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in the hyperactive/impulsive and inattentive compared to the combined ADHD subtype (p= 0.010). DAT1 is one of the most studied genes in the etiology of ADHD. It regulated dopamine transport in the synaptic space through dopamine reuptake into the presynaptic neuron. Dopaminergic system imbalance has been implicated in the pathogenesis of many neurological disorders, including ADHD, schizophrenia, bipolar disorder, and Parkinsonism (Roman et.al, 2001).

Our results agree with Agudelo et.al. (2015), who reported no association between DAT1 polymorphism with ADHD (p= 0.85) in 73 children aged between 6 and 15. Similarly, other investigators did not support an association between DAT1 polymorphism and ADHD (Holmes et.al, 2000; Muglia et.al, 2002; Swanson et.al, 2000; Todd et.al, 2001; Wiguna et.al, 2017).

On the contrary, Cornish et.al. showed a significant association between DAT10/ 10 and ADHD behavior of children in a general population study (Cornish et.al, 2005). More recently, Sery et.al. (2015) found a significant relationship between ADHD and DAT1 polymorphism (p< 0.01), where the 9/ 9 genotype seemed to reduce the risk of ADHD by about 0.4- fold (Šerý et.al, 2015). Other studies confirmed this linkage between DAT1 polymorphism and ADHD in different populations (Barr et.al, 2001; Curran et.al, 2001; Hong et.al, 2018; Stanley et.al, 2017).

Moharrari and Barabadian (2015) reported that patients with ADHD with the 10R/10R VNTR allele of DAT1 display a lower response to ADHD treatment (Moharrari and Barabadian, 2015). In addition, carriers of the 10R/10R VNTR genotype of DAT1 have been reported to have higher levels of ADHD symptoms and more serious inattention (Cornish et.al, 2005; Li and Lee, 2012). Hong et.al. (2018) observed that the 10R/10R genotype is significantly associated with ADHD (OR 1.98, 95% CI 1.080- 3.619, p= 0.025) (Hong et.al, 2018).

Two meta- analyses pointed to a modest but significant role of the 10- repeat allele of VNTR polymorphism in the DAT1 gene in ADHD. The pooled results of 18 published transmission disequilibrium test studies found that the 10- repeat allele has a small but significant role in the genetic susceptibility of ADHD (Yang et.al, 2007). Similarly, another meta- analysis found a significant but modest association between ADHD and the 10- repeat allele (OR= 1.10, 95%CI: 1.03- 1.17) (Gizer et.al, 2009).

A recent meta- analysis confirmed the presence of a weak association between the 10- repeat allele of the DAT1 3′- UTR VNTR gene and ADHD in children and adolescents (OR 1.105, 95% CI: 1.020- 1.197). However, the analysis was accompanied by high heterogeneity (Grünblatt et.al, 2019).

The DAT 10/ 10 allele correlates with increased dopamine concentrations in CSF (Horn et.al, 2013) and therefore indicates the involvement of dopamine in the neurobiology of this disorder. DAT 10/ 10 has been the initial susceptibility genotype found to relate to ADHD in family transmission studies (Cook et.al. 1995).

It is apparent in the current study that consanguinity was common in the ADHD group (36.7%), but it was also found in 26.7% of the Control group (p= 0.239). Another study from Egypt demonstrated that consanguinity had a nearly 1.5- fold increased chance of leading to ADHD (Farahat et.al, 2014). A study from Qatar showed a significant relationship between ADHD symptoms and consanguineous parents in univariate but not multivariate analysis (Bener et.al, 2008). Another Jordanian study found that the prevalence of ADHD was 34.8% among consanguineous families and the inattentive subtype was more common than others (Al Azzam et.al, 2017).

In the current study, cesarean delivery was significantly more common in the ADHD group (p= 0.003). It+ has been hypothesized that birth by cesarean section (CS) may affect psychological development in children through a variety of mechanisms, including exposure to altered microbiota (Mueller et.al, 2015) and inadequate priming of the stress response (Cho and Norman, 2013). Other studies reported inconsistent and limited results on the association between cesarean delivery and the risk of ADHD (Ketzer et.al, 2012; Silva et.al, 2014). A recent systematic review of 61 studies found that offspring born via CS had increased odds of ADHD (OR: 1.17; 95% CI, 1.07- 1.26) and autism spectrum disorders (OR: 1.33; 95% CI: 1.25- 1.41) (Zhang et.al, 2019).

