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Immunological and Bacteriological Study of Autism Spectrum Disorder

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Abstract

Autism spectrum disorder (ASD) is an intricate neurodevelopmental disorder generally demonstrated in the first few years of life and treatment to persist into adolescence and adulthood. In this study was (50) total number of ASD patients. While 30 as controls from Baghdad. All isolates were obtained from the stool of patients and number of isolates Escherichia coli was (n = 4, 25%). While the results showed serum levels of interlukin-1β and IL-17a in Autistic patients by using ELISA technique, the mean level of IL-1β and IL-17a were higher in patient group (2275.89 ± 77.38pg/ml) , (1457.72 ± 35.66pg//ml) , than control group (429.33 ± 35.84pg/ml) , (963.64 ± 8.91pg/ml) respectively, this variance was significant ((P <0.001)).

Keywords
Escherichia coli, Autistic, IL-1β, IL-17a.

Currently one of the most prominent and widely discussed human conditions
is autism. Increased prevalence has brought the attention of society in the United States, with worldwide recognition. Much discussion surrounds the
conceptualization of autism as a disability or as a set of unique skills that can be seen as strengths [1]. Although there is truth in both, there is also much verification that the life course for many individuals with autism, from infancy and into adulthood, is challenging for them and their families [2]. Autism spectrum disorder (ASD) is an intricate neurodevelopmental disorder generally demonstrated in the first few years of life and treatment to persist into adolescence and adulthood. It is characterized by deficits in communication and social interaction and restricted, repetitive patterns of behavior, interests, and activities. It is a disorder with multifactorial etiology [3]. *Escherichia. Coli is a widespread species found in the intestines of farm animals, poultry and humans. The majority of E. coli strains are non-pathogenic, but a few are very pathogenic,* [4]. Gastrointestinal (GI) dysfunction is an ASD-associated comorbidity, implying a potential role of the gut microbiota in ASD GI pathophysiology [5,6]. Several recent studies found that autistic individuals harbor an altered bacterial gut microbiota. In some cases, remodeling the gut microbiota by antibiotic administration and

microbiota transfer therapy reportedly alleviated the symptoms of ASD [7,8].

Material and methods

Eighty subjects (50 patients & 30 controls) were enrolled. Blood collected were taken from patients whom suffering from autistic(diagnostic by physicians) and normal control, the stool of some patients was also collected .The medical and social history was taken from each subject according to special protocol. This study performed during the period from October 2021 - January 2022. These subjects were selected from patients in three hospitals in Baghdad.

Whole blood samples were obtained from autistic people (Diagnostic by doctor). A syringe (5 ml) is used to collect blood from patients, and the blood is then allowed to coagulate in gel tubes for as a minimum 10 to 15 min. at room temp. &for elongated periods of time, in a fridge. The tube is then centrifuged at 2000 rpm, the supernatant (serum) is collected & it is preserved in a sterilized bottle for laboratory inquiry for the presence of total IL-6 by ELISA a sandwich test for the quantitative evaluation. IL-1β, IL-17a. heights in serum were determined by My Biosource (USA) [9].

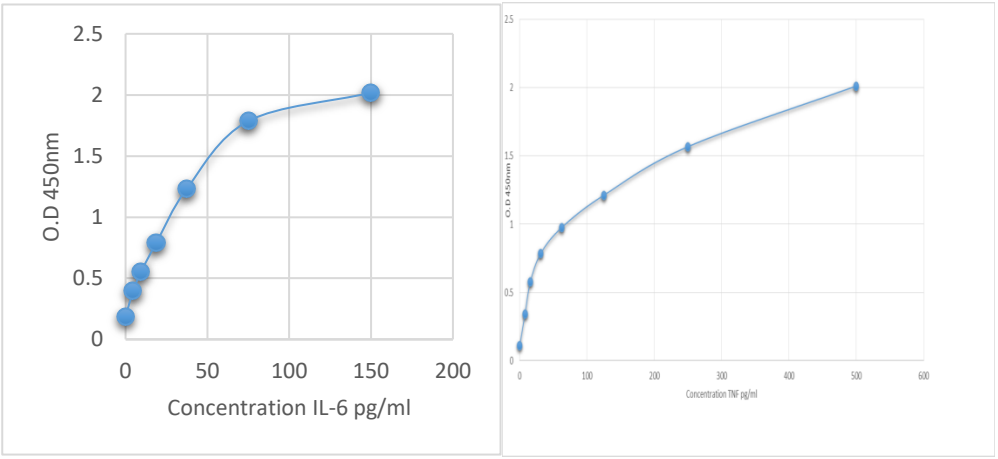

Figure (1): Standard curve of IL-1β, IL-17a. (pg/ml).

