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The association between salivary oxytocin, age, and puberty in children with and without OCD

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The oxytocin system has been thought to contribute to obsessive-compulsive disorder (OCD). Few studies, only involving adults, have investigated this hypothesis and have found inconsistent results regarding oxytocin system activity and OCD. We investigated whether salivary oxytocin concentrations differed between children and adolescents with and without OCD and qualified our comparative analysis by investigating the possible covariates age, pubertal stage, and sex. Participants included 113 children and adolescents (8–17 years) with OCD and 88 children and adolescents without any previous or current psychiatric disorder and their parents (254 parents included). Salivary oxytocin concentrations were measured in children and parents with enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed using frequentist and Bayesian approaches. We found no evidence of a difference in mean salivary oxytocin concentrations between children and adolescents with and without OCD. Bayesian analysis indicated anecdotal to moderate support for the null hypothesis. We found an association between oxytocin and age and between oxytocin and pubertal stage, which by visual inspection of plots and post-hoc tests indicated nonlinear relationships. We found no association between oxytocin concentration and sex. Our findings do not suggest elevated oxytocin concentrations in pediatric OCD. Nonlinear changes in oxytocin across development show the importance of accounting for hormonal and behavioral changes during puberty.

Keywords Oxytocin, Obsessive-compulsive disorder, Children, Adolescents, Pubertal stage, Age

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Obsessive-compulsive disorder (OCD) is a common and impairing psychiatric disorder[1] marked by persistent obsessions compulsions or both, that are time consuming, cause distress and/or impairment[2,3]. Obsessions are unwanted and repetitive thoughts, images or urges that often cause distress. Compulsions are repetitive behaviors or mental acts that the individual feels driven to perform to suppress obsessions, to follow rigid rules, to feel "complete" and/or reduce distress[2,3]. The biological mechanisms underlying OCD are not fully understood.

Oxytocin, a neuropeptide mainly synthesized in the hypothalamus, has been proposed to play a key role in OCD[4]. OCD-like behaviors in animals and humans have been observed under conditions of elevated oxytocin[4]. For example, injecting oxytocin into the central nervous system of rats and squirrel monkeys induced licking and grooming behavior – possibly analogous to cleaning compulsions[4]. Pregnant and postpartum women, groups marked by elevated oxytocin concentrations, have reported obsessions about and compulsive checking of their child's well-being[4]. Combined with the knowledge that oxytocin receptors are present in brain areas that are important in OCD this led to the hypothesis that high levels of oxytocin may intensify and prolong obsessions, discomfort, and compulsions and that oxytocin levels therefore may be elevated in patients with OCD[4]. This hypothesis has been investigated in three studies so far. The first study of 29 adults with OCD, 23 with Tourette's syndrome, and 31 controls found no differences in oxytocin concentrations in cerebrospinal fluid (CSF) among the three groups[5]. Another study of 14 adults with OCD and 26 controls found no significant difference in CSF oxytocin between the two groups[6]. A more recent study—the largest to date (44 adult patients with OCD and 44 controls) – found significantly higher concentrations of plasma oxytocin in patients with OCD[7]. Studies that investigate oxytocin concentrations in children and adolescents with OCD are needed as OCD often onsets in childhood and oxytocin system functioning changes across the lifespan[8]. Given the invasive and stressful nature of lumbar puncture for extracting CSF and the risk of selection bias in collecting plasma samples (only children without needle phobia would consent), salivary oxytocin is the most viable alternative. Salivary oxytocin has been extensively used in studies on parenting behavior and infant development[9].

Peripheral oxytocin concentrations often show high variability, which can complicate the detection of group differences[10,11]. Thus, studies that identify the sources of this variation and attempt to account for it before performing group comparisons could contribute with important knowledge. In children and adolescents, age, sex, and pubertal stage may account for variation in oxytocin concentrations as major physiological and behavioral changes occur during this stage in life and sex differences in oxytocin have been observed[8,12,13].

Thus, we investigated whether salivary oxytocin concentrations differed between children and adolescents with OCD and children and adolescents without OCD, while controlling for age, pubertal stage, and sex. We also explored whether and how these variables contributed to variation in salivary oxytocin.

Materials and methods

Study design

This is a sub-study of the TECTO trial (Treatment Effects of Family Based Cognitive Therapy in Children and Adolescents with Obsessive-Compulsive Disorder)[14]. The TECTO trial is a randomized clinical trial (RCT) comparing family-based cognitive behavioral therapy with family-based psychoeducation and relaxation training in 130 children and adolescents with OCD[14] combined with a case-control study including 90 age- and sex-matched controls without current or previous psychiatric diagnoses (described in this paper as "children and adolescents without OCD"). In the present study, we used baseline data only. Data collection started July 2018 and finished September 2022.

The TECTO trial complies to the Helsinki Declaration[15] and was approved by the ethics committee (Scientific Ethics Committees for the Capital Region of Denmark) (H-18010607). The trial was approved by the Knowledge Centre on Data Protection Compliance in The Capital Region of Denmark and was registered with clinical trials.gov (NCT03595098).

Participants and procedures

We included children and adolescents (8–17 years) and their parents as participants. Presence or absence of psychiatric diagnoses were based on the Kiddie-Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version (K-SADS-PL)[16] and the criteria for International Classification of Diseases-10 (ICD-10)[17]. All children were assessed for eligibility with full-scale Wechsler Intelligence Scales (either WISC-V[18] for children aged 16.9 years or below or WAIS-IV[19] for children above 16.9 years). The children with OCD were also assessed with the Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS)[20]. The assessors performing the K-SADS-PL were medical doctors or psychologists (and for the children and adolescents without OCD also psychology students) with experience in child- and adolescent psychiatry. All assessors performing the K-SADS-PL completed a course in the method and were provided continuous supervision. Parents or legal guardians provided written informed consent concerning their child's and their own participation.

