

Testosterone in relation to behavioral problems in pre-pubertal boys with autism spectrum disorders

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Running Headline: Testosterone and behavioral problems in ASD

Summary

Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by impairment in social communication and presence of stereotyped/restricted behaviors. Children with ASD very often demonstrate co-morbid psychiatric problems, problems known to be affected by testosterone in neurotypical populations. However, there are few reports investigating relationships between testosterone and psychiatric conditions in children with ASD. The aim of this study was to determine the relationship between plasmatic levels of testosterone and behavioral/emotional problems in pre-pubertal boys with ASD. The study sample consisted of 31 pre-pubertal boys (ages 3-10) with ASD. Parents completed the Nisonger Child Behavior Rating Form (NCBRF) to assess specific behavioral/emotional problems as observed in the previous 2 months. Plasmatic testosterone levels were determined in boys according to standardized procedures. It was found that there were positive correlations between testosterone levels and the conduct problems subscale ($p=0.034$, $r_s=0.382$) of NCBRF and also between testosterone levels and the hyperactive subscale ($p=0.025$, $r_s=0.402$) of NCBRF. Findings in this study are in line with research conducted in the neurotypical population. This is the first large study investigating testosterone-emotional/behavioral problems relating to ASD and warrants further research in this field in order to clarify the etiopathogenesis of psychiatric co-morbidities and improve their treatment.

Key words: autism spectrum disorders, testosterone, conduct problems, hyperactivity

Introduction

Autism spectrum disorders (ASD) are a set of heterogeneous neurodevelopmental conditions, characterized by early-onset difficulties in social communication and unusually restricted, repetitive behavior and interests. The worldwide prevalence is about 1% (Babinska *et al.* 2014, Lai *et al.* 2014). Children with ASD have often comorbid emotional and behavioral symptoms including irritability and aggression, hyperactivity, anxiety and depression (Gadow *et al.* 2005, Gadow *et al.* 2012, Hallett *et al.* 2013, Simonoff *et al.* 2008, Vickerstaff *et al.* 2007). Previous studies suggested that as many as three-fourths of individuals with ASD might reach the diagnostic threshold for a bona fide coexisting psychiatric disorder (Brereton *et al.* 2006), including attention-deficit/hyperactivity disorder (ADHD), anxiety disorders, affective disorders and oppositional defiant disorder (de Bruin *et al.* 2007, Green *et al.* 2000, Simonoff *et al.* 2008). These disorders may exacerbate functional impairment and core ASD features. Children with ASD very often have a unique clinical presentation of psychiatric conditions, usually accompanied by atypical symptoms (Magnuson and Constantino 2011, Simonoff *et al.* 2008), compared to neurotypical children, and it was also observed that their psychopharmacological responses may be atypical as well (Handen *et al.* 2000).

Sex differences in human behavior, personality and tendency to emotional states are well documented, and research on sex differences has found that boys/men tend to have problems that are more externalized (e.g. aggression, dominance-seeking behavior) and are less prone to internalize problems (e.g. depression, anxiety or low self-esteem) than girls/women (Feingold 1994, Kessler *et al.* 1993, Klein *et al.* 2014). It is assumed that testosterone, may play a role in the etiology of aggression (Barzman *et al.* 2013, Carre *et al.* 2011, Golubchik *et al.* 2009) and mood-related variables such as depression or self-esteem (McHenry *et al.* 2014, Parizek *et al.* 2014, Vermeersch *et al.* 2013). These studies are

conducted in neurotypical children/adults of both genders and, to our knowledge, there are just two reports employing small samples that investigated relationships between testosterone and psychiatric conditions in children with ASD (Pivovarciova *et al.* 2014, Tordjman *et al.* 1997). Moreover, neurodevelopmental disorders such as ADHD and ASD occur more often in boys than girls (Davies 2014, Lai *et al.* 2014). Thus, investigating the etiological role of testosterone in psychiatric conditions affecting children with ASD might better clarify their specific etiopathogenesis and the need for further treatment.

The purpose of this paper is to determine the relationship between plasmatic levels of testosterone and behavioral and emotional problems in pre-pubertal boys with ASD. We hypothesized that predominantly “male” behavioral problems such as conduct disorder symptoms and ADHD symptoms might positively correlate with actual levels of plasmatic testosterone in pre-pubertal boys with ASD.

