Influence of oxytocin level and oxytocin receptor gene expression on the status and severity of autism spectrum disorder in a group of Egyptian children

Shaimaa M. Hashish[a], Nahla Nazmy[a], Lubna M. Desouky[a], Amal Elsaeid[b], Hala M. Zeidan[b]

[a]Department of Human Genetics, Medical Research Institute, Alexandria University, Alexandria, [b]Department of Research on Children with Special Needs, Institute of Medical Research and Clinical Studies, National Research Centre, Cairo, Egypt

Correspondence to Hala M. Zeidan, PhD, Department of Research on Children with Special Needs, Institute of Medical Research and Clinical Studies, National Research Centre, El-Buhouth Street, PO Box 12622, Dokki, Cairo, Egypt. Tel: +20 212 235 71 676/20 120 843 4272; fax: +20 233 370 931; e-mail: halazeidan@yahoo.com

Received: 13 August 2024
Revised: 24 August 2024
Accepted: 25 August 2024
Published: 17 December 2024

Egyptian Pharmaceutical Journal 2024, 0:0–0

Background
The use of oxytocin (OXT) as a biomarker of autism spectrum disorder (ASD) and its role in the pathophysiology of ASD is still controversial and not straightforward.
Objective
To evaluate, the association of ASD risk with OXT concentration and the expression of its receptor (*OXTR*) *mRNA* in a group of children with autism, in addition, to investigating the influence of OXT and OXTR on the status and severity of the disorder in the study ASD group.
Patients and methods
This study included 30 children diagnosed with ASD, representing the cases group, and a comparable 30 neurotypical children with matched age and sex as a control group. After detailed history taking, pedigree construction, and clinical examination to exclude recognizable syndromes, OXT level and *OXTR* gene expression were assessed in all included children.
Results and conclusion
The OXT level in autistic cases was significantly lower than in controls (P=0.0001). Also, the fold change of *OXTR* was significantly lower than controls (P=0.01). Receiver operating characteristic curve analysis showed that at a cut-off point of less than 0.2806, *OXTR* showed reasonable diagnostic performance with sensitivity, specificity, positive predictive value, and negative predictive value of 53.33, 78.2, 72.7, and 63.2, respectively (P=0.0067). At a cut-off point of less than or equal to 119 pg/ml, the OXT level showed good diagnostic performance with sensitivity (86.67%), specificity (76.67%), positive predictive value (78.8%), and negative predictive value (85.2%) ($P<0.0001$). Our results proved the potential role of OXT plasma measurements and gene expression in diagnosing ASD at optimal cut-off values. These results support the use of OXT as a biomarker for ASD and also as a possible therapeutic option to improve the social behavior of ASD children.

Keywords:
autism, mRNA, oxytocin receptor gene, oxytocin

Egypt Pharmaceut J 0:0–0

1687-4315

Introduction

A complex collection of neurodevelopmental abnormalities known as autism spectrum disorder (ASD) is typified by restricted behaviors/interests and deficits in social communication and language [1,2]. ASD-diagnosed individuals may also exhibit a wide range of behaviors, such as impulsivity, aggression, self-harm, anxiety, hyperactivity, and tantrums [3]. ASD characteristics appear from early childhood before the age of two, even if these are not obvious until later in childhood. ASD is a disorder with a complex multifactorial etiology. Significant discoveries have been made in the past 10 years about genetic, epigenetic, organic/immunological deficiencies, and chemical/environmental loads as evident etiology of ASD [4,5]. The prevalence of ASD has been steadily increasing over the past 20 years, that could be due to increased awareness and improving diagnostic protocols [6]. The prevalence of ASD is about one in every 100 children worldwide [7].

Hypothalamic neuropeptide oxytocin (OXT) plays an important role in social salience and motivation [8]. Through direct neuronal modulation, neural network activity regulation, and interaction with other neurotransmitter systems via the single oxytocin receptor (OXTR), OXT controls social behavior. The brain expresses OXTR-mRNA widely and diffusely, with some regions showing especially strong expression [9]. OXT and its receptor gained a great research attention to modulate social behavior

across species, including humans. According to the literature, convincing results explained the OXT involvement in the etiology and treatment of psychiatric disorders, including ASD [10]. The diagnosis of ASD usually takes place by a pediatrician, child psychiatrist, or clinical psychologist with experience in the area of ASD [11,12]. In addition, there is a necessity to identify biomarkers for early detection and even individualized intervention of children with ASD. OXT measurement and mRNA expression of its receptor OXTR could provide an easy and cost-effective biomarker in ASD diagnosis, especially at the early childhood.

Recent data suggested that signaling dysregulation of OXT, as well as *OXTR*, have been associated with ASD; however, these data are still not fully understood [13]. Accordingly, the present study aimed to evaluate the association of ASD risk with the expression of *OXTR*-mRNA and OXT concentration in a group of selected children with ASD compared to normal developing control children of matched age range and sex, in addition to investigate the influence of OXT and OXTR on the status and severity of disorder in the study ASD group.