Inclusion criteria for children and adolescents with OCD were a primary diagnosis of OCD according to ICD-10[21], CY-BOCS total score ≥ 16, age 8 to 17 years both inclusive, and signed informed consent from parents/legal caregivers. Exclusion criteria for children with OCD were comorbid illness with pervasive developmental disorder (not including Asperger's syndrome), schizophrenia/psychosis, mania or bipolar disorder, depressive psychotic disorders, substance dependence syndrome, IQ < 70, treatment with cognitive behavioral therapy, psychoeducation with relaxation training, antidepressant, or antipsychotic medications within the last six months prior to trial entry. Inclusion criteria for children and adolescents without OCD were age 8 to 17 years both inclusive, sex and age matched with an included child with OCD (+/- 3 months of birthday) and signed informed consent from parents/legal caretakers. Exclusion criteria for children without OCD were any current or previous psychiatric disorder according to ICD-10 criteria, non-Danish-speaking, and IQ < 70. We included parents with a child participating in the study and with informed consent to participation.

Oxytocin was assessed in saliva samples from children and parents. Saliva samples were collected with Salivettes (Sarstedt, Rommelsdorf, Germany). Participants were asked to refrain from eating two hours and drinking liquids 30 min prior to sampling. A detailed description of the handling of the saliva samples are available in Appendix A. The samples were analyzed with immunoassay using Enzo® (NY, USA) oxytocin kits according to the manufacturer's instructions, and concentrations were calculated using WorkOut 2.5 (Dazdaq Solutions Ltd, Brighton, UK). The intra-assay coefficient of variation was 13% and the inter-assay coefficient of variation was 20%.

Children and adolescents self-reported their pubertal status after instruction from a trained investigator using Tanner staging[22,23]. The children and adolescents chose the most fitting pubertal stage among pictorial presentations of secondary sex characteristics on a 5 point scale ranging from 1 (pre-pubertal development) to 5 (full adult development)[22,23]. We did not use classification of the specific sex characteristics but asked the children and adolescents to report the best fitting total pubertal stage. In one post hoc analysis, we assigned parents "pubertal stage 6" indicating "adults". However, this is not a part of the original Tanner staging. Throughout the paper, we use the term "pubertal stage" to describe Tanner stage.

Statistical analysis

We registered our statistical analysis plan prior to any data analysis (date of registration June 7, 2023, https://osf.io/hmc43). Changes from the pre-registered plan are described in Appendix B. We used frequentist statistics and Bayesian statistics. Bayesian statistics has been recommended as a valuable supplement to null hypothesis significance testing (NHST), as it can quantify the degree to which the data favor the alternative hypothesis (H_1) or the null hypothesis (H_0)[24]. Traditional NHST cannot evaluate the evidence for the null hypothesis[24]. Bayesian statistics can be used to describe the probability of observing data by updating prior information about the data with the observed data. One approach to compare models in Bayesian statistics is by using Bayes Factors[24]. The Bayes factor (BF_{10}) is the ratio between the support of the alternative hypothesis (H_1) and the null hypothesis (H_0)[24,25].

We interpreted the Bayes Factors using a classification scheme by Lee and Wagenmakers[25] with a BF_{10} of 1 indicating no difference in support of the H_1- compared to the H_0. Values above 1 indicate support of the H_1, and values below 1 indicate support of the H_0. The degree of support is divided by cutoffs describing anecdotal, moderate, strong, very strong, or extreme evidence (see description of cutoffs in Appendix C). We used Bayes factor, when we tested a discrete parameter (such as OCD-status of the children). When the variables were not discrete, we used the expected log-pointwise predictive density estimated by leave-one-out cross validation (ELPD-LOO). For a more detailed description of the Bayesian methods and analyses of sensitivity to the prior distributions, see Appendix C. Analyses were performed using R (version 4.2.0 and 4.3.0 for random forest analyses).

We excluded outliers in oxytocin concentrations (values ± 3 SDs from the mean) in accordance with previous studies in salivary oxytocin[26,27] and our pre-registration, and applied a logarithmic transformation with the natural logarithm. We have added a description of the outliers in Appendix B. In our pre-registration, assuming $n = 220$ (children and adolescents with OCD: 130, healthy controls: 90), $\alpha = 0.05$ (2-sided), and $\beta = 0.80$, we noted that we were able to reliably detect a Cohen's d of at least 0.4 (calculated using r package "pwr" as a Cohens d of 0.39). Our sample size was slightly reduced by missing information and outliers, however, without major change in the effect size we could detect ($n = 201$ (children and adolescents with OCD: 113, children and adolescents without OCD: 88), $\alpha = 0.05$ (2-sided), and $\beta = 0.80$, Cohens d of 0.40). We did not adjust for multiple comparisons as the study was exploratory, but we reported all comparisons and considered the risk of false discoveries.

The following primary statistical models were run (see also Appendix E):

Model 1: Using a Student's t-test we investigated the difference in oxytocin concentrations (dependent variable) between children and adolescents with and without OCD (OCD-status; independent variable). A Bayes factor was calculated to compare H_1 (an effect of OCD-status) to H_0 (no effect of OCD-status).

