Pivotal Role of Arginine Vasopressin in the Pathophysiology of Childhood Autism and Atypical Autism

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Abstract

Neurohypophyseal hormone arginine vasopressin (AVP) and its receptors have essential roles in regulating cognition and social behaviours in mammals. This research study hypothesized that arginine vassopressin perform a role in the etiology of autism based on a verified involvement in social bonding and in the modulation of a range of socially significant behaviours in animal models. Whereas, it also has been more likely to influence male social behaviour in autism spectrum disorder as autism is 3 fold more frequent in males. The current investigation was carried out on 90 children suffering from childhood autism or atypical autism, the study included 66 males (73.3%) and 24 females (26.7%) aged 2- 7 years old. The group of atypical autism consisted of 30 children and the group of childhood autism consisted of 60 children. The childhood autism group was then divided into two subgroups according to the severity of the disease measured by CARS to mild- moderate autism group and severe autism group; each group consisted of 30 children. The present study also included 30 healthy children as the control group, they were age and sex matches. The cognitive assessment was done by using Vineland adaptive behaviour scale and the social status assessment was done by using El Shakhs socioeconomic status scale. The results show that there is a highly significant low blood levels ($P<0.0001$) between both the atypical autism group and the childhood autism group relative to the control group as regards AVP and, however there is no significant difference in its blood levels comparing the two autism groups with each others. Also the present data reveal a highly significant negative correlation ($P<0.0001$) between CARS and Vineland scores and a significant positive correlation ($P<0.01$) between CARS scores and AVP blood level. These findings suggest that AVP most probably has an important role in the pathogeneses of social behaviours core impairments in ASD.

الدور الفعال لهرمون الفاسوبرسيين فى تكوين السلوك المرضى للطفولة الذاتوية التقليدية وغير التقليدية

الطفولة الذاتوية، تبعا للدليل الدولى لتصنيف الأمراض الذى تصدرة هيئة الصحة العالمية فى إصدارة العاشر هو خلل معقد بالجهاز العصبى المركزى يبدأ فى الطفولة المبكرة ويتميز بثلاث صفات جوهرية هي مشكلة فى التفاعل مع المجتمع، وخلل فى التواصل اللفظى وغير اللفظي، ونمط ينكرر من التصرفات مع اهتمامات ضيقة ومقيدة وتظهر أعراض وعلامات الطفولة الذاتوية فى السنوات الثلاث الأولى من عمر الطفل ويستمر طوال عمره ولذلك يسبب خلل فى الحياة العائلية والخاصة للأشخاص المصابين به. ويعانى ٧٥– ٨٠% من الأطفال المصابين به من نقص فى قدراتهم العقلية. والطفولة الذاتوية غير التقليدية وتشخص بظهور أعراض غير تقليدية من حيث النوعية أو درجة الشدة أو تأخرفى بدء حدوث الأعراض أو الأثنين معا.

يلعب هرمون الفاسوبرسين دورا مهما فى التفكير العقلى والتفاعل الأجتماعى عند الأنسان وهو مهم جدا فى تحديد السلوك الإجتماعى عند الذكور الذين يزيد نسبة الإصابة بالطفولة الذاتوية عندهم بثلاثة أمثال الأناث. وشملت هذة الدراسة ٩٠ طفلا من الجنسين، ٦٦ من الذكور و٢٤ من الإناث وأعمارهم تتراوح بين ٢ و٧ سنوات قسموا الى مجموعتين، المجموعة المصابة بالطفولة الذاتوية غير التقليدية وتشمل ٣٠ طفلا والمجموعة المصابة بالطفولة الذاتوية وتشمل ٦٠ طفلا وهذة المجموعة قسمت ايضا إلى مجموعتين من ٣٠ طفلا حسب تقييم مستوبات الذاتوية لدى الأطفال لأسكوبلر إلى مجموعة شديدة المرض ومجموعة متوسطة الشدة والمجموعة الضابطة وتشمل ٣٠ طفلا من بين الاصحاء لهم نفس مستوى العمر والجنس والمستوى الإجتماعي. و تم تقييم الأداء الادراكى باستخدام مقياس فينلاند للسلوك التلازمى والتقبيم النفسى باستخدام مقياس مستويات الذاتوية لدى الأطفال لأسكوبلر والتقييم المستوى الأجتماعى والأقتصادى لعبدالعزيز الشخص. وأظهرت نتائج البحث وجود انخفاض ملحوظ فى مستوى هرمون الفاسوبرسيين فى الدم لدى جميع مجموعات الدراسة مقارنة بالمجموعة الضابطة وأظهر البحث ايضا وجود علاقة ملحوظة بين اختبار الكارز واختبار الفيلاند. ويستخلص البحث أن الخلل فى هرمون الفاسوبرسيين قد يكون من العوامل المسببة لزبادة الطفولة الذاتوية عند الأولاد.