Patients and methods

Thirty patients (25 males and five females) were included in the current study aged 3–12 years old and diagnosed with nonsyndromic ASD according to 5th Edition of Diagnostic and Statistical Manual of Mental Disorders (DSM) [1]. Childhood Autism Rating Scale (CARS) [14] was applied to evaluate ASD severity. All ASD participants were referred to the Outpatient Clinic of Neuro-Rehabilitation & Learning Disabilities, Medical Research Center of Excellence, Research on Children with Special Needs Department, Medical Research and Clinical Studies Institute, National Research Centre (NRC). A comparable group included 30 neurotypical volunteer children (24 males and six females) with matched ages and sex were enrolled in the study as a normal developing control group.

All procedures are performed in accordance with the latest version of the Helsinki Declaration of 1975. In the present study, the parents of all participants signed informed consent. The study was performed according to the ethical regulations of the National Research Centre, Egypt, and the ethical guidelines of the Medical Research Institute, Alexandria University, Egypt, the ethical approval number (E/C. S/N. T32/2020-IORG0008812).

Patients with conspicuous metabolic or progressive neurological disorders and other causes of mental retardation or delayed language development were excluded from the study. Also, we ruled out patients with a family history of autism, patients with any karyotype abnormality or syndromic cases, in addition to patients with any neurological post-vaccination complication.

All children were subjected to detailed history taking, pedigree construction, and clinical examination to exclude recognizable syndromes. In addition, cytogenetic study; karyotyping (when indicated), brain imaging, electroencephalography, intelligence quotient (IQ) testing, extended metabolic screening, and heavy metal screening were performed when indicated to exclude any other developmental disorders.

Biochemical analysis

Samples collection

Two blood samples (4 ml) were collected from each participant in the study between 10 a.m. and 3 p.m. in 5-ml vacutainer tubes containing potassium EDTA. The first sample (2 ml) was centrifuged at 1900 *g* for 20 min for plasma isolation. Plasma samples were kept at −80°C until processing. The second whole blood sample (2 ml) was processed immediately for RNA isolation using Trizol (Ambion, Life Technologies, Carlsbad, CA, USA), according to supplier's instructions. For further analysis, the extracted RNA samples were stored at −80°C.

Oxytocin measurement

The concentration of OXT was measured for each participant in plasma samples using human OXT ELISA kits (SinoGeneclon, HangZhou, China), according to the supplier's instruction.

Molecular biology analysis

The reverse transcription reaction was carried out using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Vilnius, Lithuania), following the manufacturer's instructions, total RNA concentration was 2 µg in SimpliAmp Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Singapore). Gene expression level of the mRNA of the *OXTR* gene in the blood of the selected participants was carried out using Maxima SYBR Green qPCR master mix (2×) (Thermo Fisher Scientific, Vilnius, Lithuania) in 25-µl total reaction volume. The primers sequences are; Forward, 5'-CTG AAC ATC CCGAGG AAC TG-3' and Reverse 5'-CTC TGA GCC ACT GCA AAT GA-3' [15]. The mRNA for

the *OXTR* gene was normalized to the control gene *β-actin* (housekeeping gene). The thermal cycler profile was 40 cycles of denaturation for 15 s at 95°C, annealing for 30 s at 60°C, and extension for 30 s at 72°C in the quantitative real-time PCR (qRT-PCR) (DNA Technology, Moscow, Russia) [16]. Fold change was calculated using the $2^{(-\Delta\Delta\ Ct)}$ method [17].

Statistical analysis

Data were analyzed using the statistical package for the Social Sciences (SPSS), version 22 (IBM SPSS Statistics for Windows, Armonk, NY: IBMCorp, USA). Using the Kolmogorov–Smirnov test, quantitative data that are not normally distributed was summarized by median as measures of central tendency and range as measures of dispersion while for quantitative normally distributed variables, we summarized it using mean and SD. Frequencies and percentages were used to describe the categorized variables. To study the significant association between two categorical variables and to detect significant differences in median quantitative variables between the two groups, the χ^2 test and Mann–Whitney test were used, respectively.

Results

As shown in Table 1, the selected individuals' age range was from 3 to 12 years old, the median of their age was 7 and 5 years. Males constituted the majority in both groups (83.3% and 80%). Positive consanguinity was found in 43.3% of the cases. The mean maternal and paternal age at the time of the cases' pregnancy was 33.3±6.3 and 37.6±7.2, respectively. The onset of symptoms at the age of 1–2 years and 2–3 years was in 76.7 and 23.3% of the cases, respectively.

According to the detailed history of the ASD cases we included the ASD cases who delivered at full term and average birth weight. All cases received a routine vaccination schedule without any neurological post-vaccination complications. Their natal history revealed that the cesarean section delivered 70% of the cases, while 30% were delivered by vaginal delivery. Only one case with in-vitro fertilization, no neonatal Intensive Care Unit (ICU) admission was applied that excludes any secondary causes of developmental delays, such as oxygen deprivation, severe neonatal jaundice, congenital infection, or metabolic disturbance. There was no history of maternal infection during pregnancy. Five cases with positive family history: one case with a brother with delayed language development, two cases with second-degree and third-degree relative with Down syndrome, one case with a cousin with cerebral palsy, and one case with a third-degree relative with mental retardation.

The associated symptoms were seizures (16%), with associated electroencephalographic abnormalities (13.3%). Brain imaging (MRI) was done on 24 cases out of 30, and only three cases were found (mild hypoplasia of corpus callosum, areas of demyelination, wide cistern Magna). Heavy metal analysis was done in 33.3% of cases, reflecting abnormalities in aluminum, zinc, selenium, and nickel. Karyotyping was done in almost 50% of the cases, and all were free (Table 2).

