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Salivary Oxytocin in Anxiety in Children

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ABSTRACT

Background: Anxiety disorders represent a significant group of mental health conditions among children, often characterized by substantial impairment and distress. Emerging research suggests that salivary oxytocin could be a potential biomarker for anxiety due to its role in stress regulation and social behaviour.

Objective: This study aimed to evaluate the association between salivary oxytocin levels and different anxiety disorders in children.

Methods: This case-control study included 126 children diagnosed with different anxiety disorders based on DSM-5 criteria and 60 healthy controls, aged 6-12 years. Anxiety assessments utilized multiple scales including the MINI-KID, Hamilton Anxiety Rating Scale, and others. Salivary oxytocin levels were measured using a specific ELISA kit.

Results: Salivary oxytocin levels were significantly higher in children with anxiety disorders compared to controls. ROC analysis indicated high sensitivity and specificity with optimal cutoff values for distinguishing between affected and non-affected subjects. Logistic regression highlighted the influence of several socio-demographic factors on anxiety presence.

Conclusions: Elevated salivary oxytocin levels are significantly associated with anxiety disorders in children, with high diagnostic accuracy. This study confirmed the potential of salivary oxytocin as a reliable biomarker for identifying and differentiating anxiety disorders in the pediatric population, underscoring the importance of socio-demographic contexts in its expression.

Keywords: Salivary, Oxytocin, Anxiety, Children.

INTRODUCTION

Anxiety functions as a biological alert system, marked by intense feelings of fear that prime us for action. It is often accompanied by physical symptoms such as palpitations and sweating, indicating an overactive autonomic nervous system, as well as cognitive distortions. This condition should be distinguished from a normal fear response, which is a proportional reaction to a recognized threat [1]. Globally, anxiety disorders are the most prevalent category of mental health conditions and rank as the sixth leading cause of disability, according to the Global Burden of Disease Study [2]. In children, these disorders are also widespread, with an estimated prevalence of 9% and a lifetime prevalence reaching 28.8% [3].

The Diagnostic and Statistical Manual of Mental Disorders (DSM) categorizes pediatric anxiety disorders into several types: Generalized anxiety disorder, social anxiety disorder, panic disorder, agoraphobia, separation anxiety disorder, social phobia, and specific phobia. The presence of any one of these conditions qualifies as having an anxiety disorder [4]. The onset of anxiety disorders in children and adolescents results from a complex interaction of heritable traits, developmental stages, cognitive and learning factors, neurobiological aspects including genetics, and social and environmental influences [1].

Oxytocin (OXT) is a peptide hormone involved in childbirth, breastfeeding, maternal behavior, attachment, and social interactions [5]. It is produced in the supraoptic and paraventricular nuclei of the hypothalamus and transported to the gland via the tuber cinereum and infundibulum. Oxytocin functions both as a neurohormone and a neuromodulator in many brain regions expressing OXT receptors, including the hypothalamic and extra-limbic systems. Beyond its central expression, oxytocin and its receptors are also synthesized in peripheral organs [6].

Oxytocin levels can be measured in cerebrospinal fluid (CSF), blood plasma, urine, and saliva, reflecting either the cerebral or peripheral OXT systems. Under fearful and stressful conditions, endogenous oxytocin is activated, and its levels in the central and peripheral systems can be assessed as a global marker of the oxytocin system, indicating the central activity of an individual's OXT system in response to stress. Despite the high prevalence of anxiety disorders among adolescents (11-18 years), research on the therapeutic potential and anxiolytic effects of oxytocin, as well as the impact of stress on the endogenous oxytocin system in children and adolescents, is limited. Only one study by **Bernard *et al.*** [6] has investigated this, involving healthy volunteers. In a standardized laboratory setting, the researchers found that peripheral oxytocin levels increased under psychological stress. The study also revealed that lower basal levels of oxytocin were associated with higher anxiety and insecurity during stressful situations. Also, children and adolescents with initially low basal oxytocin levels showed a greater increase in oxytocin during the Trier Social Stress Test.

Therefore, this study aimed to analyze the association between salivary oxytocin levels and different anxiety disorders in children.

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SUBJECTS AND METHODS

Study design: This case control study was conducted at Pediatric Department and Psychiatry Department in Benha University Hospitals, Benha, Egypt for 1 year duration from February 2022 to February 2023 and included 126 children with different types of anxiety disorders and 60 controls as follows: Group I included thirty-five children with generalized anxiety disorders, group II comprised twenty-five children with panic disorders, group III contained nine children with specific phobias, group IV consisted of thirty patients with social anxiety disorders, group V included twenty-seven patients with separation anxiety disorder and group VI comprised sixty healthy children as a control group.

Inclusion criteria: Patients aged 6-12 years and had anxiety disorders according to the DSM-5.

Exclusion criteria: Parental (Guardian) refusal, metabolic and endocrine diseases, presence of mental retardation, neurological disorders, acute or chronic infections and any other psychiatric disorders.

