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Association between Seminal Plasma Creatine Kinase Activity and Body Mass Index among Males Investigated for Infertility

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Abstract

Obesity is a neglected risk factor for male infertility, although its impact on male infertility is still controversial. Defects in sperm cell development may cause adverse effects on enzyme activities in the seminal fluid. The objective of this study was to assess the relationship between seminal plasma creatine kinase (CK) activity, malondialdehyde (MDA), and body mass index (BMI) among males investigated for infertility. A total of 185 men, age range 26-60yrs were randomly recruited from among subjects investigated for infertility. Anthropometric data and semen analysis were measured according to the World Health Organization guidelines. Seminal plasma CK and MDA were determined using the spectrophotometric method. Continuous data were analyzed using chi-square while Student's-test and analysis of variance (ANOVA) were used to analyze discrete variables. Pearson correlation coefficient was used to determine the association between measured variables. The results indicate that 68(36.8%) of the study participants had normal BMI while 55(29.7%) and 62(33.5%) were overweight and obese respectively. Of the 185 infertile men evaluated, 110(59.5%) were normozoospermia, 45(24.3%) were mild oligozoospermia, 12(6.5%) were severe oligozoospermia while 18(9.7%) were azoospermia. Seminal plasma CK activity and MDA concentration increased with decreasing sperm quality parameters and increasing BMI. Body mass index correlated positively ($R=0.669$, $p<0.001$) with CK activity and MDA ($R=0.619$, $p<0.001$). The findings indicate adverse effects of BMI on sperm quality parameters, CK activity, and lipid peroxidation of seminal fluid. Obesity may have a negative impact on male fertility and weight reduction among infertile men may improve fertility, general health, and well-being.

Keywords: Male, Semen, Body Mass Index, Creatine Kinase

Introduction

The declining male reproductive potentials have been attributed to several factors including infections, genetic, environmental, lifestyle behaviors, or dietary habits. The relationship between obesity and infertility has been investigated. Although the adverse effect of obesity on female fertility is well understood, studies that considered the impact of body mass index on male fertility have yielded ambiguous results (1,2). The World Health Organisation (WHO) defined overweight and obesity as abnormal or excessive fat accumulation. These conditions are impacted by several factors including environmental, hormonal, and genetics and are readily determined using body mass index (BMI) data. Subjects who are overweight and obese have a BMI ≥ 25 kg/m^2 or ≥ 30 kg/m^2, respectively (3). Significantly lower sperm quality has been reported in obese men than those with normal BMI (18.5-24.9kg/m^2). Although the prevalence of obesity among men is increasing, its impact on fertility leaves much to be desired. According to 2013 estimates, the proportion of men who are overweight is 36.9% (4) and the findings of the coexistence of infertility with obesity (5-7) are not consistent and none to the best of our knowledge has reported on the relationship between creatine kinase activity and BMI among males investigated for infertility.

Creatine kinase enzyme (CK) is required for sperm function because it catalyzes the regeneration of Adenosine triphosphate (ATP) from adenosine diphosphate (ADP) which are required for the production, transport, and utilization of energy within spermatozoa (8). The evaluation of CK in human sperm cells is an impartial indicator of sperm maturity and fertilization potential. Elevated CK values may be an indication of an increased number of functional abnormalities and increased cytoplasmic residues (9). But earlier authors have demonstrated that total creatine kinase activity and isozyme distribution are not predictors of male fertility (10). It is very important therefore to assess seminal plasma CK activity among Nigerians to ascertain its association with sperm indices and BMI. Male infertility is an important factor in the infertility of couples. Male infertility may be caused by defects in sperm cell function; be it low sperm cell count, impaired motility, or changes in morphology. Defects in sperm cell development have adverse effects on enzyme activities in the seminal fluid (11).

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The study of the association between BMI and CK activity as contributing factors to male infertility is imperative because being overweight or obese was considered evidence of wealth and affluence by some individuals in Nigeria and Africa (12). This study seeks to assess the relationship between seminal plasma CK activity and BMI among males investigated for infertility to ascertain their contributions to male infertility.

Materials and Methods

Study area and population

The study was conducted at the University of Medical Sciences, Ondo. This cross-sectional study comprised 185 men, with an age range 26-60yrs who were randomly recruited from among subjects investigated for infertility. After physical examination by the attending Physician, and the anthropometric measurements made in the clinic, the subjects were referred to the Chemical Pathology Laboratory for investigation. Control subjects were recruited from among staff and Students of the University.

