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The Association of Age and Body Mass Index with Salivary Stress Biomarkers(Alpha-amylase and Cortisol) in Infertile Men: A Cross-Sectional Study

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ABSTRACT

The purpose of the study was to determine if age and body mass index in infertile men were associated with stress levels as determined by salivary stress biomarkers (cortisol and alpha-amylase). A cross-sectional study comprising 100 males with an infertility challenge was divided into 25 normozoospermia, 26 oligozoospermia, 21 asthenozoospermia, and 28 oligoasthenoteratozoospermia. Salivary cortisol and alpha-amylase were examined using the ELISA technique .The average age of the patient was 30.85±7.03, and their average BMI was 27.604±5. 7894. The infertile duration mean was 4.7896±3. 85932. The average amylase content in the saliva was 127.3721±40.0952, while the average cortisol content was 72.67±49.09. There are no discernible variations between the comparison mean of BMI and the duration of infertility. In the current investigation, there was no discernible relationship between SAA and age, BMI, or the length of infertility. Saliva cortisol and BMI in the oligoasthenoteratozoospermia group showed a substantial negative correlation (r = 0.483 & p = 0.009), although no significant link was seen with age, BMI, or length of infertility in the other study groups. There was no significant positive or negative correlation found between SAA level and the age and BMI of the four study groups, whereas salivary cortisol showed a substantial negative correlation with BMI in OAT and a non-significant correlation with age.

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KEYWORD

Varicocele, L-carnitine, Epididymal lumen, SFA, Seminal plasma, Antioxidants, ROS

1. Introduction

Forty-five percent of cases of infertility are male (Swinburne and Bold, 2022). Obesity is currently one of the most prevalent health issues in the world (Chung, F., 2016). Research has been done on the relationship between fat and infertility. Research on male fertility produced surprising results (Ramadjue, Ga. et al. 2018). Numerous research works have indicated that obesity has a significant impact on the quality of semen (Engin-Ustun et al., 2018; Sermondade et al., 2013). Additionally, testicular function and sperm quality were impacted by a man's age (Gunes et al. 2016). Male infertility is caused by poor spermatogenesis, aberrant sperm, sperm dysfunction, and defective Sertoli and Leydig cells, all of which are more common in the testes of older men (Dong, S. et al. 2022). Parental age-related sperm volume, motility, and normal morphology have all decreased (Brahem et al. 2011; Agrowal and Sekhon, 2011). Therefore, infertile men who wish to delay fatherhood should consider age Demirkol et al., 2021.

Stress is characterized as a physiological and psychological response that primes the body to defend against external dangers or internal stressors, according to Foody et al. (2015) and Vanaelst et al. (2015). Semen quality may be impacted by stress (Lupis et al. 2014). These days, psychological evaluation and biomarker testing are the two methods most frequently used to quantify stress (Iizuka et al. 2012).. The stress biomarker is a valuable tool for assessing behavioral interventions and tracking and preventing stress-related disorders (Foody et al. 2015; Marrelli et al. 2014). According to Yia TC. et al. (2013), the salivary alpha-amylase (SAA) level is a stress marker that is extremely sensitive to stressful conditions, including psychic stress.

In addition to the salivary enzyme alpha-amylase, saliva samples can be used as non-invasive monitoring for stress analysis by quantifying the quantity of cortisol, one of the stress biomarkers (Corbalan-Tutau et al. 2014; Petrakova et al. 2015). Salivary alpha-amylase served as a stress marker for the sympathetic nervous system, and salivary cortisol level as an endocrine signal, according to Khalaila et al. (2014) and Tasaka et al. (2014).

2. Patients and Methods

The current cross-sectional survey was conducted from October 2023 to November 2023. The study included one hundred infertile males. The patient had to abstain from eating, smoking, drinking, and chewing gum for thirty minutes before providing his saliva sample, which was taken using a collection tube. The sample is pressurized for examination by freezing it at -20°C. After three to five days without sexual activity, the patients were asked directly to take a sample of semen.

Ethical approval :

The High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University's ethical committee gave the study their blessing.

Study area:

After taking a complete medical history about age, weight, the length of infertility, any prior salivary gland issues, and other factors, the study was attended by a male infertility expert at a private andrology clinic in al-Najaf.

Inclusion criteria:

An examination of the seminal fluid from male infertile individuals showed anomalies in the parameters of the sperm. It removes infertile males with azoospermia, salivary gland disorders, dental disorders, digestive tract diseases, pancreatic diseases, and other conditions from the category of men who were not fertile but showed normal seminal fluid analysis (normozoospermia).

Statistics:

Analysis of variance (ANOVA test employed to compare more than two separate groups) was used to compare the groupings. Pearson's correlation coefficient (r) was used to determine the degree of relationship between continuous variables, with a value equal to or less than 0.05.

4. Results

1: Patients' baseline characteristics who are part of the current study.

The current cross-sectional study included 100 infertile males; the results were reported as mean plus standard deviation of the mean. The age of the patient was 30.850±7.0315 years on average. Table 1 shows that the average body mass index was 27.604±5.7894 (kg\m2) and the average length of infertility was 4.7896±3.85932 (years).