METHODS:

All the participants were subjected to the following: Complete history taking including age, gender, residence, history of the current condition, developmental history including natal and neonatal history and positive consanguinity in the parents and positive family history. During the clinical examination, anthropometric measurements were taken, including weight (kg) and height (cm) to assess growth. Body mass index (BMI = weight in kg/height in m^2), weight for age, height for age, and BMI for age z-scores were calculated and recorded according to World Health Organization (WHO) standards. This was done using the AnthroPlus software for personal computers [7].

Laboratory investigations:

Assessment of saliva oxytocin level:

1. Kits: Saliva oxytocin saliva kits [Sunredbio, Shanghai, China (Catalogue No. 201-12-1047)].

2. Principle of the Assay: This salivary oxytocin ELISA kit is designed exclusively for laboratory research and is not suitable for diagnostic or therapeutic use. The stop solution in the kit alters the color from blue to yellow, and this color change's intensity is measured at 450 nm with a spectrophotometer. To determine the concentration of salivary oxytocin in a sample, the kit included calibration standards. These standards were tested alongside the samples, enabling the creation of a standard curve that plots optical density against salivary oxytocin concentration. The salivary oxytocin concentration in the samples was then calculated by comparing their optical density in relation to this standard curve [8].

3. Assay procedure: All reagents were prepared, and standards and samples were added in duplicate to a Microelisa Stripplate. Each designated well received 50 µl of standards and samples, with samples diluted as necessary. Next, 100 µl of HRP-conjugate reagent was added to each well, which was then covered and incubated at 37 °C for 60 minutes. The wells were aspirated and washed five times with 400 µl of Wash Solution. Subsequently, 50 µl each of Chromogen solutions A and B were added, mixed, and incubated for 15 minutes at 37 °C, protected from light. The addition of 50 µl of stop solution changed the color from blue to yellow. Finally, the optical density (OD) was measured at 450 nm using a microtiter plate reader within 15 minutes.

4. Calculation: To determine the concentration of the unknown sample, a standard curve was created by plotting the average OD (450 nm) for each of the six standard concentrations on the Y-axis against their respective concentrations on the X-axis. The mean OD values for each standard and sample were calculated, and all OD values were adjusted by subtracting the mean value of the zero standard. The standard curve was then constructed using graph paper or statistical software. To find the concentration in each sample, the OD value was located on the Y-axis, a horizontal line was extended to intersect the standard curve, and from the intersection point, a vertical line was drawn down to the X-axis to read the corresponding concentration.

5. Psychiatric assessment using different scales to diagnose different types of anxiety:

MINI-KID: The MINI, a brief structured diagnostic interview created by psychiatrists and clinicians in the US and Europe, is designed for DSM-IV and ICD-10 psychiatric disorders. Taking approximately 15 minutes to administer, it is intended for use in multicenter clinical trials, epidemiology studies, and as an initial step in clinical outcome tracking. The interview suite included the MINI-Screen, MINI-Plus, and MINI-Kid. Validated against other clinical interviews and expert opinion, the MINI is also noted for its potential applicability in various clinical settings [9]. The diagnostic modules of the interview include a range of psychiatric conditions such as Major Depressive Episodes, Suicidality, and Psychotic Disorders, alongside others like Anorexia Nervosa and Generalized Anxiety Disorder (GAD). It also covers various disorders across the spectrum from substance use to mood, anxiety, eating, and behavior disorders. Scoring for each module is straightforward, utilizing a 2-point rating system where '(-)' indicates absent or sub-threshold symptoms and '(+)' denotes presence. This structured approach facilitated efficient and clear diagnostic assessment.

The Hamilton Anxiety Rating Scale, developed by **Max Hamilton** in 1959 and translated into Arabic by **Fatim** in 1994 [10, 11], is designed to measure the severity of anxiety. It consists of 14 items, each scored on a five-point Likert scale ranging from 0 (Not present) to 4 (Very severe). The total score ranges from 0 to 56 and is categorized into four levels: 17 or less indicates mild anxiety, 18 to 24 indicates mild to moderate anxiety, 25 to 29 indicates moderate to severe anxiety, and more than 30 indicates severe anxiety.

Social anxiety scale for children (SASFC): This scale evaluates the social skills and interpersonal relationships of children aged 6 to 12 by assessing their interactions with their environment and peers. It measures factors such as cooperation, altruism, social participation, and emotional involvement in social events, alongside dimensions like social sensitivity, support, and isolation, to gauge their social competence and satisfaction. This scale has been developed by **Stone** [12] and was translated by **Abdelsalam** [13].

Separation anxiety disorder scale: Parents completed the Screen for Child Anxiety Related Disorders (SCARED) screening instrument, which was translated into Arabic and validated [14]. This tool identifies the presence and type of anxiety disorder based on parent-reported scores. The Arabic version of the SCARED screening tool comprises 41 items addressing various anxiety disorders, including panic, social anxiety, and generalized anxiety disorder. The tool was electronically sent to parents of study participants, who rated how accurately each item described their child using a 3-point Likert scale: "not true," "somewhat true," and "very true". Throughout the study, data anonymity and confidentiality were rigorously maintained.