Ethical consideration

Ethical approval was sought and obtained from the Ethical Committee of Ondo State Ministry of Health, Akure (Ref. NHREC/18/08/2016 dated 27th October 2021). Consent was obtained from all the participants before the commencement of sample collection.

Inclusion and exclusion criteria

Thorough physical and medical examinations were conducted on the participants by the attending physicians. Only those who met the inclusion criteria were recruited for the study. They consist of males aged 26-60 years who were investigated for infertility and referred to the laboratory for semen analyses as part of their investigation for infertility, who gave consent, without physical abnormalities or chronic illnesses were included in the study. Subjects without chronic clinical illnesses and who had their babies within the last 1 year, were included and used as controls. Individuals with known pathological or congenital conditions such as severe hypertension, diabetes mellitus, sexually transmitted diseases, testicular varicocele, and genital warts were excluded from the study. Besides, individuals currently on antioxidant food supplements, smoking cigarettes, and consuming alcohol excessively were also excluded due to their high seminal reactive oxygen species levels and possibly low antioxidant activity which might lead to decreased motility and abnormal sperm morphology.

Sample size determination

The minimum sample size required for the study was estimated using the sample size determination in the health studies formula (13), and the 14% prevalence of male infertility in the target population from the previous study(14) :

Given as: $n = Z^2P(1-P)/d^2$

n = The desired minimum sample size.

z = The standard normal deviation is usually set at 1.96 which corresponds to 95% confidence interval.

p = The prevalence of male infertility in the target population from the previous study.

q = The proportion in the target population who do not have a particular characteristic, i.e.

$q = 1 - P = 1–0.14 = 0.86$

d = Tolerable margin of error, an observed difference of 5% was taken as being significant.

Therefore, 185 subjects were enrolled for the study while 50 men with proven fertility were recruited as controls

Sample collection and processing

From each participant, a semen sample was obtained by self or assisted masturbation into a clean wide-mouthed universal container after 3-5 days of sexual abstinence. For internal quality control of semen analysis, spermiogram was carried out, according to the World Health Organization guidelines (15). Spermiograms including semen volume (mL), sperm motility (%), and abnormal morphologic features (%) were evaluated. After semen analysis, the semen sample was centrifuged at 2000 rpm for 15mins and the seminal plasma was separated. The supernatant (seminal plasma) was divided into two tubes and stored at -20°c until analysis: The first tube was analyzed for malondialdehyde (MDA) while the second tube was analyzed for seminal creatine kinase.

Semen volume, total sperm count, percentage of spermatozoa with rapid progressive motility, vitality, sperm count, and percentage of normal spermatozoa were analyzed according to WHO criteria (15). The study participants were categorized into normozoospermia (sperm count ≥15million/mL), mild oligozoospermia (sperm count >5 but <15million cells/mL), severe oligozoospermia (<5million sperm cells/mL) and azoospermia (no sperm cell) after routine semen analysis. Creatine kinase and malondialdehyde (MDA) were assayed using reagents supplied by Abbot Diagnostics, USA, and Northwest Life Science Specialties, Canada respectively.

Statistical analysis

Data generated were analyzed using Statistical Package for the Social Sciences software (version 20.0, IBM SPSS, Armonk, NY, USA). Continuous data were analyzed using chi-square while Student's-test and analysis of variance (ANOVA) were used to analyze discrete variables. Pearson correlation coefficient was used to determine the association between creatine kinase activity and BMI. The level of significance was set at a p-value less than 0.05.

Results

Table 1 shows the comparison of the demographic characteristics between the infertile group and the healthy control subjects. The mean age of the infertile group was not significantly different from the control group, but the mean weight and body mass index of the infertile group were significantly higher ($p<0.001$) than the control group. All the male subjects investigated for infertility were married while 50% of the control group was married. Some 9(4.9%) of the study participants were artisan while 176(95.1%) were civil servants. Also, only 3(6%) of the control group were artisan while 47(94.5%) were civil servants. The differences were statistically significant ($p<0.001$).

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The classification of study subjects based on BMI indicates that 68(36.8%) had normal BMI while 55(29.7%) and 62(33.5%) were overweight and obese respectively. Among the control group, 43(86.0%) had normal BMI and (14%) were overweight. The difference was statistically significant ($p<0.001$). Among the study group, 31(16.7%), 2(1.1%), and 152(82.2%) had primary, secondary, and tertiary education respectively.

