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Original article

Prevalence of *H Pylori* Infection and Related Blood Biomarker among Autistic Children in Tobruk City, Libya

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ABSTRACT

Background and aims. *Neurological disorders have been linked to gastrointestinal (GI) abnormalities including a shift in gut microbiota. Helicobacter pylori bacteria plays an important role in progression and development of neurological diseases such as autism spectrum disorder (ASD). GI abnormalities in ASD children have been reported overseas, but there were not data for Libya. Therefore, the current study aims to assess the prevalence of H. pylori infection and related Blood Biomarker such as anemia among Autistic Children in Libyan population in particular in Tobruk City.* ***Methods****. The present participants recruited from the children ASD center in Tobruk city in the east of Libya with an age range 5-12years. H. pylori antigen were measured and the Iron deficiency (ID) parameters (hemoglobin content, hematocrit, MCH concentration) were also performed.* ***Results****. Seventy-eight % 78 of cases were positive for H. pylori antigen and hemoglobin and hematocrit level were low.* ***Conclusion****. H pylori infection and ID deficiency were prevalent among autistic children, which should be targeted during autism management.*

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INTRODUCTION

Autism spectrum disorder (ASD) is a broad range of pervasive developmental disorders that characterized by symptoms, such as devaluing social bonding, repetitive behaviors or restricted interests and behaviors [1]. The prevalence of ASD has been increased dramatically world widely it is raised by 10%, 1 to 54 children by 2020 in United State [2]. Similar study from France reported that the ASD rate has been increased by 0.5% to 3.1% in Iceland [2]. At the same time, reports from Libya also has been estimated that the rate of ASD among children is increasing over time and the prevalence of ASD reached about 4 per 1000 in average aged between 2 to 5 years [3].

ASD is characterized by many different clinical end phenotypes including general gastrointestinal (GI) concerns. GI symptoms may overlap with ASD core symptoms through different mechanisms. These mechanisms include multilevel pathways in the gut–brain axis contributing to alterations in behavior and cognition. Shared pathogenic factors and pathophysiological mechanisms possibly linking ASD and GI disturbances. Metabolic activity of the microbiome and dietary components are currently suspected to be associated with alterations in behavior and cognition also in patients with other neurodegenerative diseases [4,5].

H. pylori was suggested as one of the pathogenic factor that could be directly related to dysregulation of the gut microbiome that affect behavior and cognition by gut-brain axis [5,6]. The mechanism behind these alterations attributed to the metabolic activity of H. Pylori. H. pylori could get forced to migrate to the colon under the influence of antibiotic violence leading to accumulation of excess amounts of ammonia. The accumulated H. pylori- produced ammonia conforms with the observation of elevated serum ammonia among autistic children and its toxic effect with the hypothesis of the entire interestingly, kids develop the abnormal colonic H. pylori strains trans-familial at an early

age which is the typical timing where children start to develop autistic features or loose already developed skills. In addition to iron deficiency and anemia, which indicate the infection of children, iron deficiency is also linked to H. pylori [7, 8].

High prevalence of iron deficiency (ID) and iron deficiency anemia (IDA) was reported in children with ASD. Inadequate iron intake and malabsorption were thought to cause ID in these children in gut-related diseases and conditions such as: stress, depression, Alzheimer's disease, multiple sclerosis, Parkinson's and autism spectrum disorder ASD [9,10]. This study was aimed to assess the prevalence of h. pylori infection and its related Blood Biomarker among Autistic Children in Tobruk City, Libya.

METHODS

Data collection Participants

In the present study, participants were recruited for the study consisting of 9 autistic children divided into 8 males and 1 female. All participants gave written informed consent provided by their parents and agreed to participate in the study. The study participants were enrolled in the study through the Tobruk Autism center. The center sample population consisted of children diagnosed with ASD. The diagnosis of ASD was confirmed in all study subjects using the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) and 3DI (Developmental, dimensional diagnostic interview) protocols [11]. The ages of autistic children included in study were between 5.5 to 12 years old. The total duration of the study was three months, from 1 March to 8 June 2021.

All participants were screened via parental interview for current and past physical illness. Autistic children, who have associated other physical illnesses or taking nutritional supplements, antibiotics or those who use chronic medications, were all excluded from the study to avoid any confounding factors that may affect our results. In addition, a written questionnaire was used during recruitment of volunteers to assess GI habit, dietary characteristics and antibiotic usage, and to look for any correlation between the characteristics of participants. All the persons who agreed to participate in this study gave their informed consent before their inclusion in study.

Laboratory investigation

Samples were withdrawn on 6thJune 2021, following precautions against the COVID 19 pandemic, taking into account the psychology of children and the presence of the specialist supervising the children, the director of the center and the medical staff.