Specific phobias in children: The DSM-5 defines specific phobia as an excessive, unreasonable fear of a specific object causing immediate anxiety and either avoidance or significant distress, persisting for at least six months in children. Its diagnosis excludes other causes. Severity is measured using a 10-item scale, where children rate their phobia's severity over the past week on a five-point scale from 0 (Never) to 4 (All the time), with total scores ranging from 0 to 40 to indicate the severity level.

Assessment of saliva oxytocin level: Saliva oxytocin saliva kits [Sunredbio, Shanghai, China (Catalogue No. 201-12-1047)]. This salivary oxytocin ELISA kit is designated for research use only, not for diagnostic or therapeutic procedures. It measures salivary oxytocin levels by colorimetric detection. The color changes from blue to yellow upon reaction completion, and the intensity is measured at 450 nm. The kit includes calibration standards to create a standard curve, facilitating the determination of oxytocin concentration in samples by comparing their OD to the curve. The assay procedure involves preparing reagents, adding standards and samples to wells, and processing with multiple washes and incubations before reading the OD using a spectrophotometer. The concentration of oxytocin in samples was calculated from the standard curve, with careful attention to potential variations in assay conditions affecting the results.

Ethical considerations: The study was done after being accepted by The Research Ethics Committee, Benha University. All patients provided written informed consents prior to their enrolment of their children. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring protection of their confidentiality and privacy. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Data management

Data management and statistical analysis were performed using SPSS version 28. Quantitative data were tested for normality and summarized as means and standard deviations or medians and ranges. Categorical data were presented as counts and percentages. Comparisons between groups were made using one-way ANOVA or Kruskall Wallis tests for quantitative data and Chi-square or Fisher's exact test for categorical data. ROC analyses evaluated salivary oxytocin's predictive power for anxiety disorders. Correlations and multivariate logistic regression analyses identified relationships and predictors, respectively, with significance set at p-values ≤ 0.05.

RESULTS

The mother's occupation, residency, positive family history, and presence of a risk factor emerged as significant variables. Mother's occupation showed a notable difference, particularly with a high prevalence in group VI (83.3%) compared to other groups ($P < 0.001$). Residency also presented significant differences, notably a higher proportion of rural residency was observed in group IV (83.3%) and group V (70.4%), contrasted to other groups ($P < 0.001$). Scholastic achievement significantly differed between the groups ($P < 0.001$), with group VI having the highest good achievement (73.3%) compared to other groups, while group I was the lowest, with only 11.4% having good achievement. Positive family history and the presence of a risk factor were significantly different across the groups, with group I and III having the highest percentage of positive family history (62.9%) and risk factor (77.8%). In contrast, these were completely absent in group VI (0% for both, $P < 0.001$). Other variables, such as age ($P = 0.988$), sex ($P = 0.989$), and father's occupation ($P = 0.071$), were not significant. There was a statistically significant increase in salivary oxytocin in groups I, II, III, IV and V when compared to group VI (P value <0.001) (Table 1).

Table (1): General characteristics and salivary oxytocin level of the studied groups

	G I (n = 35)	G II (n = 25)	G III (n = 9)	G IV (n = 30)	G V (n = 27)	G VI (n = 60)	P-value
Age (years)	10 ±2	10 ±2	9 ±2	10 ±2	10 ±2	10 ±2	0.988
Sex							
Males	16 (45.7)	13 (52)	5 (55.6)	16 (53.3)	13 (48.1)	30 (50)	0.989
Females	19 (54.3)	12 (48)	4 (44.4)	14 (46.7)	14 (51.9)	30 (50)	
Order in family							
First	18 (51.4)	11 (44)	4 (44.4)	10 (33.3)	16 (59.3)	33 (55)	NA
Second	8 (22.9)	6 (24)	1 (11.1)	7 (23.3)	4 (14.8)	18 (30)	
Third or more	9 (25.7)	8 (32)	4 (44.4)	13 (43.3)	7 (25.9)	9 (15)	
Father's occupation	33 (94.3)	23 (92)	9 (100)	27 (90)	24 (88.9)	60 (100)	0.071
Mother's occupation	8 (22.9)	8 (32)	1 (11.1)	5 (16.7)	8 (29.6)	50 (83.3)	**<0.001 ***
Residency							
Rural	16 (45.7)	13 (52)	5 (55.6)	25 (83.3)	19 (70.4)	22 (36.7)	**<0.001 ***
Urban	19 (54.3)	12 (48)	4 (44.4)	5 (16.7)	8 (29.6)	38 (63.3)	
Scholastic achievement							
Bad to moderate	31 (88.6)	10 (40)	5 (55.6)	22 (73.3)	19 (70.4)	16 (26.7)	**<0.001***
Good	4 (11.4)	15 (60)	4 (44.4)	8 (26.7)	8 (29.6)	44 (73.3)	
Positive family history	22 (62.9)	13 (52)	0 (0)	5 (16.7)	15 (55.6)	0 (0)	**<0.001 ***
Risk factor	21 (60)	13 (52)	7 (77.8)	5 (16.7)	14 (51.9)	0 (0)	**<0.001 ***
Salivary oxytocin (pg/ml)	20 ᵀ (11.91 - 110)	30 ᵀ (20 - 110)	30 ᵀ (20 - 60)	30 ᵀ (12.93 - 110)	40 ᵀ (12.59 - 90)	12.79 (11.4 - 40)	**<0.001 ***