According to the current international guidelines, diagnosing ADHD requires a clinical examination and reference questionnaires such as the Conners' Parent Rating Scale. We used an Arabic version of the 48- item version (CPRS- 48) introduced by Goyette et.al. (1978). According to Conners' rating scale, 49 patients (81.7%) had scores within the clinical range of the 10- item hyperactivity index (HI) subscale. This finding supports the value of HI as an overall measure of psychopathology. The HI index is used as a single scale, the Conners Abbreviated Symptom Questionnaire- ASQ) (Conners et.al, 1998).

In the current study, the aim of using CBCL was to screen for comorbid psychopathology in children with ADHD. The main finding of DSM- Oriented scores in ADHD was clinical range scores of conduct problems in 61.7% of the patients. Also, affective and oppositional defiant problems were observed in nearly half of the patients. Finding comorbid psychopathology has important implications for clinical decisions affecting these patients' management and therapeutic decisions.

Conclusion:

Due to the limited number of patients involved in this study, further studies with larger sample size are required.

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In fig. (1) lanes 1- 4 show 10R/10R genotype (480 bp), M: 50bp marker, lane 5: shows 9R/ 9R genotype (440 bp), lane 6- 8 show 10R/ 9R genotype.

Limitations:

The Covid-19 Pandemic has caused delay in the planned time to finish this work due to closure of the outpatients clinic for almost 6 months and a significant decrease in the flow of patients throughout the whole period of the pandemic.

Statistical Analysis:

Statistical analysis was done using IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi- square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was made using independent sample t- test. Comparison between 3 groups was done using ANOVA test. A p- value< 0.05 was considered significant.

Results:

The study included two groups, the ADHD group (n= 60) and a Control Group (n= 60). Table (1) shows that the two groups were comparable in baseline characteristics except for the cesarean delivery rate, which was significantly higher in the ADHD group (p= 0.003).

Table (1) Baseline characteristics of the two studied groups

		ADHD Group (N= 60)	Control Group (N= 60)	P- Value
Age (Years)		8.96±2.69	9.59 ± 2.79	0.209*
Sex (male/ female)		8/ 52	11/ 49	0.453§
Consanguinity		22 (36.7%)	16 (26.7%)	0.239§
Mode Of Delivery	Vaginal	40 (66.7%)	54 (90.0%)	0.003§
	Cesarean	20 (33.3%)	6 (10.0%)	

Data are presented as mean±SD, or number (%).
* Independent T- Test, §Chi- Square Test, p- value <0.05: Significant

According to the DSM- 5, 50 patients (83.3%) had combined ADHD, while 6 (10.0%) had predominantly hyperactive- impulsive and 4 (6.7%) had predominantly inattentive type. Genotype analysis of DAT1 VNTR between the ADHD and control groups was close to significance (p= 0.104). The 10R/ 10R genotype was the predominant one in the control group (58.3%) Table (2).

Table (2) Genotype frequency of DAT1 VNTR in ADHD and control groups

Dat1 Polymorphism	ADHD Group (N= 60)	Control Group (N= 60)	P- Value*
9R/ 9R	6 (10.0%)	6 (10.0%)	0.104
9R/ 10R	30 (50.0%)	19 (31.7%)	
10R/ 10R	24 (40.0%)	35 (58.3%)	

Data are presented as number (%), * Chi- Square Test

Among the ADHD group, 35 patients had a history of perinatal problems, and the most frequent problem was obstructed labor and fetal distress (n= 6, 10% for each). Conners' rating scale showed that 49 patients (81.7%) scored within the clinical range of the 10- item hyperactivity index (HI) subscale. Also, 38 patients (63.3%) had clinical range scores of learning problems subscale. On the contrary, the psychosomatic and anxiety subscales showed normal ranges in most cases Table (3). The CBCL scores showed that 44 patients (73.3%) scored within the clinical range of the ADHD subscale. Also, 61.7% of the patients had clinical range scores of the conduct problems subscale Table (4).

Table (3) Conner's scores of the ADHD group

Conners'	Mean±SD	Normal Range	Border- Line	Clinical Range
Conduct Disorder	61.08±8.68	32 (53.3%)	12 (20.0%)	16 (26.7%)
Learning Problems	67.38±9.17	11 (18.3%)	11 (18.3%)	38 (63.3%)
Psychosomatic	50.17±11.07	52 (86.7%)	2 (3.3%)	6 (10.0%)
Impulsive- Hyperactive	68.07 ± 8.32	7 (11.7%)	27 (45.0%)	26 (43.3%)
Anxiety	56.62±9.93	41 (68.3%)	7 (11.7%)	12 (20.0%)
10- Item Hyperactivity Index	72.62±9.81	5 (8.3%)	6 (10.0%)	49 (81.7%)

Data are presented as mean±SD, or number (%).