According to CARS scores, the autistic cases were classified as mild ASD (CARS score: less than or equal 33) (21 cases, 70%), moderate ASD (CARS score: 34–36) (nine cases, 30%), and severe ASD (CARS score: greater than 36): no cases found in our study. According to IQ, 26.7% of autistic children had an IQ less than 70, 36.7% of cases with IQ between 70 and 79, and 36.7% of cases with IQ between 80 and 89. Figure 1 illustrates the variation of IQ and its relation with CARS severity

Table 1 Demographic characteristics of study groups

Demographic characteristics	ASD (*N*=30)	Controls (*N*=30)	Test of significance (*P*)
Sex	*n* (%)	*n* (%)	
Female	5 (16.7)	6 (20)	χ^2=2.43, *P*=0.119
Male	25 (83.3)	24 (80)	
Age of children (years)			
Mean±SD	6.8±2.1	6.15±2.6	*U*=585, *P*=0.08
Median (minimum–maximum	7 (4–11.6)	5 (3–12)	
Mother age (years)			
Mean±SD	33.3±6.3	35.3±3.3	*U*=264, *P*=0.004*
Median (minimum–maximum)	32 (26–57)	36.5 (28–42)	
Father age (years)			
Mean±SD	37.6±7.2	33.2±3.4	(U=650, *P*=.007*)
Median (minimum–maximum)	36 (27–64)	34 (27–40)	

χ^2; χ^2 test; ASD, autism spectrum disorder; U; Mann–Whitney. *P value less than or equal to 0.01.

Table 2 Descriptive statistical analysis of history and clinical data of autism spectrum disorder cases

Clinical data of ASD cases	*n* (%)
Age of milestone affection (months)	
12–24	23 (76.7)
24–36	7 (23.3)
Developmental delay	
Cognitive	21 (70.0)
Social+language	19 (63.3)
Motor	1 (3.3)
GDD	1 (3.3)
Associated symptoms	
Seizures	5 (16.7)
EEG abnormalities	4 (13.3)
MRI abnormalities	3 (10.0)
Heavy metal analysis abnormalities	10 (33.3)

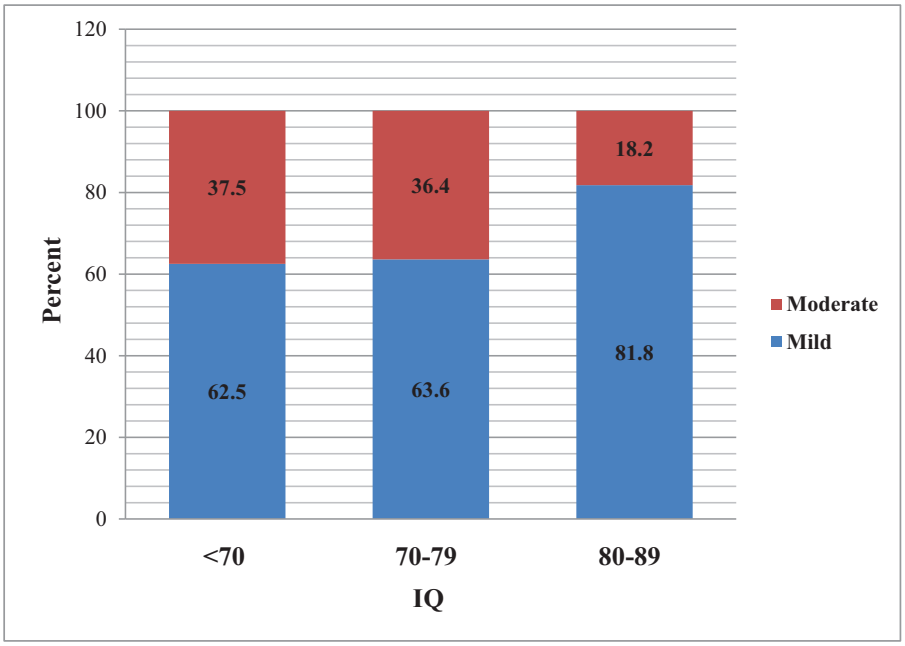
ASD, autism spectrum disorder; EEG, electroencephalography; GDD, global developmental delay.

among ASD cases. These results are represented in Fig. 1.

The OXT level in ASD cases was significantly lower than controls with a median of 87 (53–268), and 177 (62–231), respectively (P=0.0001). Also, the fold change of *OXTR* (Table 3, Figs 2 and 3) was significantly lower than controls with a median of 0.28 (0.07–5.5) and 1.39 (0.01–67.03), respectively (P=0.01).

The diagnostic values of the fold change of *OXTR* gene and OXT levels (in prediction of ASD are presented in Fig. 4 and Fig. 5; respectively). At a cut-off point of less than 0.2806, *OXTR* showed reasonable diagnostic performance with sensitivity, specificity, positive predictive value, and negative predictive value, of 53.33, 78.2, 72.7, and 63.2, respectively (P=0.0067). At a cut-off point of less than or equal to 119 pg/ml, the OXT level showed good diagnostic performance with sensitivity (86.67%), specificity (76.67%), positive predictive value (78.8%), and negative predictive value (85.2%) (P<0.0001) (Table 4). There is no statistically significant difference between the diagnostic ability of *OXTR* fold change versus OXT level in the prediction of ASD (P=0.08), as both with high specificity and sensitivity (Fig. 6).