* Significant P-value; NA: Not applicable, ᵀ Significant difference from the control group

Tension scores significantly differed between the groups (P = 0.01) with significance between groups I and II. Insomnia scores showed that group III significantly differed from groups IV and V (P = 0.032). Cardiovascular system symptoms showed that group II significantly differed from groups I and V (P = 0.008). Respiratory symptoms showed that group I significantly differed from group II (P = 0.006). GIT symptoms showed that group II significantly differed from groups III, IV, and V (P < 0.001). Additionally, group I differed from group III. Genitourinary symptoms showed that group II significantly differed from group V (P = 0.01). No significant difference was observed between the groups regarding the total HARS score. None of the other items differed significantly.

No significant differences were observed between the groups regarding total FNE (P = 0.212) and total SAD (p = 0.290). Panic disorder showed that group I significantly differed from groups II and III (P < 0.001). Additionally, group II significantly differed from groups III and V. Moreover, group III significantly differed from group IV, and group IV significantly differed from group V. Generalized anxiety disorder showed that group V significantly differed from groups I, II, III, and IV (P < 0.001). Additionally, group I significantly differed from group II. Separation anxiety disorder showed that group I significantly differed from groups II, III, and V (P < 0.001). No significant differences were observed regarding social anxiety disorder (P = 0.076) and school avoidance (P = 0.332) (Table 2).

Table (2): Hamilton anxiety rating scale (HARS), SASFC score and SCARED score in the studied groups

HARS	**G I (n = 35)**	**G II (n = 25)**	**G III (n = 9)**	**G IV (n = 30)**	**G V (n = 27)**	**P-value**
Anxious mood	3 (0 - 4)	3 (0 - 4)	3 (2 - 4)	3 (0 - 4)	3 (1 - 4)	0.062
Tension	3 (0 - 4) [2]	1 (0 - 4) [1]	3 (1 - 4)	2 (0 - 4)	2 (0 - 4)	**0.01***
Fears	2 (0 - 4)	3 (1 - 4)	2 (0 - 4)	3 (0 - 4)	3 (0 - 4)	0.438
Insomnia	2 (0 - 3)	2 (0 - 4)	3 (0 - 3) [5]	2 (0 - 3) [3]	1 (0 - 3) [3]	**0.032***
Intellectual	2 (1 - 4)	2 (0 - 4)	3 (1 - 3)	2 (0 - 4)	2 (0 - 3)	0.256
Depressed mood	2 (0 - 4)	2 (0 - 4)	3 (2 - 4)	2 (0 - 4)	2 (0 - 4)	0.107
Somatic (muscular)	1 (0 - 3)	0 (0 - 3)	1 (0 - 3)	1 (0 - 4)	1 (0 - 3)	0.232
Somatic (sensory)	0 (0 - 3)	1 (0 - 3)	1 (0 - 3)	1 (0 - 3)	0 (0 - 3)	0.879
Cardiovascular symptoms	2 (0 - 4) [2]	2 (2 - 3) [1, 5]	2 (0 - 3)	2 (0 - 4)	2 (0 - 3) [2]	**0.008***
Respiratory symptoms	2 (0 - 3) [2]	2 (2 - 3) [1]	3 (1 - 3)	2 (0 - 4)	2 (0 - 3)	**0.006***
GIT symptoms	2 (0 - 4) [3]	2 (1 - 4) [3, 4, 5]	0 (0 - 0) [1, 2]	0 (0 - 3) [2]	0 (0 - 4) [2]	**<0.001***
Genitourinary symptoms	1 (0 - 3)	0 (0 - 4) [5]	0 (0 - 4)	3 (0 - 4)	3 (1 - 3) [2]	**0.01***
Autonomic symptoms	0 (0 - 3)	0 (0 - 3)	0 (0 - 3)	0 (0 - 3)	0 (0 - 3)	0.965
Behavior at interview	2 (0 - 4)	2 (0 - 4)	2 (0 - 3)	2 (0 - 4)	2 (0 - 4)	0.988
Total score	25 (18 - 34)	25 (18 - 37)	26 (18 - 29)	25 (18 - 40)	24 (18 - 40)	0.917
SASFC						
Total FNE	9 (5 - 14)	10 (6 - 15)	8 (7 - 13)	10 (5 - 15)	9 (6 - 13)	0.212
Total SAD	9 (4 - 13)	9 (6 - 16)	7 (6 - 12)	10 (4 - 15)	8 (4 - 13)	0.290
SCARED						
Panic Disorder	6 (1 - 10) [2, 3]	14 (8 - 20) [1, 3, 5]	6 (3 - 9) [1, 2, 4]	13 (6 - 19) [3, 5]	5 (1 - 8) [2, 4]	**<0.001***
Generalized Anxiety Disorder	11 (7 - 17) [2, 5]	9 (6 - 15) [1, 5]	12 (9 - 15) [5]	10 (7 - 15) [5]	6 (2 - 9) [1, 2, 3, 4]	**<0.001***
Separation Anxiety Disorder	8 (1 - 13) [2, 3, 5]	10 (4 - 16) [1]	12 (8 - 17) [1]	10 (1 - 15)	10 (1 - 14) [1]	**<0.001***
Social Anxiety Disorder	9 (0 - 14)	8 (3 - 12)	8 (6 - 12)	10 (5 - 14)	9 (0 - 14)	0.076
School Avoidance	5 (2 - 10)	5 (1 - 9)	6 (2 - 7)	5 (0 - 9)	5 (0 - 10)	0.332