Table (4) DSM- Oriented scores of the Child Behavior Checklist of the ADHD group

DSM Scales CBCL	Mean±SD	Normal Range	Border- Line	Clinical Range
Affective Problems	67.53±9.07	18 (30.0%)	16 (26.7%)	26 (43.3%)
Anxiety Problems	66.20±7.02	19 (31.7%)	16 (26.7%)	25 (41.7%)
Somatic Problems	55.98±7.25	51 (85.0%)	5 (8.3%)	4 (6.7%)
ADHD	72.22±6.16	5 (8.3%)	11 (18.3%)	44 (73.3%)
Oppositional Defiant Problems	67.77±7.99	21 (35.0%)	10 (16.7%)	29 (48.3%)
Conduct Problems	71.57±8.84	14 (23.3%)	9 (15.0%)	37 (61.7%)

Data are presented as mean±SD, or number (%).

Table (5) Relation of DAT1 polymorphism with age, sex, consanguinity, and type of disease of the ADHD group

		Dat1 Polymorphism		P- Value
		10r/10r (N= 24)	Non 10R/10R (N= 36)	
Age (Years)		9.9±3.0	8.4±2.3	0.037*
Sex	Female	5 (20.8%)	3 (8.3%)	0.167§
	Male	19 (79.2%)	33 (91.7%)	
Consanguinity	Negative	16 (66.7%)	22 (61.1%)	0.662§
	Positive	8 (33.3%)	14 (38.9%)	
ADHD Subtype	Hyperactive/Impulsive Or Inattentive	8 (33.3%)	2 (5.6%)	0.101§
	Combined	16 (66.7%)	34 (94.4%)	

Data are presented as mean±SD, or number (%), * Independent T- Test
§Chi- Square Test, p- value <0.05: Significant

For statistical purposes, the 10R/10R genotype was compared with the other two genotypes Table (5). DAT1 polymorphism was not related to sex or consanguinity. The 10R/10R genotype was less common in the combined ADHD subtype than the hyperactive/impulsive and inattentive (p= 0.010).

Discussion:

ADHD has a high heritability rate between 70% and 80% (Faraone et.al, 2005), indicating a significant contribution of genetic variation to disease development. Genome- wide association studies (GWAS) identified 12 significant risk loci accounting for only 22% of the heritability (Posner et.al, 2020). Some copy number variants and accumulation of larger deletions and duplications influencing gene transcription are commonly found in ADHD patients (Williams et.al, 2012). Varientss of DAT1, DBH, DRD4, and DRD5 genes were critical genetic factors in the etiology of ADHD (Agudelo et.al, 2015).

This study demonstrated no significant difference between the ADHD and control groups (p= 0.104) in DAT1 VNTR. The 10R/10R genotype was the predominant one in the control group (58.3%) and was found in 40% of the ADHD children. The 10R/10R genotype was more common

Introduction:

Attention deficit hyperactivity disorder (ADHD) is one of the most common neuropsychiatric problems, with a prevalence varying from 2.2 to 17.8% (Sharma and Couture, 2014); (Thomas et.al, 2015).

The subjective nature of the diagnostic criteria may lead to misdiagnosis with other neurodevelopmental disorders (Daley, 2004). Therefore, objective markers of the disease are required to validate diagnosis, prognosis and assessment of response to pharmacological interventions. Genetic and biochemical markers have been suggested to help diagnose ADHD in childhood (Bonvicini et.al, 2018).

The DAT gene (DAT1, SLC6A3) is one of the most studied vulnerability genes for ADHD. DAT1 gene is located on chromosome 5p15.3 and contains a 40 bp variable number tandem repeat (VNTR) polymorphism in the 3'- untranslated region. The 9- repeat and the 10- repeat alleles are the most frequent (Banaschewski et.al, 2010). Many studies supported the positive association of the 10/ 10 genotype ADHD. Yet, many other studies provide negative results. A recent meta- analysis involving 61 studies reported a significant association between 10- repeat alleles and ADHD in children (OR 1.1050 95% CI 1.0203- 1.1968, p= 0.0128) (Grünblatt et.al, 2019). However, due to the high study heterogeneity, with some studies not reaching high quality, the authors recommended further analysis to achieve a robust conclusion. Therefore, this study aimed to test the possible association between the 40- bp VNTR in the 3′- UTR region of DAT1 and ADHD disease in a sample of Egyptian children.