Model 2: A backward stepwise regression explored the variance in children and adolescent's oxytocin concentrations (dependent variable) explained by OCD-status, age, sex, and age-sex interaction (independent variables). The reduced model from the stepwise regression was used to analyze the effect of OCD-status on oxytocin concentrations. We used Bayesian analysis to compare H_1 (the reduced model plus an effect of OCD-status) to H_0 (the reduced model). Additionally, we used Bayesian analysis to compare the results of the stepwise regression and thereby the significant effects of relevant variables by comparing H_1 (the reduced model) to H_0 (intercept model).

Model 3: We investigated the variance explained by child and adolescent pubertal stage by repeating Model 2 with pubertal stage instead of age.

Model 4: We explored the effect of age and sex by analyzing all participants (children and adolescents, and parents). A backward stepwise regression of a mixed effect model was performed with participant oxytocin concentration (dependent variable), age, sex, and the interaction between age and sex (fixed effects) and a "family-variable" (random effect). The family-variable (a nominal variable denoting family membership) was included because previous research has shown associations between the oxytocin concentrations of parent couples (mothers and fathers)[27] as well as parents and their children[9].

To further explore the data, we ran the following *post hoc* models and tests that were not pre-registered:

Post Hoc Model A: We explored the observed nonlinearity in the child and adolescent data by repeating Model 2 with a quadratic age variable (age^2).

Post Hoc test B: To investigate if either age or pubertal stage best accounts for variation in oxytocin levels, we compared Model 3 with OCD and Post Hoc Model A with OCD using Bayesian analysis.

Post Hoc Model C: We repeated Model 4 with pubertal stage instead of age for all participants assigning parents an adult "pubertal stage 6". Bayesian analyses were used to compare the reduced Post Hoc Model C to the reduced Model 4.

Post Hoc Model D: To further explore the adult data we repeated Model 4 in adults alone.

Post Hoc test with random forest: A random forest approach (described in Appendix D) explored non-linear associations between salivary oxytocin and relevant covariates. We explored sex, age, pubertal stage, and information on menarche status, menopause status and use of hormonal contraception.

Complete case analyses were performed. Sample selection is presented in Fig. 1. Due to missing information on pubertal stage for some children and adolescents, we refitted models without child and adolescent pubertal stage to data which excluded children and adolescents with missing pubertal stage, when comparing models with pubertal stage to models without pubertal stage. We excluded cases with less than two family members from the models that included the 'family-variable'. We report supplementary analyses (described in Appendix F) of variables that could cause variation in oxytocin but were not main objectives of this study (time of sample, psychosocial functioning, OCD severity score, social responsiveness, menarche, and menstrual cycle phase).

Results

Participant characteristics are presented in Table 1 (children and adolescents) and Table 2 (parents). The children and adolescents with OCD did not differ on age, sex or pubertal stage from the children and adolescents without OCD. We did not have information on medication use or somatic disorders on the children without OCD.

In Model 1, salivary oxytocin concentrations did not significantly differ between children and adolescents with and without OCD ($p = 0.2682$, Table 3 and in Appendix E for further details) and the Bayes factor was below 1 indicating support for the null hypothesis. However, the size of the Bayes factor showed only anecdotal support, i.e., the lowest degree of support ($BF_{10} = 0.37$, Table 3).

When including age and sex and an age-sex interaction in a model with OCD-status (Model 2) only age remained significantly associated with child oxytocin in the reduced model showing a significant positive linear relationship between age and child and adolescent oxytocin ($p = 0.0031$, Table 4, and see Appendix E) [Figs. 2 and 4 (pink line)].

The Bayesian analysis showed that the model including child and adolescent age had a better predictive performance but a large standard error (*SE*) (ELPD-LOO = -3.4, *SE* = 2.7, Table 4). When we reexamined the relationship between child and adolescent oxytocin and OCD-status while controlling for age, OCD-status did not significantly account for variation in oxytocin ($p = 0.3605$, Table 4, and see Appendix E). The corresponding Bayes factor again showed support for the null hypothesis with a value below 1. The size of the Bayes factor indicated moderate support for no difference in oxytocin concentrations between children and adolescents with and without OCD controlling for age ($BF_{10} = 0.31$, Table 3) but with considerable prior sensitivity (see Appendix C).

When we included pubertal stage and age and their interaction (Model 3) all variables except pubertal stage were dropped in the model reduction (see Appendix E). The reduced model showed a significant relationship between child and adolescent oxytocin and pubertal stage ($p = 0.0004$, Table 4 and see Appendix E) (Fig. 3).

Bayesian analysis showed a better predictive performance of a model including pubertal stage, but with a large standard error (ELPD-LOO=-6.5, *SE* 4.1, Table 4). Controlling for pubertal stage in a model with OCD-status did not change the results for OCD-status, which was still not significantly associated with variation in oxytocin ($p = 0.1477$, Table 4 and Appendix E). The corresponding Bayes factor was below 1, thus again showing support for no difference in oxytocin concentrations between children and adolescents with and without OCD controlling for pubertal stage, and the size of the Bayes factor indicated anecdotal support ($BF_{10} = 0.52$, Table 4) with considerable prior sensitivity (see Appendix C).

In Model 4, we included children and adolescents, and parents. Again, we found no association between oxytocin and the age-sex interaction and sex, which were removed from the model in the first two steps (see Appendix E). The reduced model showed a significant positive, linear relationship between participant age and oxytocin ($p < 0.0003$, see Appendix E) [Fig. 4 (blue line)].

Including quadratic age in the exploratory Post Hoc Model A showed a significant relationship between oxytocin and quadratic age [Figs. 2 and 4 (purple line); $p = 0.0496$, see Appendix E], but again sex and the age-sex interaction was dropped in the model reduction (Appendix E).

