured parameters in the non smokers.

Parameters	Correlation coefficients (r)	Parameters	Correlation coefficients (r)
Testosterone vs age	-0.08	Estrogen vs age	0.19
Testosterone vs BMI	-0.03	Estrogen vs BMI	0.15
Testosterone vs WHR	-0.06	Estrogen vs WHR	0.17
Testosterone vs estrogen	0.16	Estrogen vs testosterone	0.16

Table 4: Correlation coefficients of testosterone and estrogen with other measured parameters in the smokers.

Parameters	Correlation coefficients (r)	Parameters	Correlation coefficients (r)
Testosterone vs age	-0.02	Estrogen vs age	-0.23
Testosterone vs BMI	-0.24	Estrogen vs BMI	0.07
Testosterone vs WHR	-0.30	Estrogen vs WHR	-0.14
Testosterone vs estrogen	0.27	Estrogen vs testosterone	0.27
Testosterone vs dur. of exposure	-0.04	Testosterone vs dur. of exposure	-0.05

Table 5: Effect of marijuana on the level of measured parameters

Parameters	Mean within normal range		Percentage		Mean above normal range		Percentage		Mean below nornal range		Percentage	
	Smokers	Non-smokers	Smokers	Non-smokers	Smokers	Non-smokers	Smokers	Non smokers	Smokers	Non smokers	Smokers	Non smokers
Testosterone	6.13±2.10	6.89±1.96	94.00%	87.5%	11.02±0.73	10.90±0.76	6.00%	12.5%	-	-	-	-
Estrogen	12.45 ±15.21	9.40 ±5.32	100%	100%	-	-	-	-	-	-	-	-
BMI	22.56± 2.34	21.68 ± 1.73	88.00%	92.50%	32.99 ±2.34	-	4.00%	-	17.18± 1.59	17.79± 0.77	8.00%	7.50%
WHR	0.84 ± 0.04	0.84 ±0.03	82.00%	67.5%	0.92± 0.01	1.03± 0.12	8.00%	32.5%	-	-	-	-

DISCUSSION

Marijuana is one of the most commonly used illicit drug in Nigeria even though it is considered illegal and smokers are often treated as criminals. In the United States, people of about 12 years of age have been reported of having used marijuana and its use among young adults is particularly high.[10] It has reported that the regular use of marijuana during adolescence could be associated. with an increased likelihood of damaging consequences.[11] Brown and Dobs[12] reported that marijuana and other cannabinoids, act on the hypothalamic – pituitary – gonadal (HPG) integrity and affect reproductive function which results in infertility. Earlier studies have also reported that the effects of marijuana on fertility are mediated by central cannabinoid receptors (CB1) in the hypothalamus.[13] These cannabinoid receptors have been found in the testes[14] and ovaries[15], of experimental animals which suggests that cannabinoids could have some effect on the gonads. Kelodny and his co-workers[16] reported decreased level of testosterone in male marijuana smokers while Freidrich et al.[17] observed no significant difference in the testosterone levels of marijuana smokers and non-smokers. The results of mean ±SD of testosterone obtained in this study for subjects who smoked marijuana was 6.94 ± 2.64 ng/mL while that obtained for non smokers of marijuana was 7.37 ± 2.25 ng/mL and there was no significant difference ($p>0.05$) between the two means though the level was slightly higher in the non smokers. In a related study carried out in Edo State, mean ±SD testosterone obtained for smokers and non smokers of marijuana was 5.33±3.5ng/mL and 8.5 ± 2.4 respectively and the difference was significant ($p<0.05$).[18] The levels of testosterone obtained in these two separate studies from the same geo-political zone, the Niger Delta region are similar since they are within the normal reference range of 2.4-9.4 ng/mL.[19] However, an outstanding observation in the two studies is that the levels of testosterone was reduced in the smokers when compared with the non smokers. This may be the basis for the conclusion by Kelodny and co-workers[6] that marijuana causes a decrease in testosterone level in males and therefore could cause male infertility. Decrease in testosterone often occurs when there is inhibition of the gonadotrophin releasing hormone (GnRH) pulse generator in the hypothalamus usually caused by Δ9-THC.[20] Low or reduced testosterone level suppresses spermatogenesis and cause oligospermia.[1] However, the findings of Freidrich et al.[17] maintained that no significant difference occurs between the serum testosterone level of marijuana smokers and nonsmokers and thus infertility in marijuana smokers should not be delibrately attributed to the effect of marijuana on testosterone in male marijuana smokers.