Methodology:

The study included 60 patients with ADHD and 60 matched normal control subjects with comparable age and sex. The patients were recruited from the outpatient clinic of the Clinical Genetics Department, the National Research Center. They were selected from both sexes, ages 6 to 17, diagnosed with ADHD. The diagnosis was based on clinical phenotyping fulfilling the criteria of DSM- 5 (Vahia, 2013). The control group was also assessed with the DSM- 5 checklist and screened for cognitive disability.

Individuals with intellectual disabilities (according to the Draw a Person Test) were excluded from the study.

This study fulfills the ethical principles for medical research on human subjects according to the Helsinki Declaration of 1964 and its following revisions. The study protocol was assessed and approved by scientific ethical committee of the faculty of postgraduate childhood studies and the Medical Research Ethics Committee of the NRC. All parents of participating children (cases and controls) signed informed consent forms allowing them to participate and authorizing researchers to use their DNA samples after an explanation of the aim and possible risks of the study.

All patients were subjected to full history taking, physical examination (including anthropometric measurements to exclude genetic or neurological disorders), and psychiatric evaluation.

Psychometric Assessment:

All patients were subjected to the "Draw a Person Test", developed by Florence Goodenough in 1926, as a cognitive and intelligence test. Patients that showed intellectual disability were excluded. An Arabic version of Conners' test translated by El- Beheiry (2011) was used to assess the severity of ADHD and the most commona ssociated problems (behavioural, social and academic). This was a translation of the 48- item Parent Rating Scale version (CPRS- 48) introduced by Goyette et.al. (1978). An Arabic translation of the Childhood Behavioral Checklist (Achenbach& Rescorla, 2000), a component of the Achenbach System of Empirically Based Assessment (ASEBA). It is used to detect behavioral and emotional problems in the included children.

Extraction of genomic DNA and detection of the VNTR polymorphism: A (3- 5) ml of blood on EDITA was withdrawn from children of the two groups. Genomic DNA was extracted from peripheral blood samples following the stranded salting out procedure (Miller et.al, 1988). The detection of the 40- bp VNTR polymorphism of the DAT1 gene was performed as previously described by Sery et.al. (2015). The region in the DAT1 gene encompassing the VNTR polymorphism was amplified using the following primers: a) Forward 5'- TGTGGTGTAGGGAACGGCCTGAG- 3' and b) Reveres 5'- CTTCCTGGAGGTCACGGCTCAAGG- 3'. PCR was performed in 50µl reaction volumes containing 0.5µg genomic DNA, 10× buffer (Finzyme, Finland), 0.25 mMdNTPs (Finzyme, Finland), 2.5 pmol of each primer, and two units of Taq polymerase (Finzyme, Finland). PCR amplification will be carried out on Perkin Elmer thermal cycler (Applied Biosystem 2720, Singapore) using the following conditions: initial denaturation at 96°C for 10 min followed by 30 cycles of 40 sec at 92°C, 70 sec at 66°C, and 40 sec at 72°C, ending with a final extension of 10 minutes at 72°C. A 10µl of PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide for 1 hour at 120 volts and visualized with a UV transilluminator. An O'RangeRuler™ 50 bp DNA ladder (Fermentas, Germany) was used to determine the different repeats' exact sizes. A 440 bp fragment indicates the 9 repeat allele, 480 bp fragment indicates 10 repeat allele, 520 bp indicates 11 repeat allele, and so on.

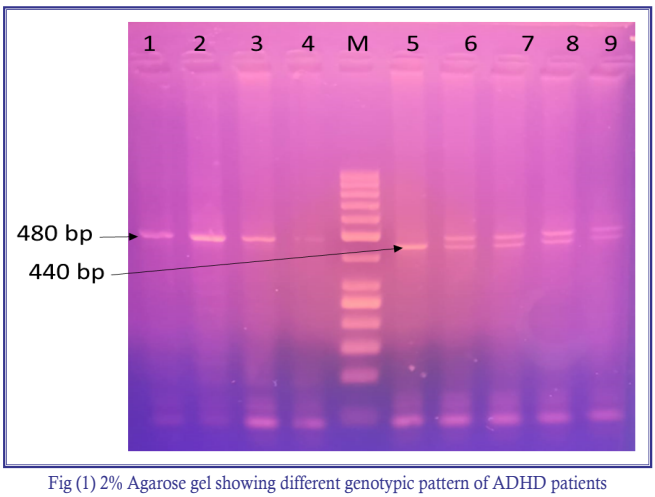

Fig (1) 2% Agarose gel showing different genotypic pattern of ADHD patients

Study of Attention Deficit Hyperactivity Disorder in a Sample of Children and the Role of DAT1 Gene Polymorphism as a Biomarker

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Summary

Introduction: Attention deficit hyperactivity disorder (ADHD) is one of the most common neuropsychiatric problems. According to the Diagnostic Statistical Manual of fifth revision (DSM-5), Attention deficit hyperactivity disorder (ADHD) is defined as a neurodevelopmental disorder characterized by inattention, disorganization, and/or hyperactivity-impulsivity. Numerous genetic factors are involved in the etiology of ADHD. The DAT gene (DAT1, SLC6A3) is one of the most studied vulnerability genes for ADHD.