Methods

Design of the study and selection of subjects

The study was approved by the Ethical Committee of the Faculty of Medicine, Comenius University (FM CU), Bratislava, Slovakia and it has been performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association. ASD children in our study were recruited after diagnostic procedures (in the Academic Research Center for Autism, Institute of Physiology, FM CU) and after confirming the diagnosis of ASD (see description of diagnostic procedures below). After one parent signs the consent form, 35 pre-pubertal boys with ASD between 3 and 10 years of age were enrolled in our study. Parents then completed the Nisonger Child Behavior Rating Form (NCBRF) and venous blood was drawn from children according to standardized procedures (see biochemical procedure below).

In order to avoid gender differences only boys were enrolled in the study. We also decided to enroll only pre-pubertal subjects (≤ 10 years old) in order to avoid large variability in sex hormone levels described in children of pubertal age (Sperling 2008).

Diagnosis of ASD and measurement of behavioral/emotional problems

The diagnosis of ASD was determined by a clinical psychologist or psychiatrist according to ICD 10 and children also underwent behavioral testing by trained specialists at the Academic Research Center for Autism, Institute of Physiology, FM CU. The diagnostic tools involved: observation of a child by the Autism Diagnostic Observation Schedule- second revision (ADOS-2) and the Autism Diagnostic Interview-Revised (ADI-R), a comprehensive interview administered to parents that provides a thorough assessment of individuals with ASD (Lord *et al.* 2000, Lord *et al.* 1994). All children enrolled to the study had to meet the criteria for ASD on both autism scales. Parents of the children with ASD completed the Nisonger Child Behavior Rating Form (NCBRF) version for parents (Aman *et al.* 1996). NCBRF is a behavior rating scale with good psychometric properties designed for children and adolescents with intellectual disabilities (Aman *et al.* 1996). This scale also has good psychometric properties and also has been widely used in clinical and research practice in populations of children and adolescents with ASD (Lecavalier *et al.* 2004). The behavior of subjects was assessed in two major areas: *social competence* and *problem behavior*. The 10 *social competence* items are distributed on two subscales: Compliant/Calm (e.g. accepted redirection, followed rules, initiated positive interactions) and Adaptive/Social (e.g. participated in group activities, shared with others, stayed on task). Items are rated on a four-point Likert scale ranging from not true (0) to completely or always true (3). The 66 *problem behavior* items are also rated on a four-point Likert scale. Raters are instructed to consider both the rate of occurrence and the degree to which the behavior was a problem over the last month. Ratings can vary from “did not occur” or “was not a problem” (0) to “occurred a lot”

or “was a serious problem” (3). There are 66 items that are distributed on six subscales: (1) Conduct Problem (e.g. explosive, easily angered, gets in physical fights, argues, violates rules, tantrums), (2) Insecure/Anxious (e.g. nervous/tense, overly anxious to please others, easily embarrassed, silent and moody) (3) Hyperactive (e.g., difficulty concentrating, easily distracted, fidgets/wiggles, overactive) (4) Self-Injury/Stereotypic (e.g., hits or slaps own head or other body parts, rocks body back and forth repetitively, self-scratching, self-biting) (5) Self-Isolated/Ritualistic (e.g., apathetic or unmotivated, has rituals, shy around others, isolates self from others), and (6) Overly Sensitive (e.g., crying, tearful episodes, easily frustrated, feelings easily hurt).

Biochemical procedure

Venous blood samples were drawn into sterile polypropylene tubes containing K2 EDTA (Sarstedt, Nümbrecht, Germany) using standardized procedures the same month of a year from 8:00 to 10:00 a.m. from all children at the Pediatric Department of Children Faculty Hospital CU in Bratislava. Whole blood samples were centrifuged for 5 min at 2000 g immediately after collection. Plasma aliquots were stored at -20°C for not longer than one month. On the day of testing, frozen samples were brought to room temperature and pipetted on to a testing plate. The ELISA assay using a commercial Testosterone ELISA kit and an Estradiol ELISA kit were used according to manufacturer's instructions (DRG Instruments GmbH, Marburg, Germany). The intra-assay coefficient of variation was 3.3% and the inter-assay coefficient of variation was 6.2%.

Statistical analysis

Statistical analyses were done using *Graph Pad Prism 5* (Graph Pad software, San Diego, USA). Correlations between hormonal levels and behavioral scores were assessed using the Spearman correlation test due to the non-parametric distribution of the sample.