Estradiol, the predominant form of estrogen reportedly plays a critical role in male sexual function. By ensuring that proper degree of libido, erectile function and spermatogenesis occurs in the males.[21] Decreased testosterone has been reported to be clearly associated with low libido in males.[22] In addition, an abnormal T/E ratio (<10) has been associated with decreased semen parameters, poor sperm concentration, motility, and morphology.[3] Tietz et al.[19] recommended etrogen level of 4-94 ng/mL as the normal reference range for serum estrogen. The estrogen concentration obtained from this study for smokers and non smokers of marijuana were 12.45 ±15.21 ng/mL and 9.40 ± 5.32 ng/mL respectvely and this value is relatively low for normal population. Increase in the levels of estrogen and testosterone thus is required to boost libido and sexual drive in males.[24] These reports confoundly supports the observation of Kelodny and his co-workers.[16] However, sperm counts, motility, morphology and viability, which are cardinal indices indicating the fertility status of males were not determined in this study making it difficult to fully ascertain the fertility profile of the study populations.

The effect of number of years of smoking on testosterone and estrogen levels (table 2) did not show consistency in the concentration of the hormones. The comparison of the means of the hormones according to the years of exposure (in classes) did not also show any significant difference ($p>0.05$) between the various classes of years of exposure and level of estrogen and testosterone in the subjects but a positive correlation was observed between testosterone and estrogen concentration ($r= 0.27$). This positive correlation beween testosterone and estrogen agrees with the works of Ramasamy et al.[25] and Schlegel[24] who reported that both estrogen and testosterone are necessary for normal libido in testosterone‐deficient men. However, while inconsistent increase in the means was observed in the levels of testosterone between the age classes as the years of exposure increases, the reverse was the case with estrogen levels. The results of human studies investigating the effects of cannabinoids on reproductive hormones have been conflicting. While lower testosterone levels were reported in chronic marijuana users compared to nonusers in some studies[6], in others, acute decreases in both LH and testosterone have been observed after marijuana smoking.[26] Research designs aimed at investigating the conflicting effect of chronic marijuana smoking had shown no significant changes in LH and testosterone levels over the period of study.[27] This inconsistent observation was also made in this study and could be attributed to reflect the development of tolerance in the users. Tolerance to chronic marijuana use has been reported in experimental animal studies.[28]

The BMI, defined as kg/m^2, is a useful tool to measure obesity. Obese men are three times more likely than healthy men of normal weight to have a sperm count of fewer than 20 million/ml, an indicator of oligospermia.[29] This study revealed that obesity was evident in 4% of the marijuana smokers in the population studied (table 5). MacDonald et al.[30] found no evidence for a relationship between BMI and total sperm concentration or total sperm count. For the whole range of BMI values, Giagulli et al.[31] reported that there is a negative correlation of BMI values to serum testosterone levels. This discovery was also made in this study where serum testosterone level was found to be negatively correlated with BMI (r= -0.24). No correlation with BMI values (r=-0.07) was found with serum estrogen levels (table 4). The BMI of marijuana smokers was also significantly higher ($p<0.05$) when compared with non-smokers. This observation agrees with the findings of previous authors.[32, 33] Gorter et al.[34] and Glass[35] had previously reported that marijuana smokers often show increase in the circulating estrogen/androgen ratio which physiologically lead to accumulation of breast tissue which is responsible for the increase in BMI in marijuana smokers. We reasoned that this condition could also be responsible for the increase in BMI in mar ijuana smokers in this population. It is of note to state that total sperm counts was not performed on the marijuana smokers who participated in this study, thus, the effect of BMI on spermatogenesis could not be determined in these subjects.

An elevated BMI value has been recognised as an established risk factor for ischaemic heart disease, stroke and many cancers.[36] Researchers has opined that as a result of the inclusion of abdominal fat deposition in the Syndrome X, Waist-Hip- ratio (WHR) should be regarded as a more sensitive indicator of obesity.[37] The mean difference in WHR obtained in this study between the marijuana smokers and non smokers was insignificant ($p>0.05$) indicating that the influence of WHR on obesity in this population may have been insignificant.

CONCLUSION

The evidence obtained from this study suggests that marijuana use could be a major cause of infertility and marijuana – related complications among young male adults in Port Harcourt, Nigeria. We recommend that a comprehensive investigation into the health effects, patterns of use and problems and especially its effects on the risks of cardiovascular, respiratory disease and cancer risks be made a priority in our health policy formulation in order to save our future generations.

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