SASFC: Social anxiety scale for children; FNE: Fear of negative evaluation; SAD: Social avoidance disorder, * Significant P-value; 1: Significant difference from Group I; 2: Significant difference from Group II; 3: Significant difference from Group III; 4: Significant difference from Group IV; 5: Significant difference from Group V.

ROC analysis was done for salivary oxytocin to differentiate between GAD and controls. It revealed an AUC of 0.876, with a 95% confidence interval ranging from 0.797 to 0.955. The best cutoff was >13.63, at which sensitivity and specificity were 90.6% and 85% respectively. ROC analysis was done for salivary oxytocin to differentiate between patients with panic disorders and controls. It revealed an AUC of 0.950, with a 95% confidence interval ranging from 0.909 to 0.991. The best cutoff was >16.75, at which sensitivity and specificity were 100% and 85% respectively. ROC analysis was done for salivary oxytocin to differentiate between patients with specific phobias and controls. It revealed an AUC of 0.930, with a 95% confidence interval ranging from 0.869 to 0.990. The best cutoff was > 16.75, at which sensitivity and specificity were 100% and 85% respectively. ROC analysis was done for salivary oxytocin to differentiate between patients with social anxiety and controls. It revealed an AUC of 0.927, with a 95% confidence interval ranging from 0.874 to 0.979. The best cutoff was > 16.75, at which sensitivity and specificity were 93.3% and 85% respectively. ROC analysis was done for salivary oxytocin to differentiate between patients with separation anxiety disorders and controls. It revealed an AUC of 0.937, with a 95% confidence interval ranging from 0.878 to 0.997. The best cutoff was >13.71, at which sensitivity and specificity were 92.6 and 85% respectively (Figure 1).