Figure 1



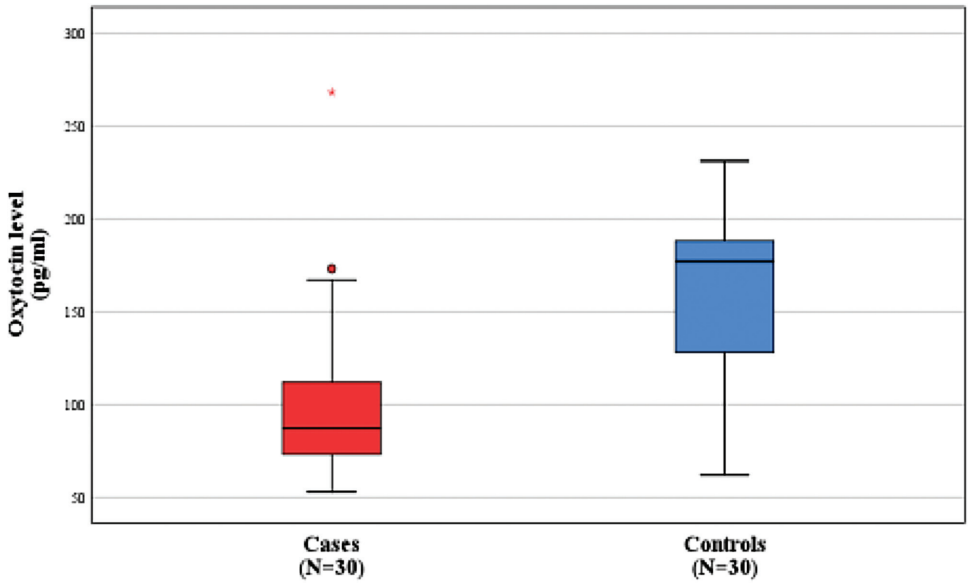
Autism spectrum disorder (ASD) cases severity related to intelligence quotient (IQ) level.

Table 3 Comparison between the study group regarding fold change of oxytocin receptor gene expression.

Parameters	ASD (*N*=30)	Controls (*N*=30)	*P* value
Fold change of oxytocin receptor gene expression			
Median (minimum–maximum)	0.28 (0.07–5.5)	1.39(0.01–67.03)	*U*=280, *P*=0.01*
Oxytocin level (pg/ml)			
Median (minimum–maximum)	87 (53–268)	177 (62–231)	*U*=136, *P*=0.0001*

ASD, autism spectrum disorder. **P* value less than or equal to 0.01.

Figure 2



Comparison between the study groups as regard to oxytocin level.

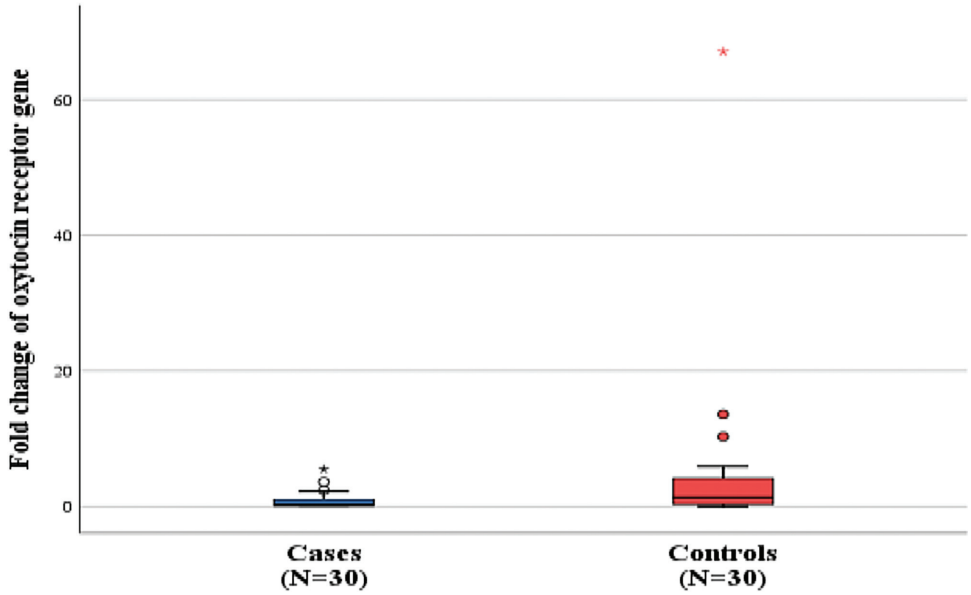
There was a significant correlation between the fold change of the *OXTR* gene and the OXT level (*P*=0.039) in autistic cases, while this correlation was insignificant in the controls (*P*=0.055).

We did not find any significant correlation between OXT level and fold change of *OXTR*, with each parameter of CARS in ASD group, except for a significant correlation of OXT level and fear or nervousness (*P*=0.032). Also, no significant statistical difference between the severity of ASD or IQ deterioration with the level of OXT or the fold change of *OXTR* gene expression values was reported (*P*=0.397, 0.594, 0.18, 0.95, respectively) as presented in Table 5.