Table 2 shows the distribution of study subjects according to the categories of sperm count. Of the 185 infertile men evaluated, 110(59.5%) were normozoospermia, 45(24.3%) were mild oligozoospermia, 12(6.5%) were severe oligozoospermia while 18(9.7%) were azoospermia. Interestingly, 2(4.0%), 9(18.0%), and 39(78.9%) among the control group were severe oligozoospermia, mild oligozoospermia, and normozoospermia respectively.

Table 1. Socio-demographic characteristics of study Participants (Mean±SD)

Variables	Men investigated for infertility (n=185)	Controls (n=50)	X^2 or P-value
Age (Years)	40.55±7.30	39.96±5.68	0.50
Weight (Kg)	78.86±11.81	68.88±6.22	0.001
Height (M)	1.71±0.08	1.75±0.04	0.6
Body Mass Index (Kg/m^2)	27.15±4.9	22.47±2.12	0.001
Marital Status			
Married	185 (100%)	25 (50%)	
Single	0 (0.0%)	25 (50%)	0.001
Occupation			
Artisans	9 (4.9%)	3 (6.0%)	
Civil Servants	176 (95.1%)	47 (94.0%)	0.001
Weight Classification			
Normal (BMI18.5-24.9Kg/m^2)	68 (36.8%)	43 (86.0%)	
Over weight (25-29.9 Kg/m^2)	55 (29.7%)	7 (14.0%)	0.001
Obese (>30.0 Kg/m^2)	62 (33.5%)	0 (0.0%)	
Educational status			
Primary	31 (16.7%)	3 (6.0%)	0.001
Secondary	2 (1.1%)	1 (2.0%)	0.512
Tertiary	152 (82.2%)	46 (92.0%)	0.001

Table 2. Distribution of males investigated for infertility and control subjects based on sperm count

Categories	Infertile males (n=185)	Control subjects (n=50)	X^2
Azoospermia	18 (9.7%)	0 (0.0%)	0.001
Severe oligozoospermia	12 (6.5%)	2 (4.0%)	0.001
Mild Oligozoospermia	45 (24.3%)	9 (18.0%)	0.001
Normozoospermia	110 (59.5%)	39 (78.0%)	0.001
Total	185 (100%)	50 (100%)	0.001

Table 3 shows a comparison of sperm indices between the infertile males and control subjects. The mean semen volume among the infertile males was significantly higher ($p<0.05$) than in the controls. The mean sperm count, percentage motility, and percentage viability among the infertile group were significantly lower ($p<0.001$) than in the controls. Conversely, mean CK activity and MDA concentration were significantly higher ($p<0.001$) in the infertile group than in the control group.

Table 4 shows the multiple comparisons of sperm indices, creatine kinase activity, and MDA concentration according to the sperm count. The differences between the categories were statistically significant ($p<0.001$). On the other hand, means creatine kinase activity and MDA concentration increased with decreasing sperm count from normozoospermia, mild oligozoospermia, and severe oligozoospermia to azoospermia. The differences between the means were significant ($p<0.001$).

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Table 3. Comparison of sperm indices, creatine kinase activity, and malondialdehyde between males investigated for infertility and control subjects

Parameters	Infertile males (n=185)	Controls (n=50)	P-value
Semen Volume (mL)	3.06±0.94	2.57±0.53	0.05
Sperm Count (x10^6/mL)	29.20±3.46	48.66±2.94	0.001
Percent Motility (%)	41.19±21.18	61.98±11.47	0.001
Viability (%)	50.64±20.91	65.64±6.76	0.001
Creatine kinase(U/L))	208.96±87.83	99.38±62.41	0.001
Malondialdehyde(nmol/L)	3.15±1.01	2.06±0.63	0.001

Table 4. Comparison of Sperm indices, creatine kinase activity, and malondialdehyde concentration among infertile subjects according to sperm concentrations

Categories of Infertile Males	Sperm Count (x10^6/L)	Creatine Kinase Activity (U/L)	Malondialdehyde (nmol/L)
Azoospermia (n=18)	0.00±0.00	311.83±15.37	4.48±.47
Severe Oligozoospermia (n=12)	3.29±.81	261.58±10.88	3.85±0.12
Mild Oligozoospermia (n=45)	9.31±2.80	241.97±7.97	3.55±0.08
Normozoospermia (n=110)	44.94±17.25	172.89±11.2	2.69±0.09
F-value	125.896	14.557	34.005
P-Value	0.001	0.001	0.001

Table 5 shows the comparison of sperm parameters, creatine kinase activity, and MDA concentration based on the body weight of subjects. The data show that the mean values of sperm motility, viability and morphology, creatine kinase activity, and MDA concentration varied with the BMI of infertile men. Sperm motility, viability, morphology, and sperm count decreased with increasing BMI ($p<0.001$), while creatine kinase activity and MDA concentration increased with increasing BMI($p<0.001$). Table 6 indicates that body mass index correlated positively ($p<0.001$) with creatine kinase activity and MDA concentration. Conversely, BMI correlated inversely with sperm count, percentage motility, and percentage viability.

