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Anandamide Level in Men with Oligoasthenoteratozoospermia

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ABSTRACT

Background: Male infertility is a relatively common medical condition. Male partners are found to be solely responsible for 20–30% of infertility cases. Oligoasthenoteratozoospermia (OAT) is the most common laboratory finding in infertile men. Endocannabinoids are endogenously produced substances, that primarily act at cannabinoid receptors (CBRs), thus reproducing some of the biological actions of the natural cannabis sativa components (the "cannabinoids"). The endocannabinoid system is involved in several physiological processes, including fertility and body weight control.

Objective: The aim of this study was to detect the level of anandamide (AEA) in patients with OAT and its relation to BMI.

Patients and methods: This study was a case control study. It was hold between June 2018 to April 2019 in the Dermatology, Venereology and Andrology Department at Aswan University Hospital. The study included 20 patients with OAT and 9 as controls. Semen analysis using CASA was done. AEA level in seminal plasma was measured using ELISA kits. BMI was calculated.

Results: AEA level in seminal plasma significantly decreased in men with ligoasthenoterat- ozoospermia than control ($P < 0.009$). AEA concentration on seminal fluid was significantly increased with an increase in BMI ($P= 0.023$). On the other hand, a significant negative relationship was present between BMI and semen volume ($P= 0.03$).

Conclusion: AEA concentration in seminal plasma was significantly decreased in patients with OAT. AEA concentration in seminal fluid was significantly increased with an increase in BMI. On the other hand, a significant negative relationship was present between BMI and semen volume.

Keywords: Anadamide, Oligoasthenoteratozoospermia, BMI.

INTRODUCTION

Infertility is the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy in one year [(1)]. Fertility rates have been declined over the last century in many developed countries [(2)]. Male infertility is a relatively common medical condition affecting up to 12% of men globally. Male partners are found to be solely responsible for 20–30% of infertility cases and contribute to roughly 50% of cases overall [(3)]. It affects approximately one in six couples (15–20%) [(4)].

It is considered a male factor when an alteration in sperm concentration and/or motility and/or morphology could be found in at least one sample of two sperm analyses, which comply with World Health Organization (WHO) 2010 guidelines, collected between1–4 weeks apart [(5)]. However, causes of approximately 50 % of male factor infertility cases are still unexplained [(6)].

Oligoasthenoteratozoospermia (OAT) is the most common finding in the era of evaluation of male infertility. In most cases the etiology of OAT can be detected by common investigative and radiological methods. When the etiology of oligoasthenoteratozoospermia could not be detected, the syndrome is termed idiopathic oligoasthenoteratozoospermia (iOAT) [(7)].

Cannabinoid signalling is mediated via two well-characterized G-protein-coupled cannabinoid receptors, CB1and CB2, which are the molecular targets of the endocannabinoids (ECS), a group of endogenous bioactive lipid mediators [(8)]. The most studied endocannabinoid is *N*-arachidonoylethanolamine (anandamide, AEA), which is found in tissues and biological fluids at nanomolar concentrations. Human spermatozoa have been shown to express functional cannabinoid receptors CB1 and CB2 and mRNA and proteins for CB1 and CB2 receptors have been reported in mature human spermatozoa [(9, 10)]. Significant levels of AEA are present in human seminal plasma mid-cycle fallopian tubal fluid and follicular fluid. This suggests that human spermatozoa are sequentially exposed to AEA during storage in the epididymis and also during their journey through the female reproductive tract, suggesting a potential modulatory role of the endocannabinoid system on human sperm functions [(11)].

Studies on the relationship between body mass index (BMI) and sperm motility have shown conflicting data. Comparing obese fertile to obese infertile oligozoospermic males found a significant negative correlation with sperm motility [(12)].

There is an increasing data on endocannabinoid control of energy homeostasis and recent evidence for

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ECS dysregulation in overweight and obesity disorders [13]. It has become apparent that human obesity is associated with activation of the ECS and with increased levels of ECS in many tissues, as well as in the circulation [14].

Current concepts in this field indicate that body weight and fat metabolism are influenced by complex ECS regulatory mechanisms that modulate energy balance, feeding behavior and peripheral lipid metabolism [15].

The aim of this study was to detect the level of anandamide in patients with OAT and its relation to BMI.

PATIENTS AND METHODS

This study was a case control study. It was hold between June 2018 to April 2019 in the Dermatology, Venereology and Andrology Department, Aswan University Hospital. The study included 20 patients with OAT and 9 as controls.

Inclusion criterion: Patients with oligoasthenoteratozoospermia.

Exclusion criteria: Systemic diseases that affect semen quality e.g. Malignancies, renal failure and liver failure. Also, any drugs that affect semen quality e.g. Chemotherapy.