Discussion

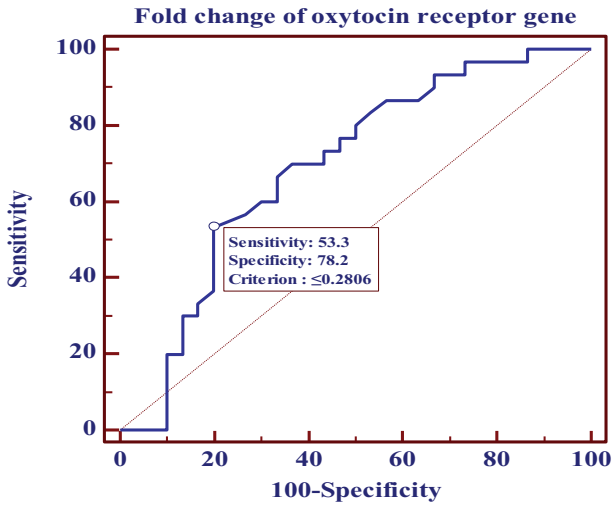
ASD is a disorder with complex etiology which includes genetic, epigenetic, and environmental. According to the clinical history of the ASD group, the cesarean section delivered is 70% of the cases. This

Figure 3



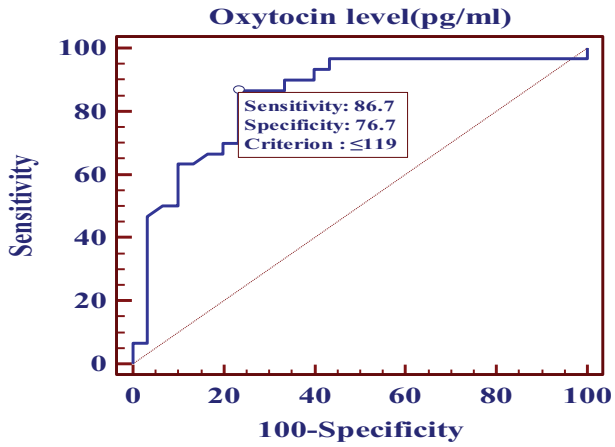
Comparison between the study groups as regard to fold change of oxytocin receptor gene expression.

Figure 4



Diagnostic ability of fold change of oxytocin receptor (*OXTR)* gene expression in prediction of autism spectrum disorder (ASD).

Figure 5



Diagnostic ability of oxytocin (OXT) level in prediction of autism spectrum disorder (ASD).

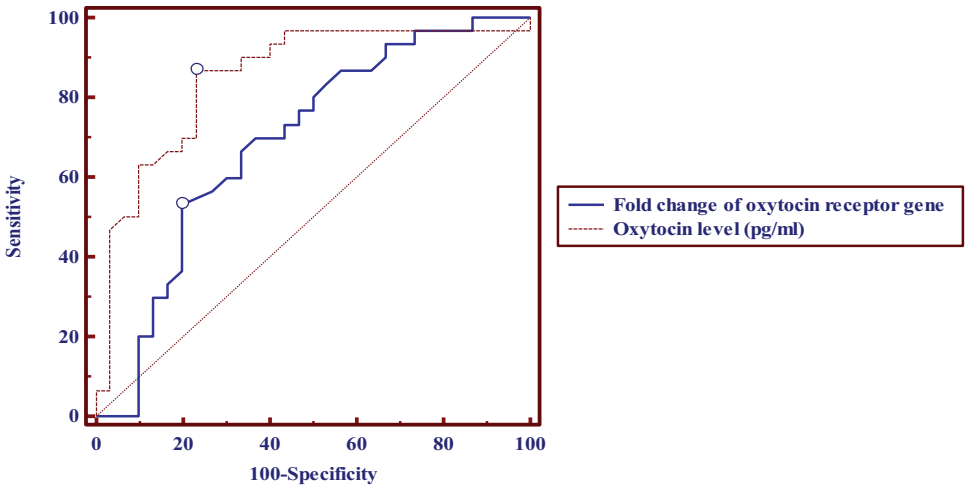
Table 4 Diagnostic ability of fold change of oxytocin receptor gene expression and oxytocin level in prediction of autism spectrum disorder

Parameters	AUC (95% CI)	*P* value	Cut-off point	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Fold change of oxytocin receptor gene expression	0.69 (0.559–0.804)	0.0067*	<0.2806	53.33 (34.3–71.7)	78.2 (61.4–92.3)	72.7 (49.2–89.6)	63.2 (46.0–78.2)
Oxytocin level (pg/ml)	0.85 (0.73–0.92)	<0.0001*	≤119	86.67 (69.3–96.2)	76.67 (57.7–90.1)	78.8 (60.8–91.2)	85.2 (65.8–95.9)

AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value. **P* value less than or equal to 0.01.

result is in agreement with Al-Zalabani *et al.* [18], who found an association between cesarean method of delivery and autism. These results support the concept of avoiding unnecessary planned cesarean section delivery. In addition, they associated the cesarean section delivery and reduced plasma levels of OXT in ASD children than that of neurotypical peers, as we found in the current study. OXT is secreted in pulses during normal birth and increased gradually to its peak in the first hour after birth.