Post Hoc test B showed that the predictive performance of the model including pubertal stage and OCD (reduced Model 3 with OCD) was slightly better than the model with quadratic age and OCD (reduced Post Hoc Model A and OCD) (ELPD-LOO = 2.9, *SE* 3.0, see Appendix C). However, the standard error of the difference in predictive performance was large.

Visual inspection of Fig. 4, illustrating the reduced Model 2 (child and adolescent age, pink line), the reduced Post Hoc Model A (child and adolescent quadratic age, purple line), and the reduced Model 4 (child, adolescent, and parent age, blue line), indicated that the models in children and adolescents cannot be extrapolated to adults and the model in all participants seemed problematic in estimating in children and adolescents.

In Post Hoc Model C, we used "pubertal stage 6" for the adults and found a significant relationship (Figure E.1 in Appendix E) between salivary oxytocin and the modified pubertal stage in the sample including children, adolescents, and adults ($p = 0.005$, see Appendix E). The Bayesian analysis showed a better predictive performance of the model with the modified pubertal stage compared to the model with age (ELPD-LOO = 5.9, *SE* 5.1, see Appendix C).

In Post Hoc Model D, including only adults, the age-sex interaction, sex, and age did not contribute to the variation in oxytocin ($p = 0.10$, see Appendix E).

Sensitivity analyses of the Bayesian analyses of the models showed a general sensitivity to priors and little difference in predictive performance between the compared models with high standard errors (Appendix C).

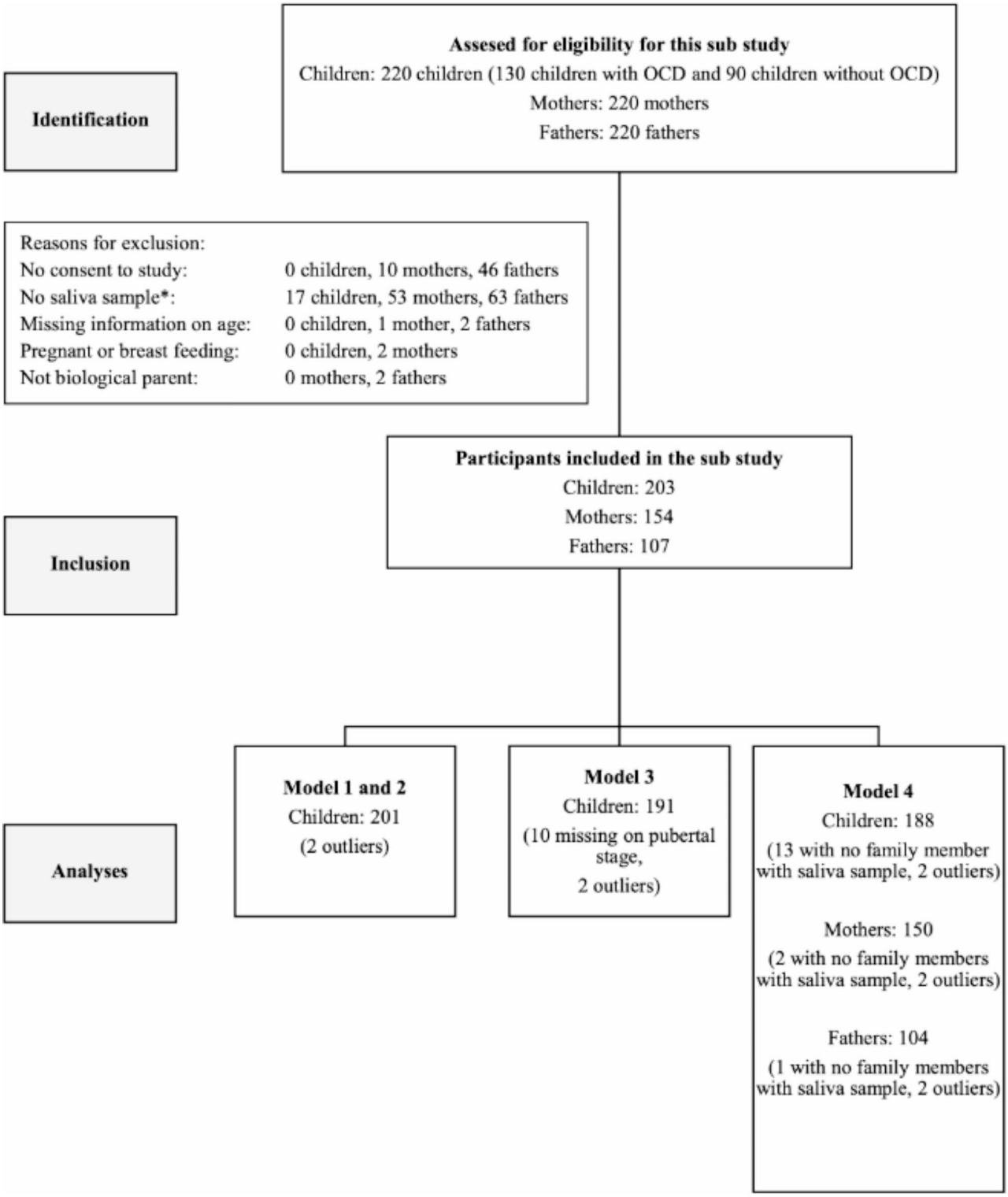

Fig. 1. Participant flow chart.*Reasons for missing saliva sample on children: 5 with too high anxiety on the day, 6 cancelled due to covid, 4 procedure mistakes, 2 with no reason registered. Reasons for missing sample on parents not registered. OCD, obsessive-compulsive disorder.