2- Patients enrolled in the current study are categorized.

Table 2 (Figure 1,2) shows that the patients were divided into 25 patients with normozoospermia, 21 patients with asthenozoospermia, 26 patients with oligozoospermia, and 28 patients with oligoasthenoteratozoospermia (OAT) based on sperm concentration, motility, and morphology. The levels of salivary stress indicators (cortisol and alpha-amylase) are displayed in Table 3.

3- Age, BMI, and Duration of Infertility are compared between study groups.

Table 4 indicates that there were no significant differences in body mass index ($p = 0.780$) or infertility ($p = 0.135$) between the study groups. However, there were significant differences in age ($p = 0.023$) between the study groups.

4- Relationship between Stress Biomarkers and Study Groups' Age, BMI, and Length of Infertility.

Table 5 shows that there was no statistically significant positive or negative link between SAA and age or body mass index in any of the study groups (normozoospermia, oligozoospermia, asthenozoospermia, and OAT) .

Table 5 shows that there was no significant positive or negative correlation between saliva cortisol and age and body mass index in normozoospermia, oligozoospermia, asthenozoospermia, or between age and infertility duration OAT. However, there was a highly significant negative correlation between saliva cortisol and BMI in OAT (r = - 0.483 & p = 0.009).

Table (1): **Patients' baseline characteristics who are part of the current study (N = 100).**

parameter	Range	Mean	SD
Age(years)	19 - 52	30.850	7.0315
BMI (kg\m2)	14.2 - 38.4	27.604	5.7894
Duration of infertility(years)	1.0 - 13.0	4.7896	3.85932

SD: standard deviation, BMI: body mass index, N = number of patients

Table (2): **Patients enrolled in the current study are categorized**

Name of sample	Number	Percentage
Normozoospermia	25	25%
Oligozoospermia	26	34.7%
Asthenozoospermia	21	28%
Oligo-astheno-teratozoospermia	28	37.3%
Total	100	100%

Table (3): **Baseline Level of Salivary Stress Biomarkers for the Study Participants (N = 100).**

Variable name	Range	Mean	S. D
Cortisol ng\ml	187.410	72.6774	49.0911
Amylase ng\ml	231.951	127.3721	40.0952

Table (4): **Age, BMI, and Duration of Infertility are compared between study groups.**

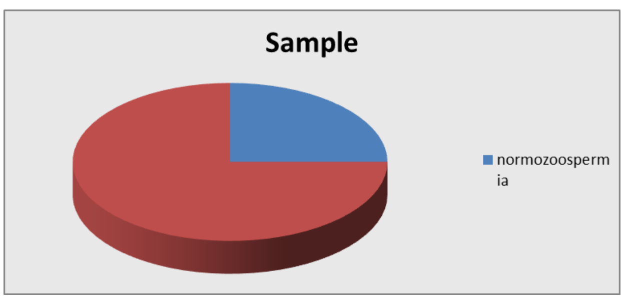
parameter	Study group	N	Mean	Chi-Square	df	Asymp. Sig.
BMI	Normozospermia	25	28.328	1.088	3	0.780 ns
	Oligozospermia	26	27.223			
	Asthenozospermia	21	26.076			
	Oligo-astheno- terato azoospermia	28	28.468			
	Total	100				
Duration of infertility	Normozospermia	25	5.3038	5.560	3	0.135 ns
	Oligozospermia	26	3.1346			
	Asthenozospermia	21	5.8410			
	Oligo-astheno- terato zospermia	28	5.0750			
	Total	100				
Age	Normozospermia	25	33.240	9.527	3	0.023 *
	Oligozospermia	26	27.692			
	Asthenozospermia	21	33.000			
	Oligo-astheno- terato zospermia	28	30.036			
	Total	100				

Ns = non-significant (p >0.05), N = number, df = degrees free, BMI body mass index, Asymp. Sig = p-value

***Table (5)*: Correlation between Stress Biomarkers and Age, BMI in the study groups**

correlation	Normozoospermia N= 25		oligozoospermia N = 26		Asthenozoospermia N = 21		OAT N = 28	
	r	p	r	p	r	p	r	p
SAA & age	0.138	0.519	0.297	o.141	0.042	0.853	-0.058	0.770
SAA& BMI	0.285	0.168	-0.151	0.463	0.104	0.653	0.099	0.616
S.cortisol & age	0.211	0.323	-0.121	0.555	-0.121	0.600	-0.289	0.138
S.cortisol & BMI	-0.299	0.147	0.027	0.896	0.421	0.057	-0.483**	0.009

N = number, r = pearson's correlation coefficient, * correlation is significant at the 0.05 level, ** correlation is significant at the 0.01level, OAT = oligoasthenoteratozoospermia, BMI = body mass index, SAA= salivary alpha amylase