Introduction:

Autism spectrum disorders (ASD) are varied class of neurodevelopmental disorders that contain core qualitative neurodevelopmental impairments in reciprocal social interaction, communication, and language, together with restricted, repetitive, and stereotype behaviours, interests, and activities (Shattuck, 2006), with an onset recognized prior to 3 years of age. ASD includes not only classical autism (autistic disorder) or childhood autism but also Asperger's disorder (high functioning) and pervasive developmental disorder not otherwise specified (PDDNOS) or atypical autism (Johnson et.al., 2007).

Autism spectrum disorders have obtained an elevating level of attention from researchers, clinicians, the media and general community over the past several decades (Minshawi et.al., 2014). The definition of autism has evolved from the very narrow view of primitive infantile autism to an expanded and more detailed description as delineated in the diagnostic and statistical manual (DSM- IV). However, as more clinical investigations and basic science research have become increasingly available over the past many years, it has become obvious that autism includes a continuum of severity and symptoms, and as an outcome, the terminology "Autism Spectrum Disorders" (ASD) has come into common usage (Bauman, 2010).

Autism has become one of the most widespread childhood epidemics in recorded history. Apart from some infectious diseases epidemics of the past, no other critical condition has ever altered so many of our children (Sears, 2010). The incidence of autism has elevated rapidly since 1970s, when prevalence evaluation suggested that 1 in 2000 children were affected. Autism rates had increased from 1 in 200 by the late 1990 to 1 in 86 by 2007 and now to 1 in 50, as published by the Centers for Disease Control and Prevention for 2011- 2012 (CDC, 2013). Atypical autism (PDD- NOS) emerges to be at least as common as childhood autism (Volkmar et.al., 2007).

Arginine vasopressin (AVP) and oxytocin (OT) are synthesized principally in magnocellular neurosecretory neurons situated in the paraventricular and supra- optic nuclei in the hypothalamus. It is now well recognized that both peptide hormones are first synthesized as large precursor proteins that are then converted by a range of post- translational processing steps to produce the biological active hormones (Altstein and Gainer, 1988).

AVP modulates male social behaviour not only via higher expression in males but also in steroid sensitive brain sexual dimorphisms in AVP neurons (Bales et.al., 2007). Investigations have mainly focused on males and shown that AVP- dependent social behaviours consist of social recognition and interaction, inter- male aggression, pair bonding and paternal protection (Ferris, 2005). Pharmacological experiments in rodents have revealed a function for vasopressin in learning and memory, aggression and associative behaviours. Increases in AVP are connected with stressful or defensive circumstances frequently in males (Tansey et.al., 2011). We now have to consider AVP an additional potent regulator of complex social behaviours in females, in particular of the fine tuned maintenance of maternal behaviour. Also, they are likely to have an important role in the healthy behaviour, especially emotional and social development of the offspring (Bosch and Neumann, 2008).

The arginine vasopressin (AVP) has been hypothesized to perform a role in the etiology of autism based on a verified involvement in social bonding and in the modulation of a range of socially significant behaviours in animal models (Tansey et.al., 2011). Variations in the vasopressin receptor have also appeared as candidate targets to clarify autism, because vasopressin regularly operates in opposition to oxytocin, an overactive vasopressin system could produce lots of the same effects that a disrupted oxytocin system triggers (Wacker and Ludwig, 2012).

Aim Of The Study:

1. To measure AVP blood levels in autistic children and whether blood AVP concentration differ between atypical autism and childhood autism.
2. To identify the ability of using AVP blood levels as an effective neurochemical marker to help in diagnosing childhood autism and atypical autism.