The random forest analysis in children and adolescents alone and in children, adolescents, and adults indicated that pubertal stage/modified pubertal stage and age were the most important variables. Furthermore, these findings both in visual presentation of the predictions and the mean squared error (*MSE*) resembled the findings from the linear models (see Appendix D).

	Children and adolescents with OCD	Children and adolescents without OCD	Test (*p*-value)
Child salivary oxytocin (pg/ml)			
Mean (*SD*)	26.61 (14.28)	25.61 (13.95)	
Missing	0	0	
n	113	88	
Age (years)			0.26_A
Mean (*SD*)	13.37 (2.83)	12.92 (2.78)	
Median (min, max)	13.81 (8.08, 17.99)	12.37 (8.09,17.83)	
Missing (*n*)	0	0	
Sex (*n* (%))			0.98_B
Female	58 (51.3%)	45 (51.1%)	
Male	55 (48.7%)	43 (48.9%)	
Missing	0	0	
Pubertal stage (*n* (%))			0.39_B
Pubertal stage 1	32 (28.3%)	23 (26.1%)	
Pubertal stage 2	13 (11.5%)	20 (22.7%)	
Pubertal stage 3	20 (17.7%)	14 (15.9%)	
Pubertal stage 4	25 (22.1%)	23 (26.1%)	
Pubertal stage 5	13 (11.5%)	8 (9.1%)	
Missing	10 (8.8%)	0 (0%)	
Nationality (*n* (%))			–
Danish	95 (84,1%)	63 (71.6%)	
Other	3 (2.7%)	2 (2.3%)	
Missing	15 (13.3%)	23 (26.1%)	
Comorbid psychiatric disorders (*n* (%))		*na*	–
Anxiety disorders ICD-10 F40-41, F93	12 (10.6%)		
Stress and adjustment ICD-10 F43	12 (10.6%)		
Asperger's Syndrome ICD-10 F84.5	14 (12.4%)		
Hyperkinetic disorders ICD-10 F90.0	13 (11.5%)		
Tic disorders ICD-10 F95	13 (11.5%)		
Other*	20 (17.7%)		
Medication (*n* (%))		*na*	–
Children receiving medication	24 (21.2%)		
Somatic disorders (*n* (%))		*na*	–
Children with a somatic disorder	32 (28.3%)		

Table 1. Descriptive characteristics of the children and adolescents included in Model 1 and model 2. Children in model 3 and model 4 are a subsample of the children. *n*, number; *SD*, standard deviation; OCD, obsessive-compulsive disorder; ICD-10, International Classification of Diseases-10, *na* refers to the information not being available. For some variables, statistical testing was not relevant or possible marked by "–". * "Other" describes all other diagnoses. For detailed information on patient medication and comorbid and somatic disorders see Appendix A. $_A$t-test, $_B$Chi-squared test.

In the additional analyses in our appendix, we found that adult females had significantly higher oxytocin concentrations during ovulation than during menstruation (estimate = 0.53, $p = 0.0008$, for more details see Appendix F). The additional analyses did not show a significant association between child oxytocin and OCD symptoms severity measured with CY-BOCS ($r = 0.003$, $p = 0.73$, for more details see Appendix F). The remaining analyses were not statistically significant.

Discussion

We evaluated the hypothesis that salivary oxytocin concentrations are elevated in children and adolescents with OCD. We included a relatively large sample of children and adolescents aged 8–17 years with and without OCD while controlling for potential variance in oxytocin concentrations related to age, pubertal stage, and sex. Overall, we found no support for the hypothesis of elevated oxytocin concentrations in OCD. Our results contrast with those of Marazziti et al. who found higher oxytocin concentrations in adults with OCD[7]. The contrasting findings could be explained by the use of different physiological fluids (i.e., saliva versus plasma). The correlation between salivary and plasma oxytocin has been found small-to-moderate (rho = 0.361)[26] raising the question of whether plasma and saliva measure the same thing[28]. A modest-to-strong positive correlation between salivary and CSF oxytocin has been found (rho = 0.711)[26] whereas a small-to-modest positive correlation has been found between plasma and CSF (rho = 0.417)[26]. Our findings are consistent with findings on oxytocin in CSF in adults with OCD

	Children and adolescents with OCD	Children and adolescents without OCD
n		
Mothers	92	58
Fathers	72	32
Mother age (years)		
Mean (*SD*)	45.72 (5.03)	45.97 (5.10)
Median (min, max)	45.61 (32.76, 56.24)	46.45 (34.97, 57.19)
Missing (*n*)	0	0
Father age (years)		
Mean (*SD*)	48.17 (5.67)	47.47 (6.78)
Median (min, max)	48.91 (33.1, 73.51)	47.08 (34.45, 62.73)
Missing (n)	0	0
Nationality Mother (*n* (%))		
Danish	77 (83.7%)	46 (79.3%)
Other	5 (5.4%)	3 (5.2%)
Missing	10 (10.9%)	9 (15.5%)
Nationality Father (*n* (%))		
Danish	57 (79.2%)	24 (75%)
Other	5 (6.9%)	1 (3.1%)
Missing (*n*)	10 (13.9%)	7 (21.9%)
Parental education mothers (*n* (%))		
Upper secondary education or below	8 (8.70%)	1 (1.7%)
Short-cycle tertiary education	14 (15.2%)	3 (3.2%)
Bachelor's degree or equivalent	38 (41.3%)	22 (37.9%)
Master's degree or above	22 (23.9%)	23 (39.7%)
Missing (*n*)	10 (10.9%)	9 (15.5%)
Parental education fathers (*n* (%))		
Upper secondary education or below	12 (16.7%)	3 (9.4%)
Short-cycle tertiary education	8 (11.1%)	3 (9.4%)
Bachelor's degree or equivalent	16 (22.2%)	6 (18.8%)
Master's degree or above	25 (34.7%)	13 (40.6%)
Missing (*n*)	11 (15.3%)	7 (21.9%)

