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Abstract: Background: Autism children were reported to have gastrointestinal problems that are more frequent and more severe than in children from the general population. Although many studies demonstrate that GI symptoms are common in autism, the exact percentage suffering from gastrointestinal (GI) problems is not well known, but there is a general consensus that GI problems are common in autism. The observation that antifungal medications improve the behavior of autism children, encourage us to investigate their intestinal colonization with yeasts. **Aim of the work:** The purpose of this work was to investigate the intestinal colonization with yeasts in autistic patients and to assess the role of yeast as a risk factor to cause autism behavior. **Patients and methods:** The study included 83 cases diagnosed as autistic children referred from the neuro-pediatric clinic and 25 normal children as a control group. All children under the study came to Phoniatic clinic, during the period from 2010 to 2012, complaining of delayed language development with autistic features. Children in this study were classified into 2 groups; control and study groups. All children were subjected to interview, E.N.T examination, language assessment, Childhood Autistic Rating Score (CARS), stool culture for *Candida albicans*, complete audiological and psychometric evaluation. **Results:** There was significant relation between the autistic children and heavy growth of *Candida albicans* in stool culture. **Conclusion:** The high rate of *Candida albicans* intestinal infection in autistic children may be a part of syndrome related to immune system disorders in these patients.

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1. Introduction:

Autism spectrum disorders (ASDs) are a group of severe neurodevelopmental conditions, referred to a broader extent as pervasive developmental disorders, characterized by a triad associating impairments in social interactions, communication deficits and restricted repetitive and stereotyped behaviors and interests with an onset in infancy or early childhood (before 3 years). The estimated prevalence of ASD was 2–5/10000 with a ratio four times higher in males than in females (*Smalley, 1997*). In the last decades, a significant increase (6–10 folds) of prevalence has been noticed, partially explained by improvements in case ascertainment, making ASD a public health priority (*Fombonne, 2003*).

Candida albicans is a yeast-like fungus which inhabits almost all humans. It lives on the moist dark mucous membranes which line the mouth, vagina and intestinal tract. Ordinarily it exists only in small colonies, prevented from growing too rapidly by the human host's immune system, and by competition from other microorganisms in and on the body's mucous membranes.

Many species are harmless commensals of hosts including humans, but other species, or harmless species in the wrong location, can cause disease.

Candida albicans can cause infections (candidiasis or thrush) in humans and other animals, especially in immunocompromised patients (*Fugelsang and Edwards, 2010*). When something happens to upset this delicate natural balance, candida can grow rapidly and aggressively, causing many unpleasant symptoms to the host. Some of the symptoms are widely known and acknowledged. Vaginal yeast infections primarily caused by candida, present the most common case in point. Thrush, the white yeast infection of the mouth and tongue which is common in infants, is another well-known example of candida overgrowth. Systemic infections of the bloodstream and major organs, particularly in immunocompromised patients, affect over 90,000 people a year in the U.S., with 40–50% mortality (*dEnfert and Hube, 2007, Tarlan and Rick, 2010*)

In recent years a minority of physicians have begun to try to persuade their colleagues, and the public, that candida may present consequences far more devastating to human well-being than vaginitis and thrush. They cite Japanese studies showing that candida is able to produce toxins which cause severe long-term disruption of the immune system and may also attack the brain. In extreme cases, they claim, severe disorders, totally resistant to conventional treatment, can occur as a result of candidiasis. These

include depression, schizophrenia and, in some cases, autism (**Bernard, 1998**).

Aim of the work:

The purpose of this work was to investigate the intestinal colonization with yeasts in autistic patients and to assess the role of yeast as a risk factor to cause autism behavior.

2. Methodology:-

The study involved 83 children, diagnosed as autism and 25 normal children as control group. Their ages ranged from 36 months to 54 months with means age (39.5 ± 6.1 months). The study group included 68 boys and 15 girls. The control group included 15 boys and 10 girls. Exclusion criteria include:

- 1- Children under antifungal treatment.
- 2- Children under cytotoxic or immunosuppressive drugs.
- 3- Children with abnormal routine laboratory investigations; blood sugar, kidney function and liver function tests.

