RESEARCH
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EFFECT OF STRESS MEASURED BY SALIVARY STRESS BIOMARKERS ON THE SPERM PARAMETERS OF IRAQI INFERTILE MEN

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Abstract:
Background: The inability to conceive after a suitable period of sexual activity without taking any contraceptive measures is known as infertility. Forty to fifty percent of cases of infertility are male-related. One important measure of the health of the male reproductive system is semen quality. Stress is bad for the body and can cause a number of health problems. Both men and women experiencing infertility without apparent explanation have had stress examined as a potential contributing factor. In roughly 30% of cases, the etiology of the illness is never identified; these cases are categorized as idiopathic.
Patients, Material and Method:100 males with the challenge of infertility were included in a cross-sectional study, and their results were split into 25 normozoospermia, 26 oligozoospermia, 21 asthenozoospermia, and 28 oligoasthenoteratozoospermia of infertile men. The ELISA technique was utilized to assess salivary cortisol and alpha-amylase, and the WHO 2021 criteria were followed to examine the semen after it had been liquefied.

Results: The mean salivary stress biomarkers (SAA and cortisol) compared a cross all research with groups reveal positive significant differences with cortisol (p= 0.022) and SAA (p = 0.016). In the current investigation there was strong positive significant association (r = 0.398 & p = 0.049) btween SAA andconcentration of sperm normozoospermia and high negative significant correlation (r = - 0.275 & p =0.006) btween SAA and motility progressive (A+B) in study groups. Saliva cortisol was significantly correlated negatively with both sperm concentration (r = 0.240 & p = 0.016) and sperm count (r = 0.255 & p = 0.010). In OAT, there was a substantial positive connection between saliva cortisol and round cells (r = 0.377& p = 0.048) and sperm count (r = 0.475& p = 0.011).

Conclusion: Saliva cortisol and SAA levels that are higher in infertile men exhibit both positive and negative correlations with sperm concentration, sperm count, and motility. In oligoastheratozoospermia, there is a positive correlation between sperm count and round cells, and a positive correlation between SAA and sperm concentration in normozoospermia.

Keyword: Correlation, Infertility, amylase, Stress, cortisol.

Introduction

Infertility is a global public health challenge that has an impact on people's social, personal, and financial lives (1). Male infertility is the inability of a male parent to conceive a child from a fertile female (21). Forty-five percent of cases of infertility are male (6). A decrease in the amount and quality of semen is often the cause of male infertility (25). About thirty percent of cases have no known cause; the etiology is categorized as idiopathic (7). Idiopathic infertility may have stress, depression, eating problems, addiction, and trouble sleeping as contributing factors (24).

Stress is defined as a physiological and psychological reaction that prepares the body to protect itself against threats from the outside or internal stressors (9&33). Prolonged stress can be harmful to one's social life or health. Stress may have an effect on the quality of semen (14). These days, the two techniques most commonly

employed to evaluate stress are psychological assessment and biomarker testing (12). In addition to the salivary enzyme alpha-amylase, saliva samples can be used as non-invasive monitoring for stress analysis by detecting the quantity of cortisol, one of the stress indicators (7); (31). Salivary cortisol level served as an endocrine signal and salivary alpha-amylase as a stress marker for the sympathetic nervous system (30;26). Relevant data regarding the activities of the central nervous system (CNS) and the hypothalamic pituitary adrenal axis (HPA) that are triggered in response to stress can be obtained from these salivary biomarkers (7).

Patients, materials and method:

The present comparative cross-sectional study was carried out between October2023 and November 2023. One hundred of infertile men were involved in the study. The saliva sample was collected by using collection tube and patient must be not eat, smoke, drink or chew gum for 30 minutes before give his saliva sample. The sample storage by freezing at (-20c°) to presserve it for study .The patients were as direct to collect the semen sample after 3 - 5 days of not having intercourse .

Ethical approval: The study was approved by the ethical committee of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al-Nahrain University

Study area:

The study was attended the male infertility consultant private andrology clinic in al-Najaf after thorough history about (age, weight, duration of infertility Any previous salivary gland problem and others).

Inclusion criteria:

Analysis of the seminal fluid from infertile males revealed abnormalities in sperm parameters. Men who were not fertile yet exhibited normal seminal fluid analysis (normozoospermia) and excludes infertile men with azoospermia, salivary gland disorders, dental disorder, disease of digestive tract, pancreatic disease and others.