Collect 18 stool samples from patients using an aseptic procedure and sterile cups from Co. (USA). in an effort to separate E. coli. Colony characteristics of the confirmed bacteria served as the basis for the initial identification of bacteria; at that moment, many biochemical assays, including [10].

Analysis of Statistical

The Analysis of Statistical system- SAS (2012) software was utilized to examine how the factors of study differed [11].

Results and Discussion

Among all studied groups, the age groups were separated into (2) groups, the major group was 3-10 years, the second group was between 11-20 years. Incidence of patients in the first age group (3-10) was 58% compared to control 56.67 %, in the second age group (11-20) incidence of patients was 42 % compared to control 43.33%. In patients' group, the most incidence of ages was in first group higher than second group ($\chi^2 = 1.28$, $P = 0.25$) also in control group ($\chi^2 = 0.53$, $P = 0.46$), non-significant difference was noticed between first and second group in both patients and control groups. The distribution of age groups is illustrated in the table (1).

Table (1): Dispersal of the study groups by age

Age	Patients No. =50		Control No. =30	
	No.	%	No.	%

Group 1 (3-10 Year)	29	58%	17	56.67%
Group II (11-20 Year)	21	42%	13	43.33%
χ^2	1.28		0.53	
P-value	0.25 N.S.		0.46 N.S.	

According to age, there was no significant variance (p>0.05) among the study groups. Early ASD diagnosis can result in early treatment [12]. The mean age of diagnosis was found to be 43.18 months (95% CI: 39.79-46.57) in the subgroup study only had children as participants, (ten years), with a range of 30.90-74.70 months. [13].

Results in table (2), showed specimen cultures revealed 16 had bacterial isolates from 18 pateints (while 2 non- culture isolate).

A total of fourteen Gram-negative bacterial isolates have been cultured from stool. The Gram-negative *E.coli* ($n = 4$, 25%) appeared with *Escherichia fergusonii, Shigella boydii, Proteus Spp* and *Serratia marcescens* ($n = 1$, 6.3%), *Pseudomonas spp.* and *Klebsiella spp. (*n = 2, 12.5%), in other hand one isolate was Gram-positive bacterial.

Table 2: Number and proportion of bacteria identified from ASD

ISOLATED	***NUMBER AND PERCENT***	
	Number	***%***
Escherichia coli	4	25
fergusonii Escherichia	2	12.5
Shigella boydii	1	6.3
Pseudomonas spp	2	12.5
Klebsiella spp	2	12.5
Proteus Spp.	1	6.3
Serratia marcescens	1	6.3
G+ve	3	18.8
culture isolated	16	
Non-culture isolated	2	
Total	18	

Out of 16 positive result, four isolates (25%) were primary identified as *E. coli.* The identification and characterization of the isolates were carried out according to certain morphological, cultural and biochemical tests. The bacterial isolates were initially cultivated on MacConkey agar and blood lactose fermentation. agar in aerobic conditions, followed by further differential diagnostic tests, to confirm this diagnosis. *E. coli* on MacConkey agar had a tiny, regular-edged appearance and was pink in color from