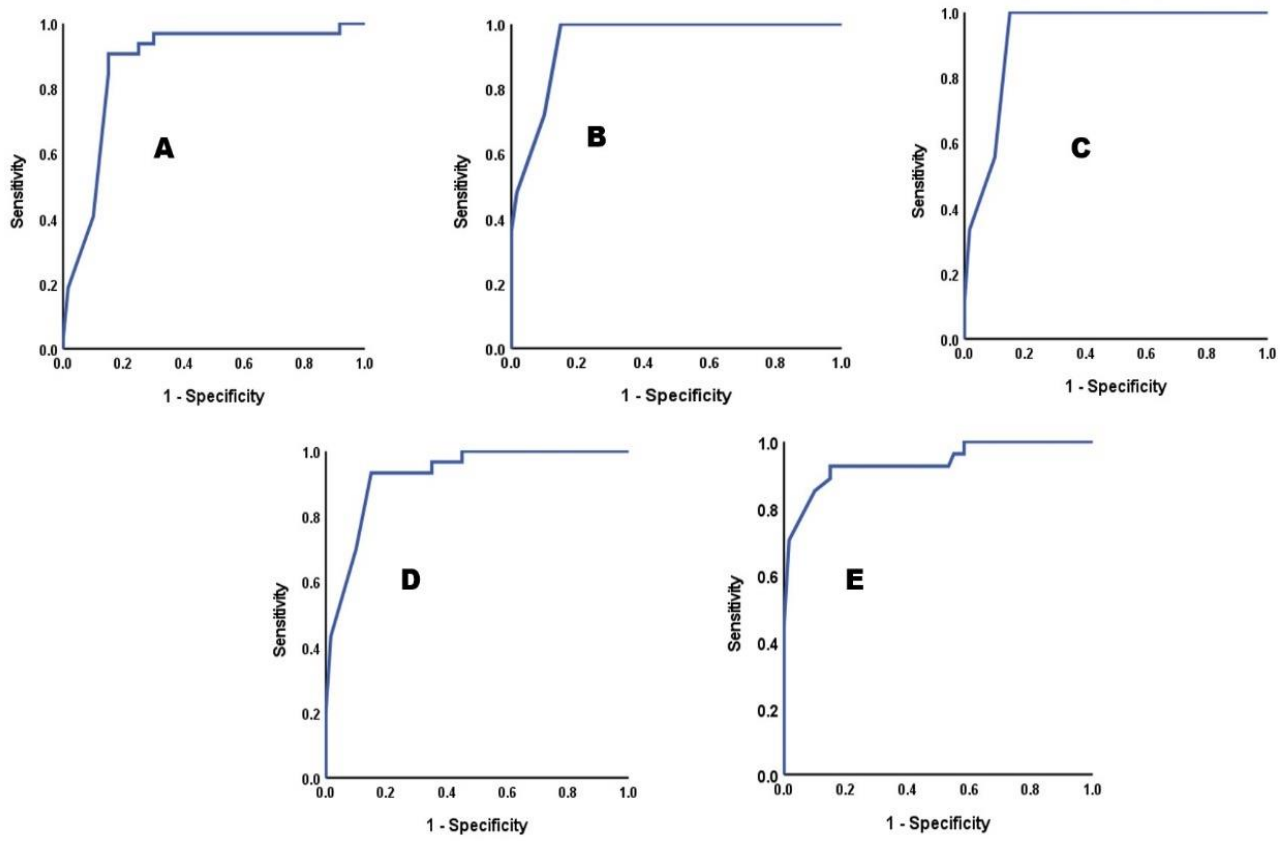

Figure (1): ROC analysis of salivary oxytocin to diagnose GAD (A), panic disorders (B), specific phobias (C), social anxiety disorders (D) and separation anxiety disorders (E).

Multivariate logistic regression analyses were done to predict different anxiety disorders. In GAD patients, controlling for age and gender, salivary oxytocin was significantly associated with increased risk of GAD (OR = 1.182, 95% CI = 1.095 - 1.275, P <0.001). In contrast, being a working mother (OR = 0.055, 95% CI = 0.019 - 0.162, P < 0.001) and good school achievement (OR = 0.041, 95% CI = 0.012 - 0.142, P < 0.001) were significantly associated with reduced risk of GAD.

In panic disorder patients, controlling for age and gender, salivary oxytocin was significantly associated with increased risk (OR = 1.221, 95% CI = 1.112 - 1.34, P <0.001). In contrast, being a working mother (OR = 0.092, 95% CI = 0.031 - 0.273, P < 0.001) was significantly associated with a reduced risk of panic disorders. In specific phobia patients, controlling for age and gender, salivary oxytocin was significantly associated with increased risk (OR = 1.185, 95% CI = 1.073 - 1.309, P <0.001). In contrast, being a working mother (OR = 0.025, 95% CI = 0.003 - 0.224, P < 0.001) was significantly associated with a reduced risk of specific phobia.

In social anxiety disorders, controlling for age and gender, salivary oxytocin (OR = 1.216, 95% CI = 1.123 - 1.318, P <0.001) and being the 3rd child or more (OR = 4.75, 95% CI = 1.561 - 14.454, P = 0.006) were significantly associated with increased risk. In contrast, being a working mother (OR = 0.038, 95% CI = 0.012 - 0.127, P < 0.001) and urban residence (OR = 0.116, 95% CI = 0.039 - 0.346, P <0.0001) were significantly associated with reduced risk of social anxiety disorders. In separation anxiety disorders, controlling for age and gender, salivary oxytocin (OR = 1.197, 95% CI = 1.11 - 1.291, P <0.001) was significantly associated with increased risk. In contrast, being a working mother (OR = 0.083, 95% CI = 0.028 - 0.244, P < 0.001), urban residence (OR = 0.243, 95% CI = 0.091 - 0.648, P <0.0001), and good school achievement (OR = 0.135, 95% CI = 0.047 - 0.389, P < 0.001) were significantly associated with reduced risk of social anxiety disorders (Table 3).

Table (3): Multivariate logistic regression analyses for prediction of different anxiety disorders

	GAD		**Panic disorders**		**Specific phobia**		**Social anxiety**		**Sep anxiety disorder**	
	OR (95% CI) †	**P**	**OR (95% CI) †**	**P**	**OR (95% CI) †**	**P**	**OR (95% CI) †**	**P**	**OR (95% CI) †**	**P**
Order in family (ref: 1st order)										
2nd	0.852 (0.305 - 2.381)	0.759	1.019 (0.313 - 3.311)	0.975	0.425 (0.043 - 4.159)	0.462	1.278 (0.407 - 4.016)	0.674	0.457 (0.131 - 1.587)	0.218
3rd or more	1.871 (0.627 - 5.582)	0.261	2.692 (0.83 - 8.735)	0.099	3.755 (0.772 - 18.275)	0.101	4.75 (1.561 - 14.454)	**0.006***	1.616 (0.507 - 5.15)	0.417
Working mother	0.055 (0.019 - 0.162)	**<.001***	0.092 (0.031 - 0.273)	**<.001***	0.025 (0.003 - 0.224)	**<.001***	0.038 (0.012 - 0.127)	**<.001***	0.083 (0.028 - 0.244)	**<.001***
Urban residence (ref: rural)	0.658 (0.279 - 1.554)	0.34	0.543 (0.21 - 1.405)	0.208	0.457 (0.111 - 1.89)	0.28	0.116 (0.039 - 0.346)	**<.001***	0.243 (0.091 - 0.648)	**0.005***
Good school achievement (ref: not good)	0.041 (0.012 - 0.142)	**<.001***	0.543 (0.202 - 1.457)	0.225	0.295 (0.07 - 1.244)	0.096	0.128 (0.047 - 0.35)	**<.001***	0.135 (0.047 - 0.389)	**<.001***
Salivary oxytocin (pg/ml)	1.182 (1.095 - 1.275)	**<.001***	1.221 (1.112 - 1.34)	**<.001***	1.185 (1.073 - 1.309)	**<.001***	1.216 (1.123 - 1.318)	**<.001***	1.197 (1.11 - 1.291)	**<.001***

OR: Odds ratio; GAD: Generalized anxiety disorders; *Significant P-value; 95% CI: 95% confidence interval; † Adjusted for age and gender.