Statistics:

The group were compared by applying analysis of variance (ANOVA test applied used to compare more than two different groups).The degree of association btween continuous variables was calculated by Pearson's correlation coefficient **(r)** and the result equal to or less than 0.05.

The Results

The current cross-sectional study included one hundred infertile men. The average age of the patients was 30.85±7.03, and their average BMI was 27.604±5.7894. The infertility duration mean was 4.78±3.85.Salivary amylase mean was 127.37±40.09, while salivary cortisol mean was 72.67±49.09.

Salivary Stress Biomarker Comparisons Among Research Groups.

Between the fourth study group, there were statistically significant changes in saliva cortisol levels (p = 0.022). Saliva cortisol levels varied significantly between study groups, with the highest levels seen in oligozoospermia, OAT, and asthenozoospermia and the lowest in normozoospermia (58.48 vs.57.48 vs.43.74 vs. 37.56 vs.). There were also significant differences in SAA (p = 0.016). Asthenozoospermia, OAT, and normozoospermia had the highest saliva alpha-amylase levels, while oligozoospermia had the lowest levels (64.95 vs. 52.50 vs. 46.25 vs. 38.69 etc.).These variations are displayed in Table 1.

Table 1 Comparison of Salivary Stress Biomarkers between Study Groups.

Salivary biomarkers	Study group	N	Mean Rank	Chi-Square	df	Asymp. Sig.
Cortisol ng\ml	Normozoospermia	25	37.56	9.666	3	0.022 *
	Oligozoospermia	26	58.48			
	Asthenozoospermia	21	43.74			
	Oligo-astheno- terato zospermia	28	57.48			
	Total	100				
Amylase ng\ml	Normozoospermia	25	46.25	10.342	3	0.016 *

	Oligozoospermia	26	38.69			
	Asthenozoospermia	21	64.95			
	Oligo-astheno- terato zospermia	28	52.50			
	Total	100				

Asymp. Sig.= p value ,N = number ,df = degree free

Correlation between Salivary Stress Biomarkers (A

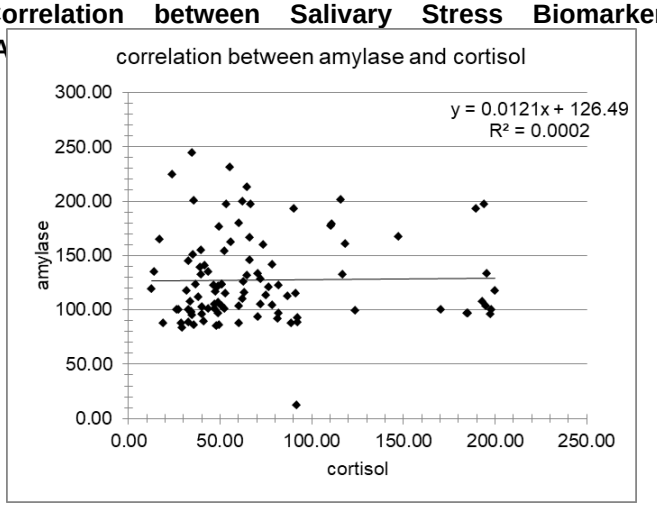

Figure 1 of the current study shows that there was no significant positive connection between saliva cortisol and saliva alpha-amylase (r = 0.015&p = 0.884).

Figure 1 Correlation btween Stress Biomarkers alpha amylase and cortisol in the Present Study (N=100).

Relationship between Sperm Parameters and Stress Biomarkers in the Current Study (N = 100)

Table 2 shows that the relationship between SAA level and motility (progressive A+B%) was extremely negative significant (r = 0.275& p = 0.006), while there was no significant positive or negative connection with other sperm characteristics. The amount of cortisol in saliva was negatively correlated with both the concentration of sperm (million/ml) (r = -0.240 & p = 0.016) and the number of sperm (million/ejac) (r = 0.255 & p = 0.010).