Figure 6



Comparison between diagnostic ability of fold change oxytocin receptor gene expression versus oxytocin level in prediction of autism spectrum disorder (ASD).

Table 5 Relation between fold change of oxytocin receptor gene expression and oxytocin level and intelligence quotient and Childhood Autism Rating Scale

Parameter	Fold change of *OXTR* gene [median (minimum–maximum)]	Test of significance (*P* value)	Oxytocin level (pg/ml) [median (minimum–maximum)]	Test of significance (*P* value)
IQ				
Extremely Low (*N*=8)	0.80 (0.07–2.24)	*H*=0.083, *P*=0.95	104 (53–268)	*H*=3.4, *P*=0.18
Borderline (*N*=11)	0.28 (0.08–5.5)		84 (58–145)	
Low average (*N*=11)	0.28 (0.065–2.4)		74 (63–167)	
Severity of ASD				
Mild (*N*=21)	0.26 (0.06–3.6)	*U*=106.5, *P*=0.594	84 (53–173)	*U*=114, *P*=0.397
Moderate (*N*=9)	0.52 (0.07–5.5)		96 (58–268)	

ASD, autism spectrum disorder; *H*, Kruskal–Wallis test; OXTR, oxytocin receptor; *U*, Mann–Whitney test.

Moreover, OXT dysregulation has been proposed to affect the brain development of infant [18].

As it is not easy to employ OXT as an ASD biomarker [19], this study aimed to evaluate the association of ASD risk with *OXTR* expression and OXT hormone level in a group of ASD participants compared to neurotypical children, as well as to investigate their effects on the status and the intensity of the disorder in a group of ASD children.

Here, we contribute a crucial developmental aspect to the developing understanding of the connections between social behavior variability and the oxytocinergic system. In the present study, there was a significantly lower level of OXT among the autistic group compared to controls (P=0.0001). Also, the median fold change of *OXTR* was significantly lower in cases than in controls (P=0.01). The potential of OXT as a blood-based biomarker of ASD was shown using receiver operating characteristic analysis. It was found that the fold change of the *OXTR* and OXT levels can significantly discriminate ASD cases (P=0.0067 and P>0.0001, respectively). OXT had excellent area under the curve values with strong specificity, sensitivity, and accuracy indices. These findings support the possibility that, at ideal cut-off values, OXT plasma tests can be used to diagnose ASD. Finally, there is no statistically significant difference between the diagnostic ability of *OXTR* fold change against OXT level in the prediction of ASD (P=0.08), as they both were with high specificity and sensitivity.

The results for lower OXT in our study agreed with other studies. In a meta-analysis done with 31 studies in 2021, John and Jaeggi [20] discovered compelling evidence that OXT levels varied between neurotypical people and children with autism (but not adults). Reduced OXT levels in autistic children indicate that the OXT system may have had a role in the emergence or expression of ASD. In addition, OXT levels were correlated with ASD symptom severity. The initial meta-analysis on a considerably smaller sample of studies indicated a suggestive difference, which is supported and strengthened by the previous results in 2016 [21] Also, compared to controls, children with ASD have reduced endogenous OXT levels; however, in adolescents and adults, this effect appears to be negligible [22]. Subgroup differences, developmental variables, or variances in intellectual development can all be responsible for variations in OXT levels between age groups [13].

Furthermore, these findings conflict with those indicating elevated OXT in individuals with ASD as Jacobson *et al.* [23] revealed that, in comparison to controls, patients with ASD had higher OXT levels. Compared to controls, children with autism showed noticeably greater levels of mRNA for stimulatory G proteins.

In a Chinese study by Yang *et al.* [24], serum OXT levels were higher in ASD participants than in controls, and there was a positive correlation found between serum OXT levels and the "adaptation to change score" of CARS evaluation and CARS total scores. In order to detect their social deficits, they went on, people with ASD may show dysregulation in OXT based on modifications in OXTR gene expression and environmental changes that affect the oxytocinergic system.

In a case–control Iraqi study, in the context of ASD patients, peripheral OXT levels and genetic changes in OXTR may serve as biomarkers of social functioning. After classifying patients with ASD into severity groups, notable variations in OXT levels, OXTR (rs2268491, C/T) genotype, and allele distributions that may be sex-dependent were observed [25].

In a study published in 2016, in comparison to the matching neurotypical control children, they

discovered that pediatric patients with ASD had considerably higher serum OXT levels. That study's stratified ASD population showed that the mild subgroup had the greatest OXT levels, while the severe group had the lowest OXT levels. This implies that individuals with milder ASD symptoms may have developed a capacity to upregulate OXT levels in order to make up for the OXTR polymorphism-induced deficit in cerebral social response skills [26].

Single nucleotide polymorphisms (SNPs) on the OXTR gene have been found to be correlated with irritability, social function, and aggression. Low CD38 expression and an absence of emotions have been connected to SNPs on the CD38 gene that have been translated from an animal model of ASD [27]. Two SNPs have been discovered to predict OXT response in patients with ASD. One SNP is on the CD38 gene, which codes for the transmembrane protein involved in OXT secretion, and the other is on the OXTR gene, which codes for the OXTR [28,29].