DISCUSSION

Anxiety disorders are among the most common psychiatric conditions affecting children, significantly impacting their developmental trajectory and quality of life. Characterized by pervasive and persistent fear or anxiety that interferes with daily activities, these disorders often manifest through various physical and psychological symptoms. The biological mechanisms underlying these conditions are complex, involving a myriad of genetic, neurobiological, and environmental factors. This study investigated the association between salivary oxytocin levels and various anxiety disorders in children at Benha University Hospitals.

Regarding salivary oxytocin, groups I, II, III, IV, and V demonstrated elevated salivary oxytocin levels, notably significant from group VI. These groups had median oxytocin levels of 20 pg/ml (range 11.91 - 110), 30 pg/ml (range 20 - 110), 30 pg/ml (range 20 - 60), 30 pg/ml (range 12.93 - 110), and 40 pg/ml (range 12.59 - 90) respectively. In contrast, Group VI exhibited considerably lower oxytocin levels, with a median of 12.79 pg/ml (range 11.4 - 40). In recent years, oxytocin has gained significant attention in psychology and behavior studies for its role as an indicator of positive emotions, with levels rising in soothing and tranquil scenarios [15]. Recognized for its pivotal contribution to social bonds, it exhibits both immediate and prolonged effects, alongside its vital functions in childbirth, breastfeeding, and the mother-child connection, typically being secreted during positive interactions [16]. Furthermore, oxytocin enhances social memory, recognition, and attentiveness, while possessing pronounced effects in reducing anxiety and stress. Unlike other welfare indicators such as cortisol, catecholamines, or alpha amylase, which are often linked to stress and adverse conditions, oxytocin is uniquely correlated with positive experiences [17]. It is worth noting that other research also reported that increased oxytocin activity may be associated with anxiety. For example, **Purba *et al.*** [18] found increased oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depressed patients, consistent with increased oxytocin production and release, which may also contribute to anxiety symptoms associated with depression [18]. Furthermore, this finding is consistent with the finding of increased oxytocin in another group of individuals with social deficits and adults with autistic spectrum disorder [19].

In the present study, the Hamilton Anxiety Rating Scale highlighted significant differences in symptoms among groups. Tension and respiratory symptoms differed notably between groups I and II. Insomnia scores were significantly different between group III and groups IV and V. Cardiovascular symptoms showed significant differences between group II and groups I and V. GIT symptoms varied substantially, with group II differing significantly from groups III, IV, and V, and group I differing from group III.

In our results, using SCARED, significant differences were found in panic, generalized anxiety, and separation anxiety disorders across groups ($P < 0.001$). Panic disorder varied notably between groups, with significant differences particularly between groups I and II, III and V, as well as IV and V. Generalized anxiety disorder differed substantially between group V and all other groups, and between group I and group II. Separation anxiety disorder also showed significant variances, especially between group I and groups II, III, and V. These variations—ranging from the focused fears in specific phobias and separation anxiety to the diffuse worries characteristic of Generalized Anxiety Disorder (GAD), and the acute panic symptoms in panic disorders highlighted the necessity of nuanced diagnostic and therapeutic strategies. Specifically, the significant disparities in panic disorder scores, which suggest distinct physiological sensitivities or cognitive interpretations of bodily sensations, necessitating interventions that target both cognitive and physiological aspects. Meanwhile, the pronounced generalized worry in GAD compared to other groups underscores the need for therapeutic approaches that address pervasive patterns of worry rather than specific fears. The unique profile of separation anxiety disorder, distinguished from generalized worry and acute panic, points towards interventions focused on attachment and developmental considerations.

ROC analysis for salivary oxytocin effectively differentiated between generalized anxiety disorders (GAD) and controls, yielding an AUC of 0.876, indicating high diagnostic accuracy. The analysis determined an optimal cutoff value of >13.63, with a sensitivity of 90.6% and specificity of 85%. This high sensitivity ensures that most individuals with GAD are correctly identified, while the strong specificity minimized false positives, reducing unnecessary anxiety and additional testing for those without the disorder. The 95% confidence interval of 0.797 to 0.955 supports the reliability of these findings.

ROC analysis for salivary oxytocin in differentiating panic disorders from controls showed an AUC of 0.950, indicating excellent diagnostic accuracy. The optimal cutoff was >16.75 pg/ml, with perfect sensitivity (100%) and substantial specificity (85%), effectively identifying all true positives while minimizing false positives. The tight confidence interval from 0.909 to 0.991 underscores the test's reliability.