Subjects And Methods

Subjects:

The present study was carried out during January 2014 to June 2016 on 90 children suffering from childhood autism or atypical autism, the study included 66 males (73.3%) and 24 females (26.7%) aged 2- 7 years old. All the diagnosed children were regularly attending the outpatient clinic of center for care of children with special needs; Institute of Postgraduate Childhood Studies, Ain Shams University, Egypt. Autistic children were divided into two main groups. The atypical autism group consisted of 30 children diagnosed with atypical autism and the childhood autism group consisted of 60 children diagnosed with childhood autism. The childhood autism group then divided into two subgroups according to the severity of the disease measured by CARS to (the mild- moderate autism group) and (the severe autism group) each group consisted of 30 children. The study also included 30 healthy children as the control group, they were age and sex matches and they are relatives of children going to pediatric surgery clinics, Ain Shams Faculty of Medicine.

1. Inclusion Criteria:
   a. Cases diagnosed with either childhood autism or atypical autism
   b. Age (2- 7) years old
   c. Medication free for at least one month
2. Exclusion Criteria:
   a. Cases diagnosed with any syndromes associated with autistic features.
   b. Cases diagnosed with other pervasive developmental disorders, Rett's disorder, Asperger disorder and Childhood Disintegrative disorder.
   c. Cases with Cerebral Palsy, CNS diseases and sensory impairments.
   d. Cases with childhood autism or atypical autism that have epilepsy

e. Cases with childhood autism or atypical autism associated with autoimmune diseases or any inflammatory medical conditions.

Ethical Consent:

Ethical approval was obtained from the Ethical Committee of National Research Center and the Ethical Committee of the Institute of Postgraduate Childhood Studies. Written informed consent was obtained from the parents after explanation of the aim of the study and its possible benefits of identifying AVP blood level associated with their autistic children and other children who have the same conditions.

Methods:

All children included in the study (subjects and control) were subjected to the following:

1. Full Psychiatric History and Complete Psychiatric Examination: Depending on the psychiatric sheet used by the outpatient clinic of center for care of children with special needs; Institute of Postgraduate Childhood Studies, Ain Shams University. Each child from the study groups received a confirmed diagnosis according to World Health ICD- 10 criteria.
2. Full Medical History and Clinical Examination: With particular emphasis on complete neurological examination, and any immune activation such as elevated temperature, infectious or inflammatory diseases, and EEG was done to exclude the presence of Epilepsy.
3. Childhood Autism Rating Scale Second Edition (CARS- 2): The scale was done by a professional Psychologist from Psychiatric Department, School of Medicine, Ain Shams University; it is subjectively rate 15 items, (relationship to people, imitation, emotional response, body use, object use, adaptation to change, visual response, listening response, taste- smell- touch response and use, fear and nervousness, verbal communication, nonverbal communication, activity level, level of consistency of intellectual response, general impressions). This second edition of CARS expands the test's clinical value, making it more responsive to individuals on the "high functioning" end of autism spectrum disorders. The clinician rates the individual on each item, using a 4- point rating scale. Ratings are based on frequency of the behavior in question, its intensity, peculiarity, and duration (Schopler et.al., 2010).
4. Vineland Adaptive Behaviour Scale (VABS) Second Edition: The scale was done by a professional Psychologist from Psychiatric Department, School of Medicine, Ain Shams University; it assesses adaptive behavior in four domains: Communication, Daily Living Skills, Socialization, and Motor Skills. It also provides a composite score that summarizes the individual's performance across all four domains (Sparrow and Crchetti, 1984).
5. Assessment Of Socioeconomic Status: Socioeconomic status for all the patients and the control children were assessed by using El Shakhs Socioeconomic Status Scale in which five primary categories were estimated and scored, education and employment of both parents and the family income and then the socioeconomic status are divided into 7 levels. The primary assumption of the scale is that the higher levels of education and employment indicate higher levels of socioeconomic wellbeing, and higher levels of poverty with low income, low education and employments indicate lower levels of socioeconomic wellbeing (Al Shakhs, 2006).
6. Determination of ArgininVassopressin (AVP) plasma level: VP plasma level was measured using an enzyme- linked immunosorbent assay (ELISA) kit (Glory Science Co., Ltd, USA) according to the manufactured procedure.
7. Principle Of The Test: Quantitative measurement of VP level in plasma sample depends on adopting purified human VP to coat microtiter plate, make solid- phase antibody, then adding VP to wells. Combining VP antibody with labeled HRP forms antibody- antigen- enzyme- antibody complex, after washing completely, TMB substrate solution is added, TMB substrate becomes blue colour at HRP enzyme- catalyzed then, the reaction is terminated by the addition of a stop solution and the colour change is measured at a wavelength of 450nm. The concentration of VP in the samples is then determined by comparing the O. D. of the samples to the standard curve.