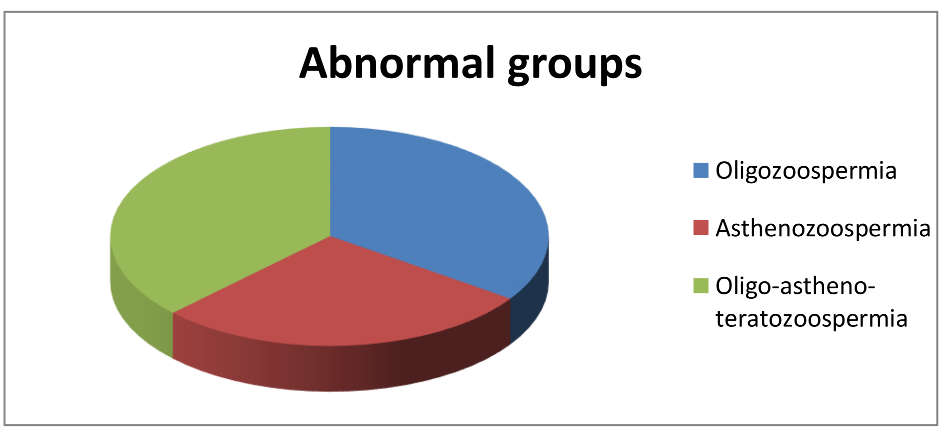


***Figure 1*: Classification of patients enrolled in the Present Study.**

5. Discussion

The current study evaluates the relationship between age and body mass index and salivary stress biomarkers, cortisol and alpha-amylase. The study assessed the sperm parameters of one hundred (100) infertile males, dividing them into four groups: oligoazoospermia, asthenozoospermia, normozoospermia, and oligo-astheno-terato-zoospermia.

.1 Age, BMI, and length of infertility were compared between research groups.

Table 4 shows that there were no statistically significant variations in BMI (P=0.780) or the length of infertility (p=0.135) among the four study groups. This result is comparable to that of (Syed Hasan Ala and Samia H. 2023) who found that sperm morphology and concentration had no significant association with BMI, and (AL Shahrains et al. 2016) who demonstrated that abnormal BMI had no significant impact on motility or progressive motility across BMI categories. Nguyen et al.'s 2007 study discovered, in contrast to these findings, that a rise in BMI was linked to an increase in infertility.

Obesity has been linked to greater rates of oligozoospermia and asthenozoospermia prevalence, according to research by Ramaraju Gaetal (2018). Additionally, the study by Samia Husain and Syed Hasan Ala (2023) discovered a correlation between low sperm motility in semen samples and high BMI. However, (Luca Boeri et al., 2018) discovered that in primary infertile males, the length of infertility had a detrimental effect on semen parameters. The majority of the four groups in the current study had BMIs below 30 (k/m2), which is in the normal range, which explains why there was no significant influence of BMI. There is discrimination as a result of the slight variation in the length of infertility across the four groups.

Table 4.6 shows that there were significant variations (p=0.023) in mean age between the four study groups. This is consistent with the findings of Mohamed and Stephen (2002), who discovered that male infertility decreased with advancing age. Semen volume, motility, and shape significantly decreased in older men, as demonstrated by Wnyne. et al. (2006). Prior research (Schwartz et al., 1983; Sharon A. Kidd et al., 2001) found that older males had lower sperm parameters than younger men. Additionally, this outcome is consistent with a 2010 study by Klemetti. et al., who found age had a significant influence on human reproduction.

2 -Salivary stress indicators (cortisol and SAA) and study groups' age and BMI were correlated.

Table 5 indicates that in the four study groups, there was no statistically significant positive or negative connection between SAA level and age and BMI. This outcome is comparable to that of (Spitzer et al.2022), who discovered no discernible relationship between SAA level and BMI and age. Saliva alpha-amylase levels and age or BMI did not significantly correlate, according to (Esmari van der Linden, 2020).

In the OAT group, there was a strong negative relationship between saliva cortisol level and BMI. (p=0.009 & r=0.483). Table 5 shows that there is, however, no statistically significant link between the saliva cortisol level and age and BMI in normozoospermia, oligozoospermia, and asthenozoospermia, as well as between age and duration of infertility in OAT. This finding is consistent with that of Spitzer .et al. (2022), who found no evidence of a significant relationship between saliva cortisol levels and age. (Crispower et al. 2006) discovered a correlation between a high cortisol level and a low BMI. However, Alain. et al. (2013) discovered a substantial negative correlation between wakefulness cortisol and BMI. Similarly, Vivek Varma et al. (1995) discovered a negative correlation between serum cortisol and both BMI and waist-hip ratio.

These findings are in contrast to those of the study by Spitzer. et al. (2022), which revealed no significant link between saliva cortisol and BMI, and the study by Andrew et al. (2020), which discovered an indirect correlation between saliva cortisol ratio and age. Conversely, the findings of (Pervanidou et al. 2013) indicated that prolonged elevation of salivary cortisol levels may result in dyslipidemia, which raises BMI, obesity, and waist-to-hip ratio.

6. Conclusion

Salivary cortisol had a strong negative correlation with BMI in OAT and was non-significant with age, while there was no significant negative or positive correlation between SAA level with the age and BMI of the four-study group.

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Author Contribution

Layla Kadhum Radeef performed the study, and Ali Ibrahim Rahim, Ula Al-Kawaz supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

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