Table 5. Comparison of sperm parameters, creatine kinase activity, and malondialdehyde based body mass index of study subjects (Mean±SD)

Parameters	Normal Weight (BMI 18.5-24.9kg/m^2) (n=68)	Overweight (25-29.9kg/m^2) (n=55)	Obese (>30.0 kg/m^2) (n=55)	F	P-value
Motility (%)	58.01±16.83	32.93±16.42^{a}	30.08±17.36^{a}	53.702	0.001
Viability (%)	64.21±15.34^{bc}	48.42±16.87	37.74±20.74	36.623	0.001
Morphology (%)	27.56±8.60^{bc}	12.02±8.71	10.89±10.01	67.382	0.001
Sperm count (x 10^6/mL)	44.52±18.70^{bc}	22.56±23.22	18.28±19.39	31.088	0.001
Creatine kinase (U/L)	106.14±73.90	251.74±82.04^{ac}	283.79±63.18^{ab}	109.122	0.001
Malondialdehyde (nmol/L)	2.22±0.75	3.58±0.78^{a}	3.78±0.63^{a}	87.730	0.001

a=Significant at P<0.05. b=Significant at P<0.01, c=P<0.001.

Table 6. Correlation between Body Mass Index and measured biochemical and sperm indices

Measured Variables	R-value	P-value
Body Mass Index and creatine kinase activity	0.669	0.001
Body Mass Index and malondialdehyde	0.619	0.001
Body Mass Index and sperm count	-0.482	0.001
Body Mass Index and percentage viability	-0.511	0.001
Body Mass Index and percentage motility	-0.542	0.001
Creatine kinase and malondialdehyde	0.830	0.001

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Discussion

Although a threefold increase in the incidence of obesity has been reported in subjects with male factor infertility, its involvement in male factor infertility is rarely investigated, thereby demonstrating the need for greater awareness in this area by caregivers (19). Although some authors have reported that obesity may be a risk factor for male infertility (20-23), conflicting reports doubting the contribution of obesity to male fertility also exist in the literature (24). This was designed to determine whether relationships exist between seminal plasma CK activity, malondialdehyde, sperm indices, and BMI among males investigated for infertility.

In this study, it was observed that seminal plasma CK correlated positively with BMI among men investigated for infertility. The seminal plasma CK activity increased with increasing BMI, increasing MDA, and inversely with sperm quality and quantity. These observations aligned with previous studies (25-29). These authors evaluated serum CK activity and correlated the activity with BMI among women with Poly Cystic Ovarian syndrome (PCOS). The observed correlation between CK activity and BMI may suggest that CK activity may be a surrogate marker for obesity and cardiovascular risk (25). Conversely, significantly lower serum CK-MB activity was reported in obese subjects and subjects with normal BMI among Nigerians in Port Harcourt (30). Obesity exacerbates adverse cardiac events in several ways including high secretion of Adipokines and other biochemical mediators (estrogen, tumor necrotic factors, interleukins, angiotensinogen, insulin growth factor, and plasminogen activator inhibitor). These adversely impact the cardiovascular system by exacerbating inflammation, clotting abnormalities, and endothelial damage. The effect of elevated CK activity on male reproductive potentials has not been sufficiently evaluated.