ROC analysis for salivary oxytocin in distinguishing patients with specific phobias from controls showed an AUC of 0.930, demonstrating excellent diagnostic accuracy. The optimal cutoff was >16.75 pg/ml, achieving a sensitivity of 100%, correctly identifying all individuals with specific phobias, and a specificity of 85%, accurately identifying most controls while allowing for some false positives. The confidence interval ranged from 0.869 to 0.990, affirming the test's reliability across different settings.

ROC analysis was done using salivary oxytocin to distinguish between patients with social anxiety and controls showed an AUC of 0.927. This value indicates excellent diagnostic accuracy, with a confidence interval from 0.874 to 0.979, suggesting the test's reliability. The optimal cutoff point identified was >16.75 pg/ml, at which the test achieved a sensitivity of 93.3%, correctly identifying a high proportion of social anxiety cases, and a specificity of 85%, effectively distinguishing most controls but with some false positives.

ROC analysis for salivary oxytocin in distinguishing patients with separation anxiety disorders from controls indicated an AUC of 0.937, showing high diagnostic accuracy. The optimal cutoff set at >13.71 pg/ml resulted in a sensitivity of 92.6%, effectively identifying the majority of true positives, and a specificity of 85%, correctly recognizing most true negatives. The 95% confidence interval from 0.878 to 0.997 underscores the test's reliability in various settings.

In the present study, multivariate logistic regression analyses indicated that higher salivary oxytocin levels significantly increased the risk of various anxiety disorders such as GAD (OR = 1.182, P <0.001), panic disorders, specific phobias, social anxiety (OR = 1.216, P <0.001), and separation anxiety (OR = 1.197, P <0.001). Conversely, being a working mother significantly reduced the risk across most disorders (e.g., GAD OR = 0.055, P < 0.001, social anxiety OR = 0.038, P < 0.001 and separation anxiety OR = 0.083, P < 0.001). Also, did good school achievement (e.g., GAD OR = 0.041, P < 0.001, separation anxiety OR = 0.135, P < 0.001). Urban residence also correlated with reduced risk for social (OR = 0.116, P <0.0001) and separation anxiety disorders (OR = 0.243, P <0.0001).

A study assessing oxytocin levels in social anxiety disorder measured plasma oxytocin in 24 patients with Generalized Social Anxiety Disorder (GSAD) and 22 healthy controls using an enzyme-linked immunosorbent assay. The results showed that, after adjusting for age and gender, higher symptom severity on the Liebowitz Social Anxiety Scale (LSAS) was correlated with higher oxytocin levels within the GSAD sample ($R^2 = 0.21$, $\beta = 0.014$, SE = 0.006, t = 2.18, P = 0.04) [20].

In another study focused on specific phobia, a double-blind, placebo-controlled trial was conducted with patients suffering from arachnophobia. Participants received intranasal oxytocin prior to exposure therapy. The findings indicated that the group treated with oxytocin had poorer outcomes, with reduced therapy credibility and perceptions of therapeutic alliance compared to the placebo group [21].

Increased risk with being the 3rd child or more could be related to dynamics within larger families, where resources (both emotional and material) are more divided, and children might compete for parental attention. Birth order could influence personality development. Later-born children might adopt more submissive roles in social settings, potentially leading to increased social anxiety. While, decreased risk with urban residence might reflect the beneficial aspects of urban environments, such as better access to healthcare services, including mental health support, and more diverse social environments. Urban settings might offer more opportunities for social engagement and activities that can buffer against anxiety through social support networks and a sense of community. The link between good academic achievement and reduced risk of separation anxiety disorders can be attributed to the fact that success at school contributes to self-esteem and personal efficacy beliefs, which can act as a buffer against anxiety. Furthermore, schools provide structured environments where children can form stable relationships with peers and adults outside the family, offering emotional support and reducing over-reliance on parents, thereby mitigating separation anxiety. Elevated oxytocin levels in patients with anxiety disorders could indicate an overactive oxytocinergic system in response to stress and social challenges. This paradoxically could exacerbate anxiety symptoms, perhaps by over-sensitizing individuals to social cues and increasing their emotional reactivity to stress. This aligns with studies suggesting that oxytocin's effects are context-dependent, enhancing the salience of social stimuli, whether positive or negative. Thus, while oxytocin might promote social bonding in some contexts, it could also heighten the perception of social threats or stress in others, contributing to anxiety [22].

Having a working mother is associated with a reduced risk of developing anxiety disorders. This finding could be interpreted through several lenses. Economically, working mothers may contribute to a more stable and resource-rich family environment, reducing stressors associated with financial strain. Psychosocially, working mothers might model coping mechanisms, independence, and social skills to their children, fostering resilience against anxiety. Moreover, children of working mothers often engage in early childcare, which can promote socialization and adaptability skills protective against anxiety.

CONCLUSIONS

Elevated salivary oxytocin levels are significantly associated with anxiety disorders in children, with high diagnostic accuracy. This study confirmed the potential of salivary oxytocin as a reliable biomarker for identifying and differentiating anxiety disorders in the pediatric population, underscoring the importance of socio-demographic contexts in its expression.

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