Table 2: Stress Biomarkers and Sperm Parameters Correlated Across Study Groups (N = 100)

Stress biomarkers		Sperm conc. Million\ ml	Sperm count million\ejac,	Progressive A+B%	Semen volume (ml)	Morphology %	Viscosity (mm)	Round cells million\ ml
Saliva alpha-amylase ng\ml	r	0.060	- 0.035	- 0.275**	-0.064	-0.043	0.139	- 0.003
	p	0.555	0.727	0.006	0.530	0.673	0.169	0.975
Salivacortisol ng\ml	R	-0.240*	-0.255*	-0.063	0.020	-0.124	-0.168	0.171
	p	0.016	0.010	0.537	0.843	0.220	0.095	0.089

N = number, r = pearson's correlation coefficient , ** correlation is significant at the 0.01 level , * correlation is significant at the 0.05 level

Stress Biomarkers and Sperm Parameters in Normozoospermia (N=25) Correlation.

Table 3 shows that the sperm concentration (million/ml) and SAA level had a positive significant association (r = 0.398& p = 0.049), but there was no significant positive or negative correlation with the other sperm parameters.

Table 3Stress Biomarkers and Sperm Parameters in Normozoospermia (N=25) Correlation.

Stress biomarkers		Sperm conc. Million\ ml	Sperm count million\ ejac,	Progressive A+B%	Semen volume (ml)	Morphology %	Viscosity (mm)	Round cells million\ ml
Saliva alpha-amylase ng\ml	r	0.398*	0.007	-0.104	- 0.079	0.256	- 0.073	0.98
	p	0.049	0.972	0.628	0.715	0.228	0.729	0.642
Salivacortisol ng\ml	R	0.012	0.000	0.045	- 0.062	0.027	0.014	- 0.017
	p	0.959	0.999	0.835	0.774	0.900	0.948	0.937

N = number, r = pearson's correlation coefficient , * correlation is significant at the 0.05 level .

Stress biomarkers and sperm parameters in oligospermia (n = 26) are correlated

Table 4 shows that the sperm characteristics in oligozospermia did not significantly correlate positively or negatively with saliva stress indicators (SAA and cortisol).

Table 4 Stress biomarkers and sperm parameters in oligospermia (n = 26) are correlated

Stress biomarkers		Sperm conc. Million\ ml	Sperm count million\ ejac,	Progressive A+B%	Semen volume (ml)	Morphology %	Viscosity (mm)	Round cells million\ ml
Saliva alpha-amylase ng\ml	R	- 0.355	0.190	-0.281	0.071	0.272	0.267	0.201
	p	0.075	0.354	0.164	0.730	0.179	0.187	0.325
Salivacortisol ng\ml	R	-0.028	-0.241	-0.136	-0.133	0.133	-0.290	-0.207
	p	0.890	0.236	0.508	0.516	0.518	0.150	0.311

N = number, r = pearson's correlation coefficient

Stress biomarkers and sperm parameters in Asthenozoospermia (n = 21) are correlated.

Table 5 shows that the sperm characteristics in asthenozoospermia did not significantly correlate positively or negatively with saliva stress indicators (SAA and cortisol).

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Table 5 Stress biomarkers and sperm parameters in Asthenozoospermia (n = 21) are correlated.

Stress biomarkers		Sperm conc. Million\ ml	Sperm count million\ ejac,	Progressive A+B%	Semen volume (ml)	Morphology %	Viscosity (mm)	Round cells million\ ml
Saliva alpha-	R	0.103	-0.324	0.154	- 0.009	0.175	0.030	0.418
	p	0.656	0.152	0.506	0.970	0.448	0.897	0.060

amylase ng\ml								
Salivacort isol ng\ml	R	0.268	0.208	-0.052	-0.282	-0.273	-0.054	- 0.270
	p	0.241	0.366	0.822	0.215	0.230	0.815	0.236

N = number, r = pearson's correlation coefficient

Stress biomarkers and sperm parameters in Oligoasthenoteratozoospermia (N=28) are correlated

Table 6 shows that there was no significant positive or negative correlation between saliva cortisol and other sperm parameters in OAT, but there was a positive significant correlation between saliva cortisol level and sperm count (million\ejac.) (r =0.475& p = 0.011) and round cells 9million\ml) (r = 0.377& p = 0.048).