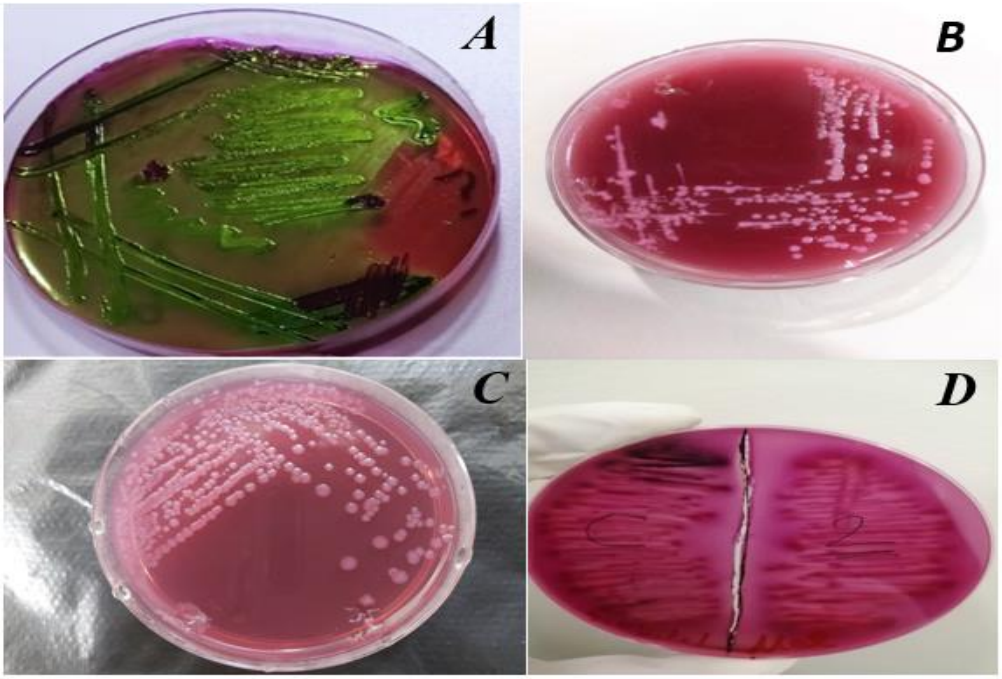

Figure (2): Colonies of E. coli on (A)Eosin Methylene Blue agar (B) SS agar(C) MacConkey Agar (D) Sorbitol MacConkey Agar

Due to the formation of -hemolysine, some isolates on blood agar created a clear, translucent region around the colonies, but on EMB agar, the growth was visible as black with a greenish-black metallic sheen [14]. All isolates passed the biochemical tests for lactose and sorbitol fermentation, and the IMVIC test revealed indole positivity. This test depends on the bacteria's capacity to break down tryptophan to form indole, which gives a red color when combined with Kovac's reagent. Positive for methyl red, a red coloration obtained after the addition of methyl red indicates an organism's capacity to acidify a phosphate buffered glucose pe Negative for the Voges-Proskauer test, which was is employed to assess the bacteria's capacity to create neutral end products (acetoin and/or diacetyl) in a phosphate buffered glucose-peptone medium. When the bacteria failed to turn bromothymel blue from green to blue as an indication of citrate consumption as an amino carbon source, the citrate utilization test was negative. Peptone-glucose buffered medium. When the bacteria failed to turn bromothymel blue from green to blue as an indication of citrate consumption as an amino carbon source, the citrate utilization test was negative.

Table 3: Biochemical tests of E.coli isolated from stool sample

Test	Catalase	Oxidase	Indole	MR	VP	Citrate use	Lactose ferment	sorbitol fermentation
Isolate								
E.coli	+	-	+	+	-	_	+	+
(+): positive result; (-): negative result; (+): Variable								

The (VITEC 2 system) methodology is a quick, standardized, and compact version of the currently used conventional methods for diagnosing and differentiating members of the

Enterobacteriaceae family [16].

Immunological markers

Serum level of IL-1βand IL-17A in patients sera ADS. The level IL-1βand IL-17A Significant increase in IL-1βserum levels in Autism Spectrum Disorder (2275.89 ± 77.38) pg /ml compared to control (429.33 ± 35.84) pg /ml Significant increase in IL-17A serum levels in Autism Spectrum Disorder(1457.72 ± 35.66) pg /ml compared to control (963.64 ± 8.91) pg /ml (P = 0.003). (see table 5,6 and figure 3).

Table-5: IL-1βserum level in patient compared to controls.