Table 2. Descriptive characteristics of parents included in model 4. *n*, number; *SD*, standard deviation; OCD, obsessive-compulsive disorder.

			t-statistics	*p*-value	Bayesian analysis
Model 1					
1. Test	H_1	OCD-status	1.111	0.2682	$BF_{10} = 0.37$
	H_0	Intercept			

Table 3. Result of student's *t*-test of salivary oxytocin levels in children and adolescents with and without OCD (OCD-status) and bayesian statistics (model 1). BF, Bayes factor; H_1, alternative hypothesis; H_0, null hypothesis.

by Leckman and colleagues[5] and Altemus and colleagues[6]. A meta-analysis pooling the two aforementioned studies in adults also found no significant difference[29], however, a more recent meta-analysis also including the study by Marazziti et al., found significantly higher levels in patients with OCD compared to healthy controls[30]. Our complementary Bayesian analyses showed only anecdotal to moderate support for the null hypothesis, even with a larger sample size than the previous studies and almost as large as the sample in the recent meta-analysis. While it may be that oxytocin concentrations do not differ between children and adolescents with and without OCD, the null findings could be due to sample sizes too small to detect a discrete group difference. Another consideration of the null findings is the heterogeneous nature of OCD with multiple different phenomenological manifestations[31] which perhaps makes assuming overall group differences in biological markers too simplistic. The heterogeneous nature of OCD could also explain the contrasting findings between our study and the study by Marazziti et al. As proposed by Leckman et al., OCD is a highly heterogenous disorder, and oxytocin could be of importance in some subtypes of OCD[4] which is also indicated by their findings of higher levels in a subgroup of OCD patients[5]. A previous study has shown different symptom dimensions in OCD and suggested

			F-statistics	*p*-value	Bayesian analysis
Model 2					
1. Test	H_1	Reduced model (age)	8.913	0.0031	H_1- H_0 ELPD-LOO (*SE*) = 3.5(2.7)
	H_0	Intercept			
2. Test	H_1	Reduced model (age) and OCD-status	0.840	0.3605	$BF_{10=}$0.31
	H_0	Reduced model (age)			
Model 3					
1. Test	H_1	Reduced model (pubertal stage)	5.384	0.0004	H_1- H_0 ELPD-LOO (*SE*) = 6.6(4.1)
	H_0	Intercept			
2. Test	H_1	Reduced model (pubertal stage) and OCD-status	2.113	0.1477	$BF_{10=}$0.52
	H_0	Reduced model (pubertal stage)			

Table 4. Results of reduced models, reduced models with OCD, and bayesian statistics of model 2 and model 3. BF, Bayes factor; H_1, alternative hypothesis; H_0, null hypothesis; ELPD-LOO, expected log-pointwise predictive density estimated by leave-one-out cross validation; *SE*, standard error.

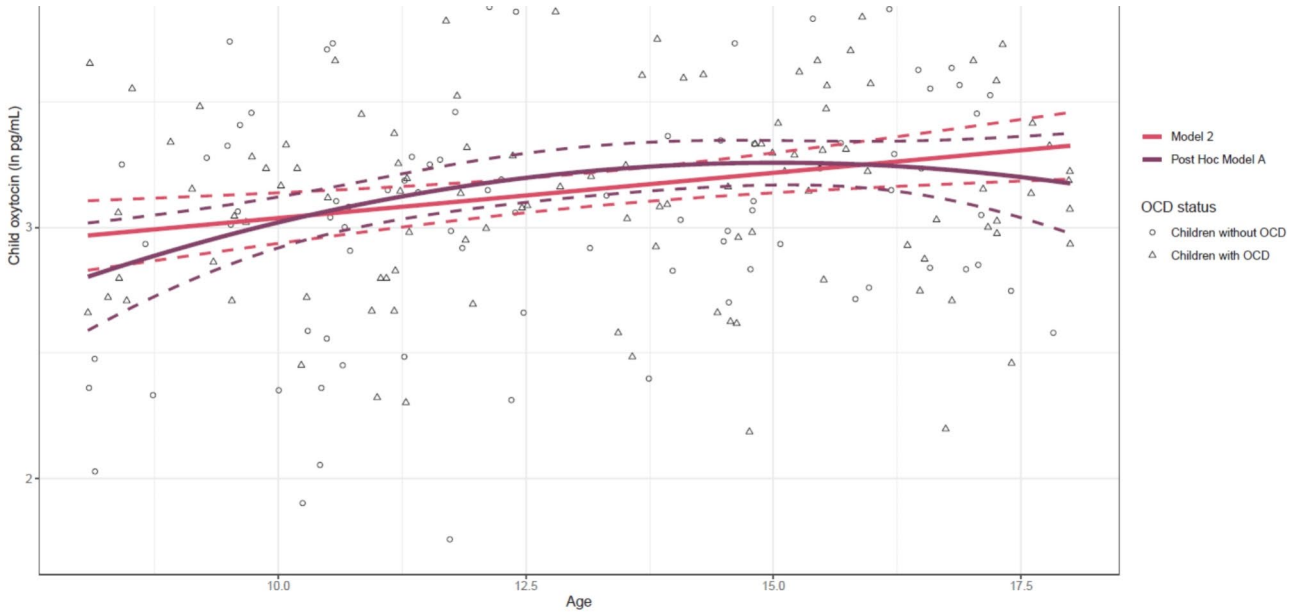

Fig. 2. The relationship between oxytocin and age predicted by the reduced model 2 with age (pink line) and the reduced post hoc model A with quadratic age (purple line). Note: The dashed lines represent the 95 confidence interval of the mean. Ln, natural logarithm.