Aim: This study aimed to test the possible association between DAT1 polymorphism and ADHD disease in a sample of Egyptian children.

Methods: The study included 60 patients with ADHD and 60 normal control subjects between (6- 17) years of age. All patients were subjected to psychometric assessment using an Arabic version of the 48-item Conners' Parent Rating Scale (CPRS-48). An Arabic translation of the Childhood Behavioral Checklist (CBCL) was used to detect behavioral and emotional problems in the included children. Genomic DNA was extracted to detect the 40-bp VNTR in the 3′-UTR region of DAT1.

Results: ADHD type was combined in 50 patients (83.3%), predominantly hyperactive-impulsive in 6 (10.0%), and predominantly inattentive disease in 4 (6.7%). The difference between the ADHD and control groups in DAT1 polymorphism was close to significance (p=0.104)..

Conclusion: Our results showed that the difference between the ADHD and control groups in the DAT1 VNTR genotype was statistically close to significant. The 10R/10R genotype was the predominant one in the control group (58.3%) and was found in 40% of the ADHD children.

Key words: ADHD, biomarker, DAT1 polymorphism, 10/10 alle, VNTR.

دراسة إضطراب فرط الحركة ونقص الإنتباه في عينة من الأطفال ودور التعدد الشكلي لجين DAT1 كدلالة حيوية

مقدمة: يعد اضطراب فرط الحركة ونقص الانتباه (ADHD) أحد أكثر المشكلات العصبية والنفسية شيوعا. وفقا للدليل الإحصائي التشخيصي للمراجعة الخامسة (DSM-5)، يعرف اضطراب فرط الحركة ونقص الانتباه (ADHD) بأنه اضطراب في النمو العصبي يتميز بعدم الانتباه، وعدم التنظيم، و/ أو فرط النشاط والاندفاع. العديد من العوامل الوراثية متورطة في اضطراب فرط الحركة ونقص الانتباه. يعد جين DAT1، SLC6A3 أحد أكثر جينات الضعف التي تم دراستها من أجل ADHD.

الهدف: هدفت هذه الدراسة إلى اختبار الارتباط المحتمل بين التعدد الشكلي لجين DAT1 ومرض ADHD في عينة من الأطفال المصريين.

الطريقة: اشتملت الدراسة على ٦٠ مريضا يعانون من اضطراب فرط الحركة ونقص الانتباه و٦٠ شخصا عاديا تتراوح أعمارهم بين (٦- ١٧) عاما. خضع جميع المرضى للتقييم النفسي باستخدام نسخة عربية من مقياس تقييم الوالدين المكون من ٤٨ عنصرا (CPRS-48). تم استخدام ترجمة عربية لقائمة مراجعة سلوك الطفولة (CBCL) للكشف عن المشكلات السلوكية والعاطفية لدى الأطفال المشمولين. تم استخراج الحمض النووي الجيني للكشف عن VNTR-′4 bp في منطقة UTR-′3 من DAT1.

النتائج: أظهرت الدراسة أن ٥٠ مريض كانوا من النوع المشترك للADHD (٨٣,٣%)، و٦ مرضى من نوع فرط الحركة و الاندفاعية (١٠,٠%)، وفي ٤ مرضى من نوع تشتت الانتباه (٦,٧%). لم يكن هناك فرق كبير بين ADHD ومجموعة الضبط في التعدد الشكلي لجين DAT1 (ع=٠,١٠٤).

الخلاصة: أظهرت نتائجنا أن الفرق بين مجموعتي ADHD والضابطة في التركيب الوراثي DAT1 VNTR كان غير ذي دلالة إحصائية (p=0.104) كان النمط الجيني 10R/ 10R هو السائد في المجموعة الضابطة (٥٨,٣%) ووجد في ٤٠% من الأطفال ADHD.

الكلمات المفتاحية: فرط الحركة ونقص الإنتباه، دلالة حيوية.

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