Blood collection

Blood samples were collected by a qualified lab technician into 5-ml blood collection tubes containing EDTA. Immediately after collection, blood was centrifuged at 4˚C at 3000 g for 20 minutes. The plasma was decanted, dispensed into four 0.75 ml aliquots (to avoid multiple freeze-thaws cycles) and stored at −80˚C until analysis.

We defined anemia as the state in which the hemoglobin concentration based on this WHO report [12], anemia was defined as hemoglobin concentration <11.0 g/dL in children.

Hematological Analysis was based on the instruction manual of the hematology analyzer (Sysmex Kx21). Total red blood cell (RBC) count, hemoglobin content (Hb; g/dL), hematocrit, total number of white blood cells (WBCs) lymphocyte (LYM) count and platelet (PLT) count were assessed. Corpuscular hemoglobin (MCH; pg), MCH concentration. Commercially available ELISA kits were used to measure Serumfree testosterone (supplied by ALPCO, USA).

Commercially available colorimetric assay kits were used for assays of the serum levels of ammonia (supplied by Biodiagnostics, Egypt), The H. Pylori IgG antibodies were identified by using the enzyme linked immunosorbent assay. All serum samples were tested qualitatively for the presence of H. Pylori IgG antibodies using a bioelisa Helicobacter IgG kit19 following the instructions given by the manufacturer (Biokit, S.A. Barcelona, Spain).

Statistical analysis

Data were analyzed using IBM SPSS version 22, The associations of the levels of H. pylori infection and anemia biomarkers were assessed throughout using the frequency and percentage and compare them with the normal value of each marker**.**

RESULTS

The present study included 9 autistic children. Table 1 shows the distribution of age and gender among children with ASD. The range age of male participants 5.5 to 11 years old, whereas the only female was 8 years old. Regarding to the gender, 89% was male whereas, 11% was female (figure 1).
The present study included 9 autistic children. Table 2 shows distribution of age among children with ASD. The range age of male participants 5.5 to 11years old, where the only female was 8 years old. Regarding to the gender, 89% was male whereas, 11% was female (table2).

Table 1. Distribution of age among autistic children

Variables		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	5.5 to 7	5	55.6	55.6	55.6
	8 to 11	4	44.4	44.4	100.0
	Total	9	100.0	100.0	

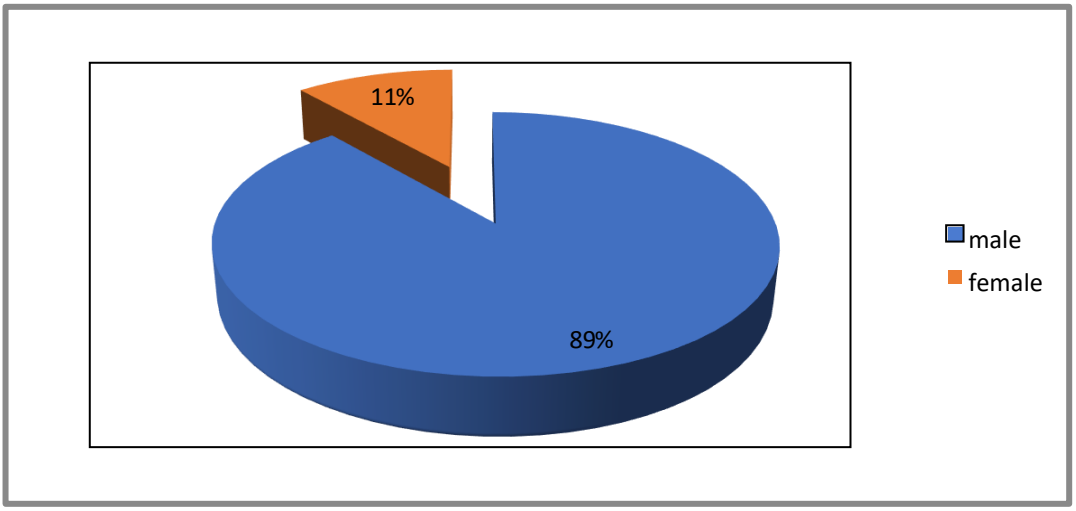

Figure 1. Distribution of gender among autistic children.

Figure 2 represents the baseline blood biomarker of autistic children compare to the normal value. The level of hemoglobin, hematocrit and MCV (Figure 3 a, b and c) were all lower or almost closes to the lowest value of the biomarker suggested that the participants suffered from anemia currently or in the nearest to the recent time.

Table 3. Hemoglobin value in autistic children compare with normal value.