Results

The aim of this study was the determination of the relationship between plasmatic levels of testosterone and behavior subscales of NCBRF in pre-pubertal boys with ASD. Positive correlation between total plasmatic testosterone levels and conduct problem subscale of NCBRF ($p=0.034$ $r_s=0.382$) was observed and a positive correlation between total testosterone and hyperactive subscale of NCBRF ($p=0.025$, $r_s=0.402$) was found. No other correlations with other social competence and problem behavior subscales of NCBRF and testosterone levels were found (see correlation matrix in Table 1. and Fig.1 a, b).

Discussion

Many children with ASD demonstrate co-morbid psychiatric problems, problems known to be affected by steroid hormones according to research done in a neurotypical population. Although there are lines of research supporting idea about testosterone involvement in the ethiopathogenesis of ASD, studies about the relationship between testosterone and psychiatric co-morbidities in the ASD population are few (Pivovarciova *et al.* 2014, Tordjman *et al.* 1997). Our study is the first large study investigating correlations between plasmatic testosterone levels and behavioral problems in prepubertal boys with ASD.

In our study it was found that there was positive correlation between total plasmatic testosterone levels and the conduct problem subscale of NCBRF. Conduct disorders and aggressive behavior have been related to increased prenatal and postnatal testosterone levels, in the neurotypical population of children (Barzman *et al.* 2013, Golubchik *et al.* 2009) and adults (Bailey and Hurd 2005, Carre *et al.* 2011, Dabbs *et al.* 1987, Montoya *et al.* 2012). Aggression is generally more often present in boys than in girls in the neurotypical population (Lansford *et al.* 2006) although gender has consistently not been associated with aggression in children with ASD (Kanne and Mazurek 2011, Murphy *et al.* 2009). To our knowledge, there

are two brief reports discussing the question of whether higher testosterone levels are related to aggressive symptoms in children with ASD, comparing them with neurotypical children and ASD children without aggression. Tordjman *et al.* (1997) measured plasma testosterone in nine patients with ASD compared to a group of neurotypical children. The nine adolescent children with ASD were divided into three groups comprised of (1) those aggressive against others, (2) those who are self-mutilating, and (3) a group that had the withdrawal characteristic of ASD. The group that exhibited aggression against others had higher testosterone levels than any of the comparison subjects. However, the other autistic patients showed normal adrenal androgen levels. Similarly, in our recent initial report (Pivovarciova *et al.* 2014), only one of the three pre-pubertal boys with ASD who engaged in a specific type of aggression had an abnormally high level of plasmatic testosterone compared to a group of neurotypical control children; the rest of the ASD patients had no significant difference in testosterone levels compared to the control group. In both of these studies higher testosterone levels and aggressive behavior in children with ASD were observed comparing to neurotypical children or other children with ASD.

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder that is characterized by a combination of severe inattention, extreme impulsiveness across the variety of domains, and hyperactivity (Tonhajzerova *et al.* 2014). In a neurotypical population, the lines of evidence support the idea that elevated androgen levels (especially prenatally) may confer increased vulnerability to ADHD (Davies 2014). ADHD is generally more often present in boys than in girls in a neurotypical population although gender has consistently not been associated with ADHD in children with ASD (Brereton *et al.* 2006, Simonoff *et al.* 2008). In our study, testosterone levels were found to be positively correlated with the hyperactive subscale of NCBRF. According to NCBRF, hyperactive subscale items (e.g. difficulty concentrating, easily distracted, fails to finish things he/she starts) are

symptoms often related to ADHD. To our knowledge, there are no studies investigating a relationship between actual testosterone levels and ADHD symptoms in ASD children. However, there are studies conducted in children with ASD that show a positive relationship between prenatal levels of testosterone and ADHD symptoms in these children (de Bruin *et al.* 2006, Romero-Martinez *et al.* 2013). Moreover, it was reported that high intrauterine testosterone levels may be partially involved in the development of both disorders: ADHD and ASD that may point to common pathways in etiopathogenesis of both disorders (Davies 2014, Romero-Martinez *et al.* 2013).