Patients were subjected to full clinical history taking, full general examination and full andrological examination. Semen analysis using computer assisted semen analysis (CASA) according to World Health Organization (WHO) guidelines **(WHO, 2010)**[5] was done. Semen samples were assessed for anandamide using enzyme linked immunoassay (ELISA). BMI was calculated.

Ethical and patients' approval:

An approval of the study was obtained from Aswan University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation.

Statistical analysis

Data collected and arranged in excel data sheet. Data analyzed using SPSS version 22. Continuous variables presented as mean ± standard deviation (SD) and compared using student's T test. Categorical factors presented as frequencies and percentages and compared using Person's Chi- square test. P value ≤ 0.05 considered significant.

RESULTS

The mean age of the included men in the present study was 39.25 ± 8.08 years for cases and 36.67 ± 14.61 for controls. P=0.629, which was considered not statistically significant (Table 1).

Socio-demographic data differences between cases and controls (Table 2). In the present study we found a significant decrease in semen parameters in smokers (**P=0.015***). Semen parameters differences between cases and controls showed that there was statistically significant difference between cases and controls as regards Conc./ml **(p < 0.001**)**, total sperm count **(p < 0.001**)**, progressive motility **(p < 0.001**),** total motility **(p < 0.001**)** and morphology **(p < 0.001**)** as shown in table (3).

We calculated seminal plasma concentrations of AEA and studied relationship between it and semen quality. We found that AEA seminal plasma concentrations were significantly lower in cases (147.18 ± 51.28) as compared to controls (196.4 ± 13.22) **(P=0.009**)** (Table 4).

We studied also, relationship between anandamide concentrations in seminal plasma and BMI and found that it was 136.41 ± 63.2 for normal, 178.79 ± 12.67 for overweight and 191.8 ± 7.97 for obese (**P=0.023*),** which is considered statistically significant (Table 5). The same relation was significant among cases as shown in (Table 8). Therefore, AEA concentration on seminal fluid is significantly increased with an increase in BMI.

In the present study we found a significant increase in patients with varicocele within cases **(p=0.002*)** (Table 2). In comparison between varicocele and control group as regards semen parameters and AEA level, there was statistically significant relationship regarding conc./ml **(P < 0.001**)**, total sperm count **(P = 0.006*),** progressive motility **(P < 0.001**)**, total motility **(P < 0.001**)**, morphology **(P < 0.001**)** and AEA level **(P=0.007*)** (Table 6).

In comparison between different BMI groups and semen parameters (volume, conc./ml, total sperm count, progressive motility and total motility), we found a significant negative relationship between BMI and semen volume **(P=0.030*)** (Table 7).

Table (1): Descriptive data for cases and controls regard age.

	Cases (n=20)	Control (n=9)	P. value
	Mean ± SD	Mean ± SD	
Age (years)	39.25 ± 8.08	36.67 ± 14.61	0.629

P-value < 0.05 was considered statistically significant.

Table (2): Socio-demographic data differences between cases and controls.

	Cases (n=20)		Control (n=9)		P. value
	No.	%	No.	%	
Occupation					
Worker	17	85	8	88.9	0.779
Not worker	3	15	1	11.1	
Special habit					
Non smoker	11	55	9	100	**0.015***
Smoker	9	45	0	0	
Type & duration of infertility					
No infertility	4	20	6	66.7	**0.007****
Not married	2	10	3	33.3	
1ry	9	45	0	0	
2ry	5	25	0	0	
Other Diseases					
No	15	75	6	66.7	0.254
Diabetic	1	5	0	0	
ED	2	10	0	0	
ED+HTN	0	0	1	11.1	
ED+HTN+DM	0	0	1	11.1	
Hypothyrodism	1	5	0	0	
Psoriasis	0	0	1	11.1	
Varicose veins	1	5	0	0	
Local Examination					
Normal	8	40	9	100	**0.002***
Varicocele	12	60	0	0	

P-value < 0.05 was considered statistically significant.

Table (3): Semen parameters differences between cases and controls.

	Cases (n=20)	Control (n=9)	P. value
	Mean ± SD	Mean ± SD	
Semen volume (ml)	1.75 ± 0.85	2.21 ± 1.2	0.247
Conc.(10^6 per ml)	11.91 ± 3.11	45.69 ± 19.87	**<0.001*****
Total sperm count (10^6 per ejaculate)	20.1 ± 9.3	103.7 ± 96.45	**<0.001*****
Progressive motility (PR,%)	19.12 ± 10.47	43.8 ± 7.32	**<0.001*****
Total motility (PR+NP, %)	36.41 ± 17.39	72.61 ± 7.96	**<0.001*****
Morphology (normal forms,%)	2.77 ± 0.73	13.39 ± 2.4	**<0.001*****

P-value < 0.05 was considered statistically significant.