Statistical Analysis:

Data obtained from the research was organized, tabulated and analyzed through IBM personal computer. Statistical analysis was performed using the SPSS statistical package software for Windows version 20 (SPSS Inc., Chicago, Illinois, USA). Parametric variables among the controls and the studied patients groups were analyzed using two tailed unpaired t- test. Qualitative variables were assessed by Chi-square test. A P- value< 0.05 was considered significant difference and P<0.005 was considered highly significant difference (SPSS, Statistical package for social science, 1999).

Results:

Table (1) Comparison between the control group and the autism groups (atypical autism and childhood autism) according to their scores in the psychological tests.

Groups Markers	Control	Autistic Groups			
		Atypical Autism		Childhood Autism	
			P- value		P- Value
Number (Male/Female)	30 (15/ 15)	30 (21/ 9)		60 (45/ 15)	
Mean Age/ Year	6.6±0.7	5.11±1.5		6.1±2.1	
Mean CARS	20.1±1.15	29.9±2.12a	0.00	36.9±3.6ab	a& b: 0.00
Mean Vineland Score	87.0±5.36	63.2±14.26a	0.00	43.4±15.7ab	a& b: 0.00
Mean Social Status	39.1±6.1	45.3±11.6a	0.014	42.8±10.0	0.086

Data were expressed as means ± standard deviation (SD)

a: Significance at P>0.05 vs control. b: Significance at P>0.05 vs Atypical Autism group.

Table (1) shows a highly significant difference between the autistic groups (atypical autism and childhood autism groups) and the control, and also between the childhood autism group and the atypical autism group (P< 0.0001) in respect to both CARS and Vineland scores. Regarding the social status scores the data also show a significant difference between the atypical autism group and the control group (P= 0.014) and no significant difference between the childhood autism group compared to both atypical autism and the control groups.

Table (2): Comparison between the atypical autism group and the childhood autism groups (mild- moderate autism and severe autism) according to their scores in the psychological tests.

Groups Markers	Autistic Group				
	Atypical Autism	Childhood Autism			
		Mild-Moderate	P- Value	Severe	P- Value
Number (Male/Female)	30 (21/ 9)	30 (23/ 7)		30 (22/ 8)	
Age/Year	5.11±1.5	5.7±2.0		6.4±2.2	
CARS	29.9±2.12	33.83±1.51a	0.00	39.93±2.36ab	a& b: 0.00
Vineland Score	63.23±14.25	51.4±15.65a	0.00	35.53±11.22ab	a& b: 0.00
Social Status	45.33±11.65	44.3±10.74	0.679	41.4±9.19	0.117

Data were expressed as means ± standard deviation (SD), a: significance at P>0.05 vs Atypical Autism group. b: significance at P>0.05 vs mild- moderate Autism group.

Table (2) shows a highly significant difference between the mild-moderate autism group and the atypical autism group (P< 0.0001) and also show a highly significant difference P< 0.0001) between severe autism group and both atypical autism and mild- moderate autism groups regarding to both CARS and Vineland scores. There is also no significant difference between all autistic groups regarding social status scores.

Table (3) Comparison between the autism groups (atypical autism and childhood autism) and the control group regarding to AVP blood levels.

Groups Markers	Control	Autistic Group			
		Atypical Autism		Childhood Autism	
			P- Value		P- Value
Number (Male/Female)	30 (15/ 15)	30 (21/ 9)		60 (45/ 15)	
Age/Year	6.6±0.7	5.11±1.5		6.1±2.1	
AVP (ng/L)	83.03±11.21	41.9±14.02a	0.00	43.5±16.2a	0.00

Data were expressed as means ± standard deviation (SD), a: significance at P>0.05 vs control. b: significance at P>0.05 vs Atypical Autism group.

Table (3) shows that there is a highly significant difference (P<0.0001) between both atypical autism and childhood autism groups relative to the control group as regards AVP. While, there is no significant difference in AVP blood levels comparing the two autism groups with each others.