IL-1β	**Patients (Number =20)**	**Control (Number =15)**
Mean ± S.D.	2275.89 ± 77.38	429.33 ± 35.84
***P*-value**	**< 0.0001****	

Over 40 years ago, the first theory relating immune system malfunction to autism spectrum disorder (ASD) was put forth [17,18]. The autistic phenotype and other features of impaired immunity, such as cytokine levels and secretion, have been linked in a number of studies. Immune system dysfunctions have lately been noted in ASD [19,20]. Studies have shown a robust inflammatory state strongly connected with ASD in recent years; this inflammatory condition is frequently associated with immune system malfunction [21]. According to experimental research, cytokines, such as TNF-, play significant roles in the emergence of ASD and are linked to its identification [22]. Recent evidence points to a potential inflammation pathway linking associated ASD with the activity of T helper 17 (Th17) lymphocytes and their effector cytokine interleukin-17A (IL-17A). Interlukin-17A has been implicated from human studies and elevated IL-17A levels in the blood have been found to correlate with phenotypic severity in a subset of ASD individuals. Additionally, antibody blockade to inhibit IL-17A signaling was found to prevent ASD-like behaviors in offspring exposed to MIA. Therefore, IL-17A dysregulation may play a causal role in the development of ASD[23]. This theory of immunological alteration is based on the knowledge that the brain is able to recognize cytokines, such as the proinflammatory cytokines IL-1a, IL-1β, TNF-α, and IL-6, as molecular signals of sickness. Altered cytokine profiles have been consistently linked to ASD in children during this period. In high-functioning male children with ASD, the plasma levels of IL-1β, IL-1 receptor antagonist (IL-1RA), IL-5, IL-8, IL-12(p70), IL-13, and IL-17 are elevated relative to matched controls [24].

These researchers suggest that this difference in cytokine levels inflammatory response [25] showed no correlation between the cytokine level and autism severity which agreed with our results as there was no difference in the level of both cytokines depending on the ASD severity [26].

Table (6): Serum levels of IL-17in patients associated to control.

IL-17 (pg /ml)	**Patients (No. =20)**	**Control (No. =15)**
Mean ± S.D.	**1457.72 ± 35.66**	**963.64 ± 8.91**
***P*-value**	**0.002****	

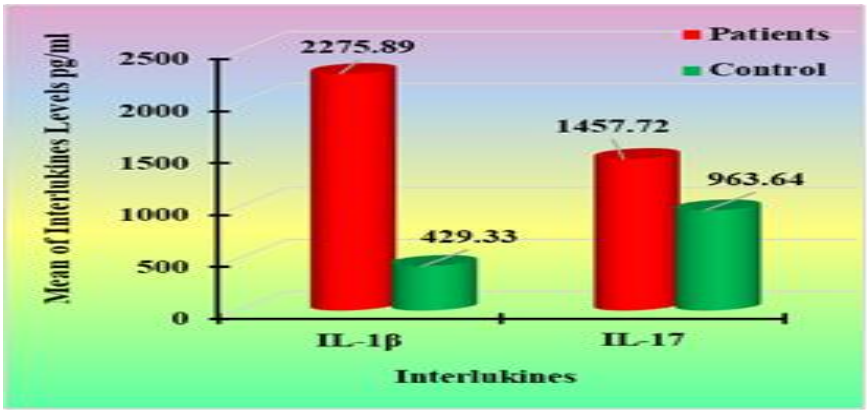

Figure (3): IL-1β and IL-17level in autistic patients compared to healthy controls.

It is believed that ASD causes a disruption in the blood-brain barrier's integrity as well as an increase in neuro-inflammatory processes. By allowing unfettered entry of pro-inflammatory signaling chemicals generated by circulating monocytes [27], the barrier membrane's enhanced permeability allows for the modulation of brain activity. In addition to elevated expression of IL-1β, as identified by Suzuki and colleagues, IL-6, IL-12, TNF-α and IL-23 are also elevated in ASD compared to healthy controls, suggesting a dysregulated immune response [27].

Microglia are then encouraged to activate & proliferate, which disrupts neuronal plasticity and causes social interaction, communication, and behavioral problems. [28] As a result, peripheral cytokines can communicate with the brain through the blood-brain barrier, which is crucial for brain development [29]. TNF- was chosen specifically because it stimulates NF-B nuclear translocation in primary cortical neurons and the innate immune system's traditional NF-B signaling cascade [30].

Conclusions

IL-B1 and IL-17A levels are pointedly higher in autistic patients than in healthy controls. In people with autism, there are notable correlations between immunological signs.

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