Statistic		Frequency	Percent	Valid Percent	Cumulative Percent	Normal value
Valid	less than 11.5	4	44.4	44.4	44.4	
	less than 12	5	55.6	55.6	100.0	11.5 -14.5
	Total	9	100.0	100.0		

Table 3b. Hematocrit value in autistic children compare with normal value

Statistic		Frequency	Percent	Valid Percent	Cumulative Percent	Normal value
Valid	less than 36	4	44.4	44.4	44.4	
	less than 37	5	55.6	55.6	100.0	36-44
	Total	9	100.0	100.0		

Table 3c. MCV value in autistic children compare with normal value

Statistic		Frequency	Percent	Valid Percent	Cumulative Percent	Normal value
Valid	less than 80	1	11.1	11.1	11.1	
	less than 85	6	66.7	66.7	77.8	
	less than 90	2	22.2	22.2	100.0	80-95
	Total	9	100.0	100.0		

Figure 2 presents the high prevalence of *H pylori* antigen among autistic children, in which 78% were positive for H. pylori antigen among the included autistic children.

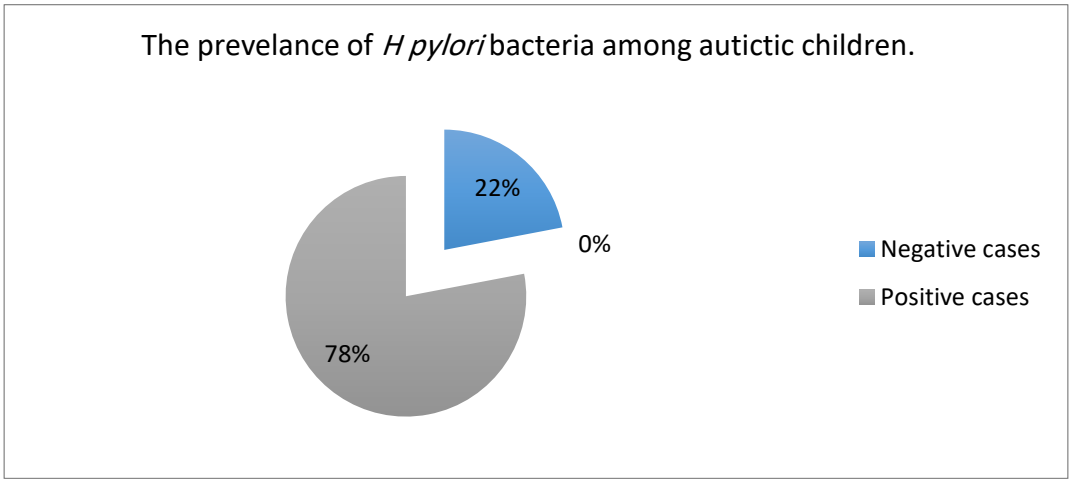

Figure 4. Prevalence of H pylori antigen among the autistic children

DISCUSSION

This study presents, to the best of our knowledge, the first report on an establishing level of iron deficiency and identify the pathogenesis profile and other biomarker related to children with autism in Tobruk city and in Libya in general. The results indicate that the prevalence of ASD in male 8 (89%) more than female 1 (11%) and it is in correlation with the hypothesize that said autism may be the result of disrupted hormonal balance during prenatal developmental periods. Considering gender differences in cognitive profile in normal population, typical male cognitive profile is described as less emotional and more systematic. On the other hand, females are more emotional and less systematic, cognitive profile of ASD patients is supposed to be more systematic and less empathic than in males from normal population It is, thus, logical that testosterone as a male sex hormone organizing the brain structure is suggested to play a role in the pathogenesis of ASD and to be responsible for the extreme male brain characteristics in autism [12-14].

Iron deficiency (ID) is a main nutritional health problem among children leading to insufficient iron that is an important factor to maintain normal cellular function. In addition, central nervous system processes are highly dependent on iron-containing enzymes and proteins. ID deficiency one of the essential cause of increasing the risk of psychiatric disorders, including developmental disorder and ASD. Low serum ferritin levels (figure3) in ASD children may be a sign of iron deficiency and an early precursor of iron deficiency anemia. These findings suggest that food selectivity is more common in children with autism than it is in typically developed children. These results suggest that ferritin levels should be measured in children with ASD as part of routine investigation. Furthermore, the current study reveals the association between autism and ID (figure3) and this finding is compatible with previous studies [15-21].

The frequent occurrence of GI issues in ASD patients imply the possible involvement of the gut microbiota in gastrointestinal pathophysiology of ASD. H pylori was suggested as one of the pathogenic factor that could be directly related to dysregulation of the gut microbiome that affect behavior and cognition by gut-brain axis. Thus, it is important to detect the change of gut microbiota in ASD children [22]. The high prevalence of H. pylori (78%) among ASD children was reported in this study and this was in agreement with other report from different region [23].

CONCLUSION

In conclusion, the identification of blood biomarkers and H. pylori infection related to autism would be advantageous for clinical diagnosis and therapeutic intervention in this disease.

Conflict of interest

There are no financial, personal, or professional conflicts of interest to declare.

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