Table (4) Comparison between the atypical autism group and the childhood autism groups (mild- moderate autism and severe autism) regarding to the AVP blood levels

Groups Markers	Autistic Group				
	Atypical Autism	Childhood Autism			
		Mild- Moderate	P- Value	Severe	P- Value
Number (Male/Female)	30(21/ 9)	30(23/ 7)		30(22/ 8)	
Age/Year	5.11±1.5	5.7±2.0		6.4±2.2	
AVP (ng/L)	41.9±14.02	43.0±16.33	0.789	44.1±16.5	0.572

Data are expressed as means ± standard deviation (SD). a: significance at P>0.05 vs Atypical Autism group. b: significance at P>0.05 vs mild- moderate Autism group.

Table (4) shows that there is no significant different between all autism groups as regards both AVP.

Table (5) Spearman correlation between the measured psychological tests and AVP blood levels in the atypical autism group.

	CARS		Vineland		Social Status	
	r	P- value	r	P- value	r	P- value
CARS	1.0	-	- 0.026	0.89	- 0.474	0.008
Vineland Score	- 0.026	0.89			0.354	0.055
Social Status	- 0.474	0.008	0.354	0.055		
Vp Ng/L	0.124	0.513	- 0.012	0.95	- 0.144	0.447

R: Correlation Coefficient

Table (5) and Figure (1) show significant negative correlation between CARS and Social Status scores (P= 0.008).

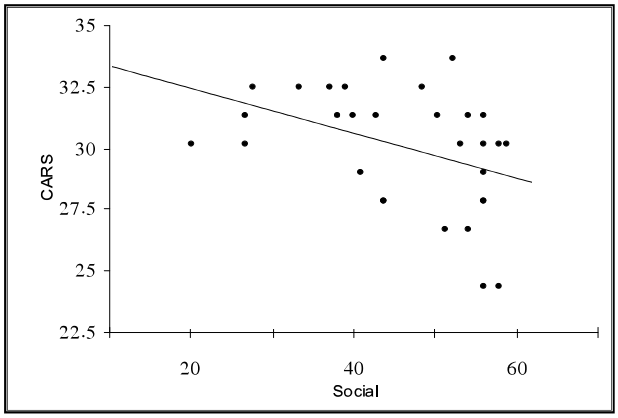

Figure (1) Correlation between the measured psychological tests and AVP blood levels in the atypical autism group.

Table (6) Spearman correlation between the measured psychological tests and AVP blood levels in the childhood autism group

	CARS		Vineland Score		Social Status	
	r	P- value	r	P- value	r	P- value
CARS	1.0	-	- 0.568**	0.00	- 0.25	0.054
Vineland Score	- 0.568**	0.00			0.285*	0.027
Social Status	- 0.25	0.054	0.285*	0.027		
Vp Ng/L	0.323*	0.012	- 0.187	0.152	- 0.131	0.317

R: Correlation Coefficient

Table (6) and Figure (2a, b) show a highly significant negative correlation (P<0.0001) between CARS and Vineland scores and a significant positive correlation (P<0.01) between CARS scores and VP level.

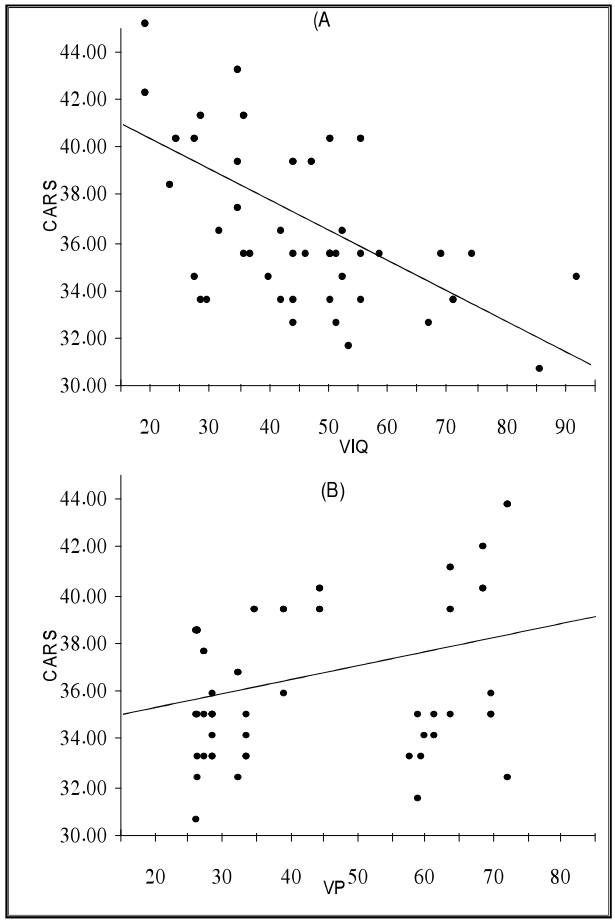

Figure (2a, b) Correlation between the measured psychological tests and AVP blood levels in the childhood Autism group, Correlation between CARs scores and Vinland scores, Correlation between CARs scores and AVP blood levels.