Table (4): Anandamide levels in cases and controls.

	Cases (n=20)	Control (n=9)	P. value
	Mean ± SD	Mean ± SD	
Anandamide (µg/kg)	147.18 ± 51.28	196.4±13.22	**0.009****

P-value < 0.05 was considered statistically significant

Table (5): Anandamide distribution between BMI groups.

	BMI			P. value
	Normal weight (n=13)	**Overweight (n=10)**	**Obese (n=6)**	
	Mean ± SD	Mean ± SD	Mean ± SD	
Anandamide	136.35 ± 63.2	178.79 ± 12.67	191.8 ± 7.97	**0.023***

P-value < 0.05 was considered statistically significant.

Table (6): Comparison between patients with varicocele and control group regarding semen parameters.

	Control (n=9)	Varicocele (n=12)	P. value
	Mean ± SD	Mean ± SD	
Semen volume (ml)	2.21 ± 1.2	1.60 ± 0.82	**0.185**
Conc.(10^6per ml)	45.69 ± 19.87	12.05 ± 3.68	**<0.001*****
Total sperm count (10^6 per ejaculate)	103.7 ± 96.45	18.4 ± 7.11	**0.006****
Progressive motility (PR,%)	43.8 ± 7.32	16.63 ± 11.91	**<0.001*****
Total motility (PR+NP, %)	72.61 ± 7.96	34.24 ± 21.81	**<0.001*****
Morphology (normal forms,%)	13.39 ± 2.4	2.68 ± 0.69	**<0.001*****
Anandamide (µg/kg)	196.4 ± 13.21	144.23 ± 50.13	**0.007***

P-value < 0.05 was considered statistically significant.

Table (7): BMI groups and distribution of semen analysis parameters.

	Normal weight(n=13)	Overweight(n=10)	Obese(n=6)	P. value
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Semen volume (ml)	2.39 ± 1.13	1.64 ± 0.48	1.25 ± 0.76	**0.030***
Conc.(10^6per ml)	23.36 ± 21.38	22.16 ± 16.74	20.68 ± 21.84	0.963
Total sperm count (10^6per ejaculate)	63.9 ± 92.1	34.28 ± 20.25	26.9 ± 35.06	0.419
Progressive motility (PR,%)	29.15 ± 16.59	25.66 ± 16.55	23.5 ± 8.62	0.730
Total motility (PR+NP, %)	50.7 ± 23.53	45.69 ± 25.93	44.28 ± 17.13	0.813
Morphology (normal forms,%)	5.88 ± 5.41	6.99 ± 5.77	4.92 ± 4.23	0.745
Anandamide (µg/kg)	136.41 ± 59.41	178.79 ± 12.67	191.8 ± 7.97	**0.023***

P-value < 0.05 was considered statistically significant.

Table (8): BMI groups and distribution of semen analysis parameters among cases.

Cases	BMI in cases			P. value
	Normal weight (n=9) Mean ± SD	**Overweight (n=6)** Mean ± SD	**Obese (n=5)** Mean ± SD	
Semen volume (ml)	2.13 ± 0.91	1.65 ± 0.53	1.2 ± 0.84	0.137
Conc.(10^6per ml)	37.26 ± 40.81	72.75 ± 69.68	45.05 ± 24.99	0.391
Total sperm count (10^6per ejaculate)	23.93 ± 8.55	20.45 ± 9.89	12.81 ± 6.54	0.095
Progressive motility (PR,%)	22.51 ± 13.61	15.62 ± 13.44	25.57 ± 14.23	0.469
Total motility (PR+NP, %)	52.36 ± 30.66	28.53 ± 16.77	38.74 ± 11.68	0.181
Morphology (normal forms,%)	2.6 ± 0.73	2.65 ± 0.87	3.2 ± 0.46	0.319
Anandamide (µg/kg)	106.15 ± 51.46	173.03 ± 6.48	190.02 ±7.47	**0.001****

P-value < 0.05 was considered statistically significant.

DISCUSSION

In most cases of male infertility, OAT is found in there semen analysis. The etiology of OAT can be detected by common investigative and radiological methods [16]. When the etiology cannot be detected, this condition is named iOAT [7]. The most accepted pathogenesis that have been claimed lead to OAT is increase in ROS concentration and subsequent increase in OS which has negative effects on spermatogenesis [17]. ROS induce sperms damage by several mechanisms, such as lipid peroxidation, mitochondrial damage, apoptosis and DNA damage [7]. Excessive and uncontrolled production of ROS by seminal leukocytes or abnormal sperms result in an OS status causing impaired sperm viability and motility and increased mid-piece sperm defects impairing sperm capacitation and acrosome reaction [18].