that this could help our understanding of OCD including the underlying neurobiology[32]. Oxytocin could be linked to specific OCD dimensions e.g., the observed aggressive and sexual behavior in animals after oxytocin administration which could support a link between higher oxytocin and "disturbing thoughts" in OCD. A focus on trans-diagnostic mechanisms have also been suggested as important in OCD research[31] which could also be relevant in the research of oxytocin and OCD. Oxytocin has been implicated in regulation of anxiety and interpersonal behaviors[33]. One research group investigated a theory of oxytocin as a biochemical signal of safety with anxiolytic effects and associated with behaviors, such as family accommodation, which is common in OCD and anxiety disorders[34]. They investigated 50 children with clinical anxiety and found negative associations between salivary oxytocin, anxiety levels, and subscales of family accommodation[34]. Family accommodation is frequent in children with OCD[35] and associated with a poor treatment outcome[36]. A focus on oxytocin's relation to family behaviors, like family accommodation, rather than the overall OCD diagnosis could help understand poor treatment outcomes.

Our findings indicate that peripheral oxytocin concentrations increase with age from 8 to 17 years. This finding is consistent with findings from a study of children and adolescents (7–16 years) with clinical anxiety[34]. Another study in children (5–90 months of age) concluded that oxytocin decreases from 5 months to 90 months[37]. However, a scatter plot visualizing this data suggests an elbow shaped curve may fit this data better than a straight line[37]. Several of our analyses (scatter plot, quadratic age, and pubertal stage) supported a nonlinear relationship with oxytocin concentrations slightly increasing from 8 to 15 years, and from pubertal stage 1 to 4, followed by a slight decrease. The exploration of the relationship between age and oxytocin in our parent sample

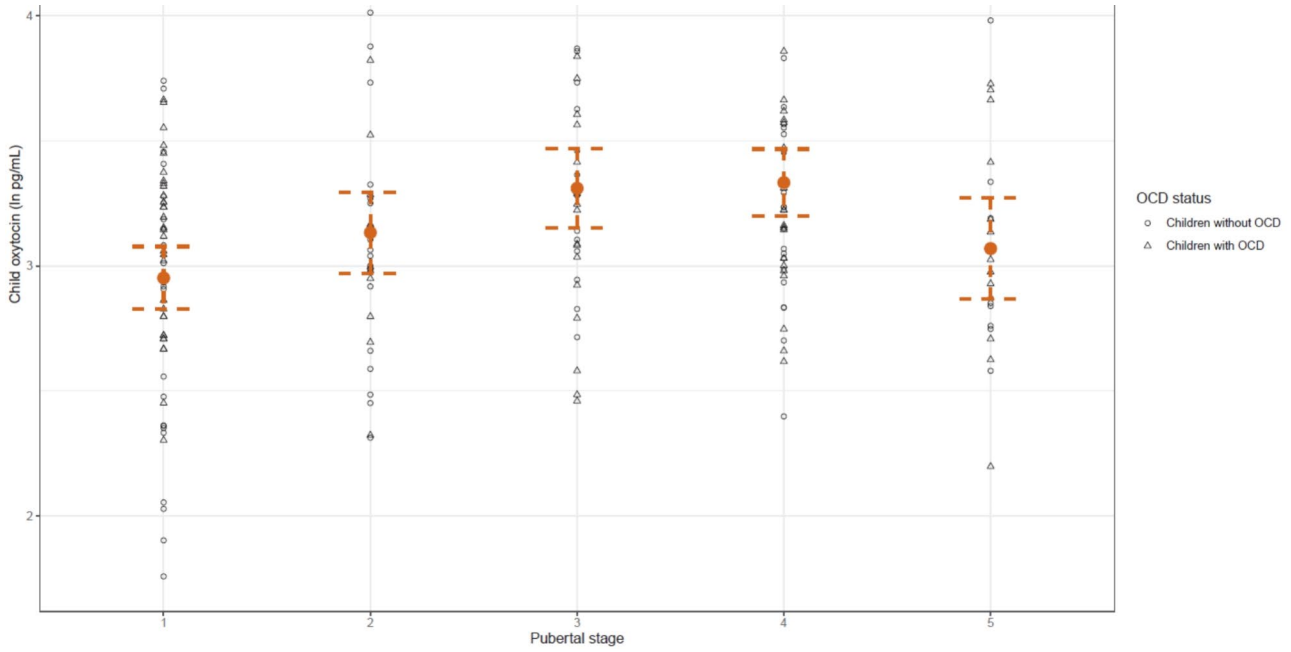

Fig. 3. The relationship between oxytocin and age predicted by the reduced model 4 with age of all participants (blue line), the reduced model 2 with age in children (pink line) and the reduced post hoc model A with age and quadratic age in children (purple line). *Note*: The dashed lines represent the 95 confidence interval. Ln, natural logarithm.