Our results need to be interpreted with regard to the limitations of the current study. This is a pilot study with a minimal number of subjects. In the future, relationships between testosterone and behavioral/emotional problems should be studied in females with ASD as well as with other age categories in order to generalize our findings (Freitag 2014). Another limitation pertains to the instrument used. Although there were significant advantages over other methods, a standardized behavior rating scale of present symptoms is limited by the range of items included. For example, there is large under-recognition of co-morbidities (e.g. anxiety and depressive symptomatology) in ASD children due to atypical symptoms and lack of specific assessment tools for co-morbidities in these individuals (Magnuson and Constantino 2011), which can contribute to the aforementioned limitation. Along the same lines, the results were dependent on the accuracy of information obtained from informants (parents). In future research, assessment of particular problem behavior in real time based on experimental design during functional behavioral analysis might be useful in order to obtain more detailed qualitative and quantitative characteristics such as function, frequency, intensity, and duration of aggressive episodes (Iwata *et al.* 1994, Mace *et al.* 1986). In future research it is necessary to study relationships between testosterone and co-morbidities in a more comprehensive way, taking into consideration complex androgen activity (15) and not

only total plasmatic testosterone levels (e.g. sensitivity of androgen receptor, activity of enzymes involved in testosterone metabolism). Moreover, it is very unlikely that testosterone solely plays a role in the etiology of co-morbidities and the potential relationship between testosterone and other hormones (cortisol, oxytocine, estradiol) might be taken into consideration.

Conclusions

Despite the limitations to the findings, the results have important implications. Psychiatric conditions are very frequent and unique in children/adults with ASD. These disorders may exacerbate functional impairment and core ASD features and may have a serious impact on children with ASD as well as their families and society. Considerable evidence exists that testosterone plays a role in the etiopathogenesis of psychiatric conditions in neurotypical populations. However, there is insufficient information about this relationship in children with ASD. Our study is the first bigger study investigating the potential relationship between testosterone and emotional/behavioral problems in ASD and warrants further research in this field in order to clarify the etiopathogenesis of psychiatric co-morbidities and improve consequent treatment and prevention (Klasen *et al.* 2014).

Acknowledgment

This study emerged as a part of the Autism research project held at the Academic Research Center for Autism (ARCA) at the Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia, supported by grants: University Science Park for Biomedicine Bratislava (ITMS 26240220087) and APVV 0254-11.

Conflict of interest:

On behalf of all authors, the corresponding author states that there is no conflict of interest.

TABLE

Tab. 1. Correlation matrix table (including correlation coefficients r_s) with the variables analyzed in groups of boys with ASD, p -values less than 0.05 were considered significant.

	Compliant/calm	Adaptive social	Conduct problems	Insecure/Anxious	Hyperactive	Self- injury/ stereotypic	Self- isolated/ritualistic	Overly sensitive
Testosterone levels (nmol/l)	-0.303 ($p=0.098$)	-0.082 ($p=0.661$)	0.383 ($p=0.034$)	0.194 ($p=0.295$)	0.402 ($p=0.025$)	0.180 ($p=0.333$)	0.061 ($p=0.746$)	0.343 ($p=0.059$)

FIGURE CAPTION:

Fig1 Correlation graphs (including correlation coefficients r_s) with the variables analyzed in pre-pubertal boys with ASD, p -values less than 0.05 were considered significant. There are total testosterone levels on axes x and scores in problem behavior subscales of NCBRF (conduct problems, hyperactive) on axes y. **A)**: Positive correlation between total plasmatic testosterone levels and **total scores on** conduct problem subscale ($p=0.034$ $r_s=0.382$) of NCBRF, **B)**: positive correlation between total plasmatic testosterone and **total scores on** hyperactive subscale of NCBRF ($p=0.025$, $r_s=0.402$).

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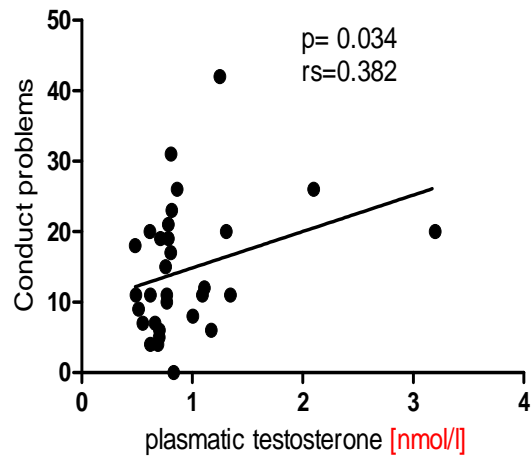
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Fig. 1.

A)



B)