Smoking tobacco may lead to decreased spermatogenesis by several mechanisms. There are 4700 different chemicals have been identified in tobacco smoke several of which have known effects on spermatogenesis and alter blood and seminal fluid heavy metal concentrations [19]. Furthermore, tobacco smoke contains polycyclic aromatic hydrocarbons (PAH) and other chemicals known to cause mutagenesis, apoptosis, and cell death in rapidly dividing cells, leading to decreased rates of cell division and spermatogenesis. Tobacco smoke may increase carbon monoxide levels and induce relative hypoxia within the testes [20].

AEA is the most studied endocannabinoid. It is the substance of our interest in this research. The ECS is deeply involved in the control of several physiological processes, including fertility [21]. ECs are endogenously produced substances act at CBRs producing some of the biological actions of the natural cannabis components (the "cannabinoids"). This is the cause of their nomenclature [22].

This study was conducted to evaluate the level of AEA in seminal plasma in patients with OAT and to evaluate its relation to BMI.

Twenty patients and nine controls were recruited for this study. BMI was calculated for all study subjects. The mean age of the included men in the present study was 39.25 ± 8.08 for cases and 36.67 ± 14.61 years for controls.

In the present study, we found a significant decrease in semen parameters in smokers (P=0.015). This finding is in agreement with a meta-analysis of more than 2500 men from five separate studies revealed a significant decrease in sperm concentrations of current smokers compared to those who had never smoked [23]. Smokeless tobacco has also been associated with a decrease in sperm quality and oligoasthenozoospermia or azoospermia in a dose-dependent fashion [24]. In contrast, **Rybar *et al.*** [25] failed to confirm a relationship between smoking and sperm quality.

In this study, we calculated seminal plasma concentrations of AEA and studied relationship between it and semen quality. We found that AEA seminal plasma concentrations were significantly lower in cases (147.18 ± 51.28) when compared to controls (196.4 ± 13.22) (P=0.009). In agreement with our study, **Amoako and colleagues** have shown that AEA seminal plasma concentrations were lower in men with asthenozoospermia and OAT when compared to controls [26].

We studied also, relationship between anandamide concentrations in seminal plasma and BMI and found that it was 136.41 ± 63.2 for normal, 178.79 ± 12.67 for overweight and 191.8 ± 7.97 for obese (P=0.023). Therefore, AEA concentration on seminal fluid is significantly increased with an increase in BMI. In agreement with our study, **Bellocchio *et al.*** [27] showed that human obesity is associated with activation of the ECS and with increased levels of ECs in many tissues, as well as in the circulation. In addition, obese menopausal women were found to have increased plasma AEA and 2-AG levels compared to lean women of similar age [28]. In contrast, **Engeli and colleagues** have shown that, there is no relationship between plasma AEA and 2-AG and obesity or body fat distribution [(14)].

In the present study, we found a significant increase in patients with varicocele within cases (p=0.002). This result is in accordance with **Punab *et al.*** [(29)] who found that 30-40% of cases have varicocele. In addition, **Esteves *et al.*** [(30)] reported an incidence of 21.9% of varicocele in 2,875 analyzed subjects. Regarding varicocele, in comparison between varicocele and control group concerning semen parameters, there was statistically significant relationship as regards semen parameters (volume, conc./ml, total sperm count, progressive motility, total motility and morphology) and AEA level. **Agarwal and colleagues** in a systematic review and meta-analysis concluded that varicocele has a significant risk factor that negatively affects semen quality [(31)].

In the present study, we found a significant negative relationship between BMI and semen volume. This is in agreement with **Eisenberg *et al.*** [(32)] who documented that men with high BMI are at higher risk of low semen volume. This finding correlates with the finding of **Chavarro *et al.*** [(33)]. In contrast to our study, **Alshahrani *et al.*** [(34)] found no significant relationship between BMI and semen volume. **MacDonald *et al.*** [(35)] showed in a metanalysis of about thirty relevant studies that there was no significant relationship between BMI and semen volume.

CONCLUSIONS

Our study suggests that smoking and varicocele are considered risk factors for the development of OAT. AEA concentration in seminal plasma is significantly decreased in patients with OAT. AEA concentration on seminal fluid is significantly increased with an increase in BMI. In addition, a significant negative relationship is present between BMI and semen volume.

This study has some limitations that it was conducted on relatively small sized sample of patients and control subjects.

RECOMMENDATIONS

- It is recommended to stop smoking especially for patients who seek for fertility.
- It is recommended to treat varicocele for patients who seek for fertility.
- Further studies with larger sample size including patients and controls are needed for more precise evaluation.
- Further studies are required to study possible use for anandamide and its receptors in the treatment of infertility and obesity.

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