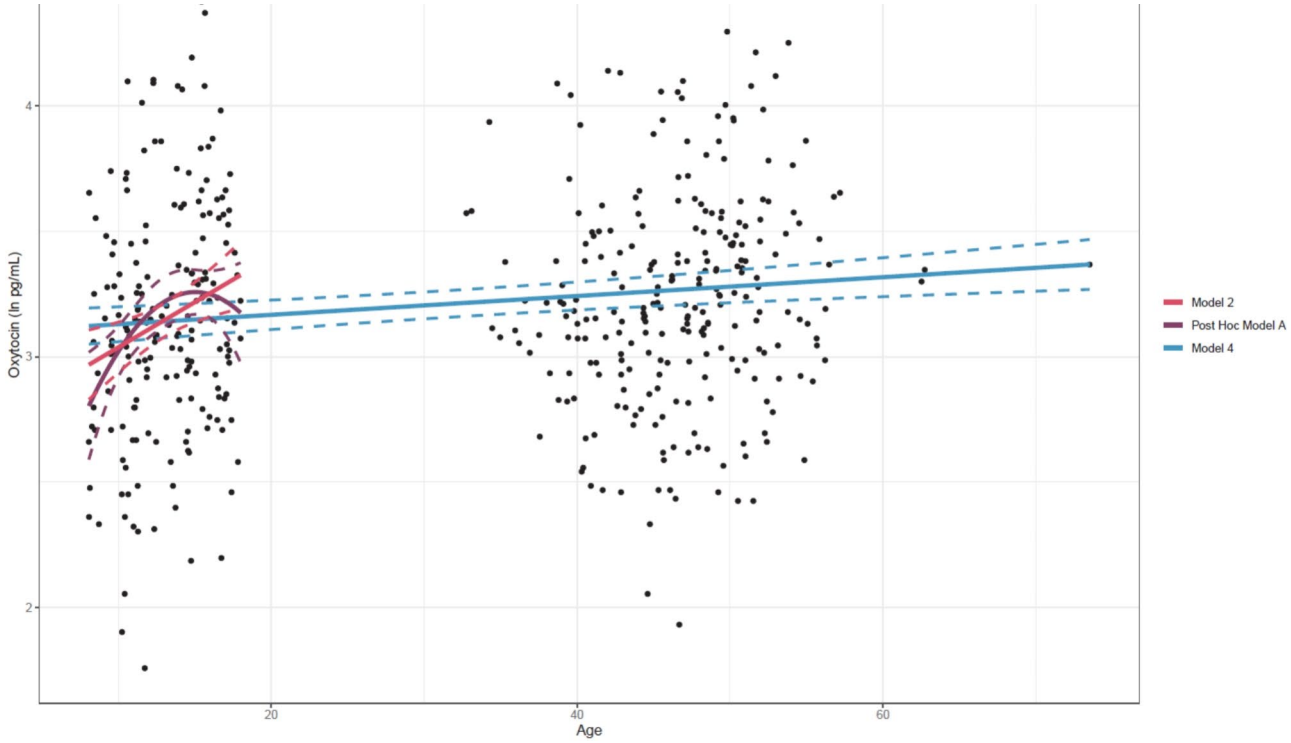

Fig. 4. The relationship between oxytocin and pubertal stage predicted by the reduced model 3 with pubertal stage. *Note*: The dashed lines represent the 95 confidence interval of the mean. Ln, natural logarithm.

(age range ≈ 33–73 years) indicated that oxytocin increases sometime after puberty, which is indicative of its coupling to sex hormones. The importance of pubertal stage could support that hormonal or behavioral changes during puberty cause important variation in oxytocin activity[13]. Our exploration with random forest analyses resembled the results from the models using quadratic age and pubertal stage suggesting that these models capture the main signal in this age range.

Our results did not indicate that sex accounts for variation in oxytocin levels in children and adolescents or adults. Previous findings on sex differences in oxytocin in adults are mixed[38–41]. The contrasting results could be due to studies investigating different ages and sex differences may only occur in some age ranges.

This study has both strengths and limitations, which should be considered when interpreting our results. Our sample is relatively large compared to prior research in the field and used frequentist and Bayesian statistics providing additional information regarding relative evidence for null hypotheses. Other studies has found an association between salivary and CSF oxytocin[26], however, whether salivary and other peripheral measures of oxytocin reflect central oxytocin concentrations under basal conditions is unclear[10,28,42]. Thus, inferences about central oxytocin activity based on peripheral measures might not be reliable. However, saliva sampling is a noninvasive and feasible method in youth. We included a clinically representative group of children and adolescents with OCD with multiple comorbidities, however comorbidity can cause variability in oxytocin[29]. We used a cross-sectional design which is a limitation when making inferences about oxytocin changes across development where a longitudinal approach would be more precise. We used no correction for multiple testing, thus statistical significance should be interpreted carefully. With the goal of transparency, we pre-registered our analysis plan. Pubertal stage was assessed without clinical examination, which decreases the accuracy[43]. Yet, pubertal stage has not previously been examined in relation to oxytocin.

Conclusion

Salivary oxytocin does not appear to be related to OCD in children and adolescents. Rather than focusing on group differences, future studies on oxytocin and OCD may focus on more specific components of OCD like family factors or family behaviors and specific features or symptom dimension of OCD that are plausible influenced by oxytocin. Oxytocin concentration fluctuations across development suggest that hormonal or behavioral changes during puberty may influence oxytocin system activity. Future studies across a wider age range and using nonlinear models may increase the understanding of oxytocin concentrations across the lifespan.

Data availability

After publication of all results from the TECTO trial, we plan to make a depersonalized data set publicly available on the EU ZENODO database.

Code availability

Analysis scripts from R are available on request to the authors.

Biological material availability

The saliva samples have been dispersed after the final analysis to follow our protocol and the biological material is therefore no longer available.

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Author contributions

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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