The children in this study underwent CRAS testing and stool culture for *Candida albicans*. The study composed of 2 groups; control group; 25 normal children without autistic feature and study group; 83 children diagnosed as autism and were subdivided into 3 subgroups according to stool culture result for *Candida albicans* growth, which included no growth group, minimal growth group, and heavy growth group.

All patients were subjected to:

- Patient interviews (personal history, family history of consanguinity, hearing loss, DLD), prenatal, perinatal, postnatal period and developmental history.
- E.N.T examination,
- Language evaluation (eye contact, response to examiner, eye head coordination), assessment of passive and active vocabulary.
- Childhood Autism Rating Scale (CARS) and the degree of autistic disorders were done as 30 serving as a cut off for a diagnosis of autism, mild-moderate autism (30-37) and severe autism (> 37) (**Eric et al., 1988**).
- Psychometric evaluation, using Stanford–Binet Intelligence Scales (**Terman et al., 1960**). & Vineland Adaptive Behavior Scales (**Sparrow et al., 2004**).
- Neurological examination.
- Audiological evaluation including (Immittancemetry, tympanometry & acoustic reflex) and auditory brain stem evoked potentials.

- Stool culture on Sabaroud Dextrose Agar (SDA) for isolation of *Candida species* and identification as follows:

1. Random stool samples were collected in sterile containers.
2. Immediate culture on SDA plate was done and incubated at 37°C.
3. Cultures were examined after 24-48 hours of incubation for candida growth characterized by paste like colonies.
4. Gram stained smears were done using (Gram stain from EDM Company) and examined by ordinary light microscope for the Gram-positive yeast cells.
5. Germ tube test was done for identification of *C. albicans* using human serum. Small colony was taken by sterile loop and emulsified in 0.5 ml serum, then incubated at 37°C for 2-4 hours and examined microscopically for the germ tube formation which is continuous with the cell (a cell wall separates the tube from the cell in *C. tropicalis*). The ability of *C. albicans* to produce a pseudo germ tube in serum is shared only with *C. stellatoidea* which is very much less common (**Mackie & McCartney, 1989**).

We considered the stool samples yielded no colonies or scanty small colonies (like that of the control group) as negative growth. Samples yielded colonies all over the lines of plating out were considered as heavy growth. Samples yielded colonies more than scanty and less than heavy were considered as minimal growth.

3. Results:

The demographic characteristics of the study and control groups are summarized in table (1). The study group included 83 children, 68 males (81.9 %) and 15 females (18.1 %), with mean age 47.44 ± 7.41 months. The control group included 25 children, 15 males (60 %) and 10 females (40 %), with mean age 44.19 ± 6.25 months. Stool culture on Sabaroud Dextrose Agar (SDA) was done for all children, 68 (81.9 %) cases were positive for *Candida albicans* growth and 15 (18.1 %) cases were negative in study group. Seven (28 %) cases were positive for *Candida albicans* growth from the control group.

Cases with negative growth of *Candida albicans* were statistically significantly increased ($P < 0.001$) in control group when compared with autistic group; while there was statistically significant increase ($P < 0.001$) in cases of heavy growth of *Candida albicans* in autistic group compared with the control group.

Characteristics of the autistic group as regards the gender and degree of autism were summarized in table 2. There were statistically non-significant

differences between males and females as regards the severity of autism ($P < 0.05$).

Relations between stool culture results and severity groups of autism were summarized in table 3. Number of patients with negative stool culture growth was statistically significantly increased ($P = 0.027$) in mild-moderate group compared with severe group; while there was statistically non-significant difference in number of minimal and heavy stool

culture cases in both mild-moderate and severe autism ($P = 0.873$ & 0.064 , respectively).

There was non-significant negative correlation between age and gender, and the total score of CARS test ($R = -0.105$, -0.044 & $P = 0.343$, 0.636 respectively), while there was significant positive correlation between stool culture results of the patients and the total score of CARS test ($R = 0.110$ & $P = 0.210$) (Table 4).