In this study, semen quality and quantity correlated inversely with increasing BMI. Also, seminal plasma CK activity increased with decreasing sperm count, sperm viability, and sperm motility. These observations are partly consistent with the previous study (31). In a systemic review with meta-analysis, the authors concluded that there was a relationship between BMI and sperm quality, suggesting obesity may be an impediment to male infertility. Obesity is regarded and considered as a risk factor for female infertility, but its effects on male factor infertility is rarely considered. This is particularly important because of the observation of a three-fold increase in the incidence of obesity among infertile men (32). Also, significantly higher spermatozoa CK activity was reported among men with idiopathic normozoospermic obese subfertile men than in control subjects (33). The authors attributed the elevated CK activity to oxidative stress. Similarly, some authors have reported an association between elevated seminal plasma CK activity and abnormal sperm function (34, 35). The levels of CK in the seminal fluid are higher than those present in serum. This means that CK may be synthesized and secreted locally within the germinal system glands. Creatine kinase activity in human sperm may be an objective indicator of sperm maturity and fertilizing potential (36). Since some authors have suggested that abnormal sperm function may be associated with spermatogenesis and the release of immature spermatozoa from the germinal epithelium (37), it is imperative to evaluate seminal plasma CK activity as a marker of male infertility. Also, since the decline in male reproductive potential was associated with an increased incidence of obesity it can be suggested that obesity may be considered when evaluating men for infertility.

There are indications that excessive weight plays a significant role in fertility, mediators of obesity that may impact the male reproductive system include chronic inflammation, oxidative stress, and abnormal protein concentrations. Obesity may also disrupt male fertility and reproductive potential via changes in the hypothalamic-pituitary-gonadal axis, and dysregulation of both testicular steroidogenesis and the metabolic system (31). Specifically, obesity and its underlying mediators may negatively impact semen parameters, including sperm concentration, motility, viability, and normal morphology (31). The need to maintain a healthier weight is vital to preventing infertility (38, 39). The observed deteriorating male reproductive health indices since the last 5–6 decades ago from different parts of the world have been related to increased incidence of modifiable lifestyle factors including obesity. The role of lifestyle factors in the etiology of infertility has generated a growing interest among scientists and several studies have provided evidence of an association between lifestyle and infertility (16, 39). Obesity may cause hypogonadotropic hypogonadism and hyperestrogenism. This may have “adverse effects on sperm quality, sperm mitochondrial activity, increased sperm DNA damage and oxidative stress” (40). Some authors have reported that obesity might cause a decline in the quality and quantity of spermatozoa (31). Conversely, no significant differences in the levels of seminal CK activity and sperm quality parameters among normozoospermic, moderate, and severe oligozoospermia were previously reported (9). These authors also reported an insignificant correlation between seminal CK activity and sperm quality parameters.

Seminal plasma CK activity was associated with seminal plasma MDA and MDA levels correlated with BMI among men evaluated for infertility. A previous study reported that the MDA concentration was significantly higher in the seminal plasma of obese infertile subjects than in non-obese infertile men (40). Also, elevated MDA concentration was significantly associated with poor sperm quality parameters. It was concluded that elevated lipid peroxidation and MDA levels in obese males may lead to the high susceptibility of sperm to ROS-induced damage.

Obesity can lead to an increase in the size or number of adipocytes resulting in both physical and hormonal alterations. Physical alterations might lead to an increase in scrotal temperature, an increased risk of sleep apnea, and erectile dysfunction. Also increased fat distribution in the upper thighs, suprapubic area, and scrotum as well as a sedentary lifestyle (often associated with obesity) may lead to raised testicular temperature (41). A moderate physiological increase in scrotal skin temperature was associated with significantly decreased sperm quality (42). The stimulation of lipid peroxidation product (MDA) in the seminal plasma is one of the processes by which oxidative stress adversely affects sperm function.

The sperm cells are particularly susceptible to ROS–induced damage because of the presence of high polyunsaturated fatty acid (PUFA) in the spermatozoa plasma membrane. Obesity has been associated with poor sperm quality, adverse effects on sperm mitochondrial activity, elevated sperm DNA damage, and elevated seminal oxidative stress (40).

Conclusion

The findings indicate adverse effects of BMI on sperm quality parameters, CK activity, and lipid peroxidation of seminal fluid. The means CK activity and MDA concentration were significantly higher among infertile men than in the control subjects. The evaluation of seminal plasma CK activity may be an additional biochemical marker in the evaluation of male fertility. Obesity may have a negative impact on male fertility and weight reduction among infertile men may improve fertility, general health, and well-being.

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Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

Author contribution

This work was conducted and approved in collaboration with the authors. MAE designed the study; MAE, EMB contributed to literature search; EMB, data collection, experiments, MAE, EMB data analysis, EMB drafted the manuscript; MAE wrote the final manuscript; MAE proofread the manuscript.

Ethical Issue

Ethical approval was sought and obtained from the Ethics Committee of Ondo State Ministry of Health, Akure (Ref. NHREC/18/08/2016 dated 27th October 2021). Consent was obtained from all the participants before the commencement of sample collection.

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