Table 6 Stress biomarkers and sperm parameters in Oligoasthenoteratozoospermia (N=28) are correlated

Stress biomarkers		Sperm conc. Million\ml	Sperm count million\ejac,	Progressive A+B%	Semen volume (ml)	Morphology %	Viscosity (mm)	Round cells million\ml
Saliva alpha-amylase ng\ml	r	-0.061	-0.059	-0.172	0.032	-0.192	0.019	0.002
	p	0.758	0.766	0.382	0.870	0.329	0.925	0.991
Saliva cortisol ng\ml	R	-0.131	o.475*	-0.084	0.187	-0.264	0.171	0.377*
	p	0.505	0.011	0.669	0.340	0.175	0.384	0.048

N = number, r = pearson's correlation coefficient , * correlation is significant at the 0.05 level

Discussion

The current study evaluates the relationship between semen and sperm parameters and salivary stress biomarkers, specifically cortisol and alpha-amylase. One hundred (100) infertile men were split into four groups based on the parameters of their sperm: normozoospermia, oligoazoospermia, asthenozoospermia, and oligo-astheno-terato-zoospermia. The study assessed the results of these groups. taken into account their age, BMI, and length of infertility. Few studies have examined the connection between male sperm characteristics and salivary stress biomarkers (cortisol and alpha-amylase); instead, the majority of earlier studies used questionnaires to examine the impact of psychological stress on infertility.

1 Comparing the four study groups' salivary stress biomarkers.

Salivary alpha-amylase (SAA) levels changed significantly (P = 0.016) and cortisol levels differed significantly (p = 0.022) among the research groups. Table 1 shows that oligozoospermia (58.48) and OAT (57.48) had higher mean saliva cortisol levels than the other groups. The mean saliva alpha-amylase was higher in the asthenozoospermia (64.95) and OAT (52.50) experimental groups. This result is in line with studies by (2) and (32), which indicated a negative correlation between infertility and increased cortisol levels, respectively, and substantial differences between the study groups.

2 Salivary Stress Biomarkers (SAA and Cortisol) Correlation in the Current Investigation.

shown in Figure 4.3, there was no statistically significant positive association found between saliva cortisol and SAA level (r=0.15 & P=0.884). This result is consistent with (3). However, salivary cortisol and SAA level were determined to have a weak positive significant correlation by (2).

3 Correlation between Sperm Parameters in the Current Study (N=100) and Salivary Stress Biomarkers (Cortisol and SAA).

Table 2 of the current investigation shows a highly negative significant connection (r=0.275, p=0.006) between the salivary (SAA) level and progressive motility (A+B). The negative significant finding is comparable to observation (29) that found a negative correlation between the level of SAA in infertile individuals and sperm progressive motility, concentration, and positivity with immotile sperm. The data we observed show a negative connection between the SAA level and all sperm characteristics, with the exception of progressive motility (2). In contrast to this outcome, the research by (8)

discovered no significant link with sperm concentration, sperm count, motility, round cells, or viscosity, but rather a negative correlation between SAA level and semen volume.In both normozoospermia and oligozoospermia, there is a negative association between the SAA level and sperm count, and in oligozoospermia, there is no significant correlation with sperm cell activation (2). The results of this study showed a strong negative association between saliva cortisol level and sperm count (r=0.255, P=0.010) and sperm concentration (r=0.240, p=0.016). however, as table 2 shows, there is no discernible positive or negative association with the other sperm parameters.

Similar to the results of this study, (2) discovered a strong negative link between saliva cortisol and sperm count in normozoospermia, and (4) discovered a negative correlation between greater cortisol levels and sperm count and volume. Additionally, the results of (5) demonstrated that males who suffer from anxiety and depression have reduced testosterone and sperm quality. However, the research by (23) discovered that there is no relationship between saliva cortisol and normal morphology or semen volume.In contrast to this finding, the research by (13) was unable to establish a clear connection between stress and infertility. According to Spitzer TL. et al. (2022), salivary cortisol levels rose along with the number of motile sperm and total sperm count.However, the study by (19) discovered that stress-related disorders in males decrease sperm count, motility, and modify sperm morphology and ejaculation. Additionally, the results of (2) indicated no negative link between saliva cortisol and sperm count and sperm cell activation in oligzoospermia. The majority of earlier studies had looked at the relationship between stress and male infertility, including how stress affects sperm quality and quantity. For example, a study by (11) suggested that stress may increase the incidence of idiopathic male infertility in men because it has an impact on sperm quality. (16) and (18) found that men's sperm count, motility, and morphology can all decline as a result of stress. However, a different study by (15) discovered a negative relationship between sperm concentration, motility, and the proportion of normal morphology with perceived stress. This study is thought to be the first to look into the relationship between all sperm parameters (concentration, motility, viscosity, semen volume, round cell, morphology, and sperm count) and the salivary stress biomarker (Cortisol).