Table (1): Demographic data

Group Parameter		Autism n= (83)		Control (n= 25)		P value
		No	%	No	%	
Gender	Male	68	81.9%	15	60.0%	0.025
	Female	15	18.1%	10	40.0%	
Stools examination	Negative growth	15	18.1%	18	72.05	0.000
	Minimal	22	26.5%	4	16.05	0.058
	Heavy	46	55.4%	3	12.0%	0.000
Age (months) (mean \pm SD)		47.44 \pm 7.41		44.19 \pm 6.25		0.031
Score (mean \pm SD)		40.23 \pm 8.29		19.12 \pm 3.26		0.000

Table (2): Relation between gender and autism severity.

Gender Autism degree	Male n= (68)	Female (n= 15)	P value
Mild -moderate	32 (47.1%)	9 (60.0%)	0.065
Severe	36 (52.9%)	6 (40.0%)	0.065
Total (n= 83)	68 (81.9%)	15 (18.1%)	

Table 3: Relations between stool culture results and severity groups of autism.

Autism severity Stools culture	Mild-moderate n= (41)		Severe (n= 42)		P value
	No	%	No	%	
Negative growth	10	24.4%	5	11.9%	0.027
Minimal	11	26.8%	11	26.2%	0.873
Heavy	20	48.8%	26	61.9%	0.064

Table (4): Correlations between CARS score and other parameters.

Parameter	R	P value
Age	- 0.105	0.343
Gender	- 0.044	0.636
Stool examination	0.110	0.210

4. Discussion:

Exact etiology of autism remains largely unknown, although it is likely to result from a complex combination of environmental, neurological, immunological, and genetic factors. Strong genetic links have been shown for cases with Fragile X, neurofibromatosis, and chromosomal abnormalities (Rutter, 2000, Wiznitzer, 2004, Cohen et al., 2005).

Dykens and Volkmar, 1997 noted that there was also evidence that a genetic link to autism may be a result of a weakened immune system. One of the questions raised in early literature from the 1960's about autism was the possibility of an infectious etiology to the syndrome. Although this notion has advanced, concerns remain that a child's weakened immune systems and susceptibility to psychological illness may contribute to the disorder. These early

studies portrayed concerns regarding pre- and post-natal infectious diseases and the impact on children's immune systems as a result of these infections. Studies were conducted that investigated whether children who experienced pre- or post-natal infections and/or a suppressed immune system developed autistic disorders (*Kennedy et al., 2004*).

Recently, increasing research has focused on the connections between the immune system and the nervous system, including its possible role in the development of autism. These neuro-immune interactions begin early during embryogenesis and persist throughout an individual's lifetime, with successful neurodevelopment contingent upon a normal balanced immune response. Many immune aberrations consistent with a non regulated immune response, which so far, have been reported in autistic children could participate in the generation of neurological dysfunction characteristic of autism (*J Leukoc Biol., 2006*).

Gastrointestinal (GI) symptoms have been described in a number of autism patients, in whom symptoms include abdominal pain, bloating, diarrhea, and constipation (*Horvath et al., 1999, Afzal et al., 2003*). The exact prevalence of GI symptoms in autism is unknown. Two retrospective studies, which analyzed representative populations of children with autism, reported GI symptoms in 20% of young children previously diagnosed with autism (*Fombonne et al., 2001*). In contrast, prospective reports from pediatric gastroenterology and general autism clinics have described GI symptoms in 46–84% of patients with autism (*Horvath et al., 1999*). However, prevalence estimates from population-based epidemiologic studies are largely lacking.

Further investigation of gut-brain interactions in this cohort of children with autism and GI symptoms is necessary to clarify the potential links with the intestinal pathology and the effect on it. It is interesting that mucosal lymphocytes isolated from the duodenum, ileum, and colon as well as peripheral lymphocytes of autistic patients with GI symptoms showed increased, spontaneous production of proinflammatory, intracellular cytokines, most notably TNF, when compared with aged-matched controls, including those with similar symptoms of constipation (*Ashwood et al., 2004, Ashwood and Wakefield 2006*). These data support the hypothesis that there is mucosal immune deregulations with a proinflammatory lymphocyte cytokine profile in autism children. These findings have since been confirmed in peripheral blood, where proinflammatory cytokines were increased upon stimulation with dietary proteins in similarly affected autistic children compared with controls (*Jyonouchi et al., 2005*).