We found no significant statistical difference between the severity of ASD or IQ deterioration with the level of OXT or the fold change of *OXTR* gene expression values. In contrast, Kobylinska *et al*. [30] demonstrated a negative relationship between the severity of ASD and plasma OXT levels, supporting the involvement of oxytocinergic mechanisms in ASD. In addition, Carson *et al*. [31] found robust negative correlations between anxiety symptoms in children and both plasma and cerebrospinal fluid OXT concentrations. These findings support the hypothesis that OXT plasma levels in ASD could predict social adaptability. Children with higher plasma OXT concentrations might have a higher potential to respond to therapy.

However, there is evidence to support the hypothesis that intranasal OXT (IN-OXT) may enhance reciprocal social behaviors by improving social orienting and the relevance of social rewards in ASD. These findings also corroborate the link between OXT and ASD [32,33]. On the contrary, OXT had a small and nonsignificant impact on core symptoms in the ASD population, according to a meta-analysis published in 2019 [34]. As for the reduction of core social symptoms in adult men with ASD, Yamasue *et al*. [35] concluded that longitudinal IN-OXT alone at the same dose and duration is not recommended. However, the results do not rule out the possibility that intranasal administration of OXT combined with behavioral interventions produces clinically satisfying effects on the core symptoms of ASD. On the other hand, the small sample size in the current study is considered as a limitation of the study. Future studies on a large sample size of children containing different age groups are required to study the effect of OXT on the severity and social behavior of ASD cases and its sex differences. In addition, future longitudinal studies are needed to assess the stability of OXT and OXTR levels over time and their correlation with symptoms severity.

Conclusion

We detected a statistically significant association between plasma OXT levels and peripheral transcriptional activity of the *OXTR* gene and ASD. Our results proved the potential role of OXT plasma measurements and gene expression in diagnosing ASD at optimal cut-off values.

Our understanding of the impacts of the peripheral OXT system is expanded by these findings, which also offer new genetic prospects for investigations aimed at unraveling the intricate etiology of ASD and its relationship to OXT signaling.

Acknowledgements

Nil.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th edn. Arlington, VA: American Psychiatric Publishing; 2013:13.

2 American Speech-Language-Hearing Association. Autism Spectrum Disorder. Available at: https://www.asha.org/practice-portal/clinical-topics/autism/#collapse_8. [Accessed April 30, 2023].

3 Trajkovski V. Health condition in persons with autism spectrum disorders health condition in persons with autism spectrum disorders. J ReAttach Ther Dev Diversit 2019; 1:112–124.

4 Lopes LT, Rodrigues JM, Baccarin C, Oliveira K, Abreu M, Ribeiro V, *et al.* Autism spectrum as an etiologic systemic disorder: a protocol for an umbrella review. Healthcare (Basel) 2022; 10:2200.

5 Cheroni C, Caporale N, Testa G. Autism spectrum disorder at the crossroad between genes and environment: contributions, convergences, and interactions in ASD developmental pathophysiology. Mol Autism 2020; 11:69.

6 Hodges H, Fealko C, Soares N. Autism spectrum disorder: definition, epidemiology, causes, and clinical evaluation. Transl Pediatr 2020; 9 (Suppl 1):S55–s65.

7 Zeidan J, Fombonne E, Scorah J, Ibrahim A, Durkin MS, Saxena S, *et al.* Global prevalence of autism: a systematic review update. Autism Res 2022; 15:778–790.

8 Rigney N, de Vries GJ, Petrulis A, Young LJ. Oxytocin, vasopressin, and social behavior: from neural circuits to clinical opportunities. Endocrinology 2022; 163:bqac111.

9 Wang P, Wang SC, Liu X, Jia S, Wang X, Li T, *et al.* Neural functions of hypothalamic oxytocin and its regulation. ASN Neuro 2022; 14:17590914221100706.

10 Siecinski SK, Giamberardino SN, Spanos M, Hauser AC, Gibson JR, Chandrasekhar T, *et al.* Genetic and epigenetic signatures associated with plasma oxytocin levels in children and adolescents with autism spectrum disorder. Autism Res 2023; 16:502–23.

11 Hyman SL, Levy SE, Myers SM. Identification. Evaluation, and management of children with autism spectrum disorder. Pediatrics 2020; 145:1.

12 American Pschological Association. Diagnosing and managing autism spectrum disorder (ASD). 2017. Available at: https://www.apa.org/topics/autism-spectrum-disorder/diagnosing

13 Moerkerke M, Peeters M, de Vries L, Daniels N, Steyaert J, Alaerts K, *et al.* Endogenous oxytocin levels in autism-a meta-analysis. Brain Sci 2021; 11:1545.

14 Conners CK, Sitarenios G, Parker JD, Epstein JN. The revised Conners' parent rating scale (CPRS-R): Factor structure, reliability, and criterion validity. J Abnormal Child Psychol 1998; 26:257–268.

15 Moezzi L, Keshavarz Z, Ranjbaran R, Aboualizadeh F, Behzad-Behbahani A, Abdullahi M, *et al.* Fetal RHD genotyping using real-time polymerase chain reaction analysis of cell-free fetal DNA in pregnancy of RhD negative women in South of Iran. Int J Fertil Steril 2016; 10:62–70.