Discussion:

Results of the present study boost previous studies indicating that CARS total score changes significantly by diagnostic group, with patients diagnosed with childhood autism having significantly higher (P< 0.0001) total CARS scores than individuals with atypical autism, who both in turn had significantly high (P< 0.0001) scores than those with healthy control. The clinically significant differences reported in CARS total scores among the diagnostic groups are congruent with the results of Chlebowski et.al. (2010) who support the utilization of the CARS as a stable measure of autism severity.

The current results also showed that there are a significantly lower scores in Vinland Adaptive Behaviour Scale with the elevation of the disease severity, as the current study found a greatly significant (P<0.0001) low scores comparing the childhood autism group with the atypical autism group and comparing both of them with the control group. Also, a high significant (P<0.0001) low scores has been detected comparing the severe autism group with the mild- moderate autism group and comparing both to the atypical autism group. These results are in agreement with Paul et.al. (2004) who reported that individuals with PDD- NOS have a higher score than those with autism in Vinelnad Adaptive Behaviour Scale. This is also in respect with the results in the current study, as a significantly (P<0.02) negative correlation between CARS and Vinland IQ in childhood autism group has been recorded. Also, the study of Perry et.al. (2005) mentioned that there is a moderate negative correlation of CARS scores and developmental levels (both cognitive and adaptive).

The present results revealed that all patients from all autistic groups, atypical autism (46.7%), mild- moderate autism (56.7%), and the severe autism groups (40%) are of high middle social status (49- 58). These findings are in concert with the study of Durkin et.al. (2010) using area-based standards of socioeconomic status which constitute that the prevalence of autism increased with SES in a dose- response aspect.

In the current study, mean plasma levels of AVP were highly significantly decreased (P< 0.0001) in both atypical and childhood autism groups compared with healthy control group, and there were no significant change of AVP levels comparing all autistic groups with each others. These results are in accordance with two previous studies; Al-Ayadhi (2005) who reported that there is a statistically significantly lower plasma level of vasopressin in autistic children as compared to the controls and there is no significant correlation between the degree of autism, or the age of the affected children and plasma levels of vasopressin, and El-Masry et.al. (2010) who also mentioned that, vasopressin plasma levels are significantly lower in autistic children as compared to the control group and there are no significant correlations between the degree of autism and levels of vasopressin.

The significant decrease in plasma AVP with all our autistic study groups compared to the control could be attributed to the change of other neurochemicals which are well known to have a role in the pathophysiology of autism as GABA neurons which exert inhibitory effects on neurons that are directly activated by AVP receptors (Huber et.al., 2005). This is also consistent to the current results in the same study which recorded a significantly positive correlation between AVP levels and CARS scores in childhood autism group.

It was suggested that AVP may have a role in the symptoms of autistic disorders and both human and animal studies suggested a key role for AVP in the regulation of male social behaviours (Ferris, 2005). One position advanced to demonstrate the marked male prevalence of ASD which is that the female neuroendocrine system presents "protection" against autistic traits. According to this view; the processes mediated the lack of reliance on AVP making being a girl a protective factor against manifesting autistic- like behaviour. (Carter, 2007). AVP levels showed positive correlation with repetitive behaviour symptoms in girls with ASD, but were negatively associated with them at a trend level (prior to adjustment for many comparisons) in boys with autism. There was a significant variation between the association of AVP and self- injurious behaviour scores in boys and girls with ASD (Miller et.al., 2013).

Conclusions:

1. AVP low blood levels in the present study suggest that AVP most probably appears to be involved in the pathophysiology of ASD especially the social behaviours impairments which are one of the core symptoms in ASD.
2. The insignificant different in AVP blood levels between the childhood autism group and the atypical autism group may confirm that why social impairments are the same core deficits in both atypical autism and childhood autism.

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