4 Correlation between sperm parameters in the normozoospermic group and salivary stress biomarkers (SAA and cortisol)

Table 3 shows that there was no association between SAA level and other sperm parameters, but there was a positive significant link between SAA level and sperm concentration (million/ml) ($r = 0.398$ & $p = 0.049$).This conclusion is in contrast to that of (29), who discovered that there was a positive association with immotile sperm and a negative correlation with sperm concentration and percentage progressing motile sperm. (23) discovered that there was no correlation between changes in semen parameters and the SAA level. However, in normozoospermia, the study by (2) discovered a negative connection between SAA level and sperm cell activation and count.

Additionally, in line with the findings of this study, (9) discovered a negative link between semen volume and SAA level but no significant correlation with the other sperm parameters. Table 4 indicates that there was no link found in the current investigation between the characteristics of sperm and the saliva cortisol level.This result is consistent with that of (23), which found no evidence of a significant relationship between semen volume, salivary cortisol, and normal morphology. However, in normozoospermia, the study by (2) discovered a strong negative connection between saliva cortisol and sperm count as well as sperm cell activation.

The results of this study point to a favorable relationship between sperm concentration and (SAA) levels in normozoospermic people. The elevated stress levels that individuals endured throughout the testing procedure may be the cause of the observed increase in SAA. Patients were asked to provide saliva samples while they awaited the results of the semen analysis, which was probably a stressful and anxious time for them. Previous study (20) suggests that SAA may be more sensitive to stress than cortisol and may respond more quickly to psychological stressors, which lends support to this argument.

5 Alpha Amylase and Cortisol Correlation with Sperm Parameters in Oligozygospermia

As shown in Table 4, there was no discernible positive or negative connection between sperm parameters and salivary stress biomarkers (SAA & cortisol). This finding is consistent with that of (2), who discovered no discernible relationship between saliva cortisol and sperm motility or count in oligospermia. In contrast, a study by (2) discovered a negative association between the number of sperm cells and the SAA level. The relationship between sperm parameters and salivary stress indicators in infertile men has not been extensively studied. Without breaking down into smaller subgroups, the majority of research examine how stressors like anxiety and depression affect certain sperm parameters in infertile men.

6 Asthenozoospermia: Alpha Amylase and Cortisol Correlation with Sperm Parameters

Table 5 demonstrated that there was no significant relationship between sperm parameters and salivary stress biomarkers (SAA & cortisol). This is the initial investigation on this correlation. The majority of studies solely look at how stress affects sperm motility, and in young, infertile males, sperm motility is adversely connected with SAA level. This outcome differs from the current study's outcome. This study's findings are consistent with the research by (8), which revealed no significant link between sperm parameters. Additionally, (23) study from 2022 discovered that cortisol had no relationship with semen volume or normal morphology.

7 Correlation between sperm parameters in oligo-astheno-terato-zoospermia and salivary stress indicators (SAS and Cortisol)

Table 6 indicates that there was no significant link between the OAT group's sperm parameters and SAA level. Table 6 shows that there were significant positive relationships between saliva cortisol level and round cells (r=0.377 & p=0.048) and sperm count (r=0.475 & p= 0.011). This study was thought to be the first to investigate the relationship between sperm parameters in OAT and salivary stress indicators. The findings of (23) that an increase in salivary cortisol is linked to a greater total sperm count and total motility are similar to the positive correlation between saliva cortisol and sperm count.. According to multiple research (17–21), changes in cortisol release brought on by prolonged stress are linked to increased inflammation. This could account for the study's finding that saliva cortisol and round cells in OAT are positively correlated.

Conclusions: The study's findings show a substantial correlation between elevated stress biomarkers and semen quality, as evidenced by salivary levels of the emotional stress enzyme alpha-amylase and the physical stress hormone cortisol. Sperm concentration and count in the current investigation demonstrated a significant negative correlation with saliva cortisol. There was a significant negative correlation between saliva alpha amylase and sperm motility (progressive A+ B). A significant positive correlation was observed between the concentration and level of SAA in normozoospermia.There was a clear positive correlation between saliva cortisol, sperm count, and round cells in the OAT. There was no correlation between sperm parameters and salivary stress markers (SAA& cortisol) in either asthenozoospermia or oligozoospermia. More research with a larger sample size is needed to validate the results of this study.

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