16 Saleh MS, Galal AF, Sallam SF, Mowaad NA, Zeidan HM, Hashish A, *et al.* Using 6-CIT, P300 encephalography, and pro-inflammation assessments for screening age-related cognitive decline and exploring associated risk factors in Egyptian elderly. Egypt J Neurol Psychiatry Neurosurg 2024; 60:28.

17 Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nature Protocols 2008; 3:1101–1108.

18 Al-Zalabani AH, Al-Jabree AH, Zeidan ZA. Is cesarean section delivery associated with autism spectrum disorder? Neurosciences (Riyadh) 2019; 24:11–15.

19 Freeman SM, Palumbo MC, Lawrence RH, Smith AL, Goodman MM, Bales KL. Effect of age and autism spectrum disorder on oxytocin receptor density in the human basal forebrain and midbrain. Transl Psychiatry 2018; 8:257.

20 John S, Jaeggi AV. Oxytocin levels tend to be lower in autistic children: a meta-analysis of 31 studies. Autism 2021; 25:2152–2161.

21 Rutigliano G, Rocchetti M, Paloyelis Y, Gilleen J, Sardella A, Cappucciati M, *et al.* Peripheral oxytocin and vasopressin: biomarkers of psychiatric disorders? A comprehensive systematic review and preliminary meta-analysis. Psychiatry Res 2016; 241:207–220.

22 Ghazy AA, Soliman OA, Elbahnasi AI, Alawy AY, Mansour AM, Gowayed MA. Role of oxytocin in different neuropsychiatric, neurodegenerative, and neurodevelopmental disorders. In: Pedersen S.H.F. Eds Reviews of Physiology, Biochemistry and Pharmacology. Reviews of Physiology, Biochemistry and Pharmacology. Cham: Springer; 2022.

23 Jacobson JD, Ellerbeck KA, Kelly KA, Fleming KK, Jamison TR, Coffey CW, *et al.* Evidence for alterations in stimulatory G proteins and oxytocin levels in children with autism. Psychoneuroendocrinology 2014; 40:159–169.

24 Yang S, Dong X, Guo X, Han Y, Song H, Gao L, *et al.* Serum oxytocin levels and an oxytocin receptor gene polymorphism (rs2254298) indicate social deficits in children and adolescents with autism spectrum disorders. Front Neurosci 2017; 11:221.

25 Al-Ali Z, Yasseen AA, Al-Dujailli A, Al-Karaqully AJ, McAllister KA, Jumaah AS. The oxytocin receptor gene polymorphism rs2268491 and serum oxytocin alterations are indicative of autism spectrum disorder: a case-control paediatric study in Iraq with personalized medicine implications. PLoS One 2022; 17:e0265217.

26 Abdulamir HA, Abdul-Rasheed OF, Abdulghani EA. Low oxytocin and melatonin levels and their possible role in the diagnosis and prognosis in Iraqi autistic children. Saudi Med J 2016; 37:29–36.

27 Jensen AR, Lane AL, Werner BA, McLees SE, Fletcher TS, Frye RE. Modern biomarkers for autism spectrum disorder: future directions. Mol Diagn Ther 2022; 26:483–495.

28 Frye RE. Social skills deficits in autism spectrum disorder: potential biological origins and progress in developing therapeutic agents. CNS Drugs 2018; 32:713–734.

29 Kosaka H, Okamoto Y, Munesue T, Yamasue H, Inohara K, Fujioka T, *et al.* Oxytocin efficacy is modulated by dosage and oxytocin receptor genotype in young adults with high-functioning autism: a 24-week randomized clinical trial. Transl Psychiatry 2016; 6:e872.

30 Kobylinska L, Panaitescu AM, Gabreanu G, Anghel CG, Mihailescu I, Rad F, *et al.* Plasmatic levels of neuropeptides, including oxytocin, in children with autism spectrum disorder, correlate with the disorder severity. Acta Endocrinol (Buchar) 2019; 5:16–24.

31 Carson DS, Berquist SW, Trujillo TH, Garner JP, Hannah SL, Hyde SA, *et al.* Cerebrospinal fluid and plasma oxytocin concentrations are positively correlated and negatively predict anxiety in children. Mol Psychiatry 2015; 20:1085–1090.

32 Spanos M, Chandrasekhar T, Kim SJ, Hamer RM, King BH, McDougle CJ, *et al.* Rationale, design, and methods of the Autism Centers of Excellence (ACE) network Study of Oxytocin in Autism to improve Reciprocal Social Behaviors (SOARS-B). Contemp Clin Trials 2020; 98:106103.

33 Le J, Zhang L, Zhao W, Zhu S, Lan C, Kou J, *et al.* Infrequent intranasal oxytocin followed by positive social interaction improves symptoms in autistic children: a pilot randomized clinical trial. Psychother Psychosom 2022; 91:335–347.

34 Wang Y, Wang MJ, Rong Y, He HZ, Yang CJ. Oxytocin therapy for core symptoms in autism spectrum disorder: An updated meta-analysis of randomized controlled trials. Res Autism Spectrum Disord 2019; 64: 63–75.

35 Yamasue H, Okada T, Munesue T, Kuroda M, Fujioka T, Uno Y, *et al.* Effect of intranasal oxytocin on the core social symptoms of autism spectrum disorder: a randomized clinical trial. Mol Psychiatry 2020; 25:1849–1858.