In some studies, circulating antibodies to food substances, namely the casein and gliadin, have been found (*Lucarelli et al., 1995, Vojdani et al., 2002*). However, these antibodies are also found with similar frequency to that in the general population. Furthermore, antibodies to neuronal-specific antigens in the sera of children with autism could cross-react with dietary peptides, including milk butyrophilin, Streptococcus M protein, and *Chlamydia pneumoniae*, suggesting that bacterial infections and milk antigens may modulate an autoimmune response in autism (*Vojdani et al., 2002*).

Knivsberg et al., 2002 reported that exacerbation of GI and behavioral symptoms in autism induced by certain foods, particularly those containing gluten and casein, has been shown through dietary intervention and their removal from the diet. Autistic children on gluten and casein-free diets also showed significantly lower eosinophil infiltrate in intestinal biopsies compared with those on a conventional diet (*Sandler et al., 2000*). The significance of this finding is still unclear. However, it has been proposed recently that immune responses associated with allergy may contribute to the pathogenesis of autoimmune diseases of the CNS in humans and in animal models (*Pedotti et al., 2003*).

Zagon and McLaughlin, 1991 hypothesized the increased passage of exorphins and/or opioids from the diet such as gliadomorphin and casomorphin into the body, where they may interact with the CNS, could play a role in inducing the behavioral features of autism. Opioid peptides and opioid receptors are important modulators of neural development, influencing migration, proliferation, and differentiation within the CNS. Peripherally, opioid peptides are contained and/or produced by the gut, lung, placenta, testis, lymphoid tissue, and immune cells, but also another important source of opioids is from the diet. The endogenous opiates and endorphins can directly influence the immune response, enhancing generation of cytotoxic T cells and NK cells, and antibody synthesis and act as chemoattractants for monocytes and neutrophils (*Weigent and Blalock, 1997*).

Wakefield et al., 2002 has been hypothesized that an excess of opioid peptides will have detrimental effects on brain development and behavior, and that autism may result from abnormal levels or activity of opioid peptides. Casomorphine-7, an opioid exclusively of dietary origin, has been shown to be present in patients with psychoses including autism. Indeed, the beneficial effects on autistic behavior following dietary exclusion therapy are thought, in part, to be a result of reduced opioid intake. Furthermore, therapeutic trials using the oral opioid antagonist naltrexone in some patients with

autism have shown improvements in behavioral characteristics such as repetitive stereotypes, hyperactivity, social contact, and self-injurious behavior (*Symons et al., 2004*).

In our study there was increased rate of infection by *Candida albicans* in autism versus control group, 68 (81.9 %) cases versus 7 (28 %) cases respectively. This in agreement with *Horvath and Perman (2002)*, who reported that there was increased rate of positive fungal culture for yeast in the duodenal juice (43%) of children with autism undergoing endoscopies more than had the age-matched controls with other gastrointestinal problems requiring endoscopies (23%).

Also, in our study there was statistically significant increase ($P < 0.001$) in cases of heavy growth of *Candida albicans* in autistic group compared with the control group, and this is near to the study of *Campbell (1983)*, who reported that autism was associated with GIT infection with *Candida albicans*; a sign of impaired immune functions resulting in the overgrowth of yeast in the body.

The survey by the Autism Research Institute of over 25,000 parents' reports that parents find antifungal to be one of the most effective medications for improving behavior. It is possible that children with autism are more sensitive to even a normal level of yeast. Also, it is possible that antifungal have other effects, such as reducing inflammation (*Edelson, 2010*).

Whiteley et al., 1999 supported our results. They reported; there was a decrease of autistic symptoms after the patient is placed on a gluten and/or casein free diet. Both gluten and casein can increase quantities of yeast in the gastrointestinal tract of patients, which increase autism symptoms.

MacFabe et al., 2007, reported that propionic acid (C₃H₆O₂) can induce an "autistic-like state" in laboratory rats. Propionic acid is produced in the human body through the breakdown of amino acids. It is also a rather common food preservative. *Reichelt and Knivsberg (2009)* hypothesized *Candida albicans*, the yeast which when present in excess has been to be correlated with autism, produces ammonia (NH₃) as a metabolite. If propionic acid presents with ammonia metabolites in the gastrointestinal tract, it could be converted to beta-alanine (C₃H₉NO₂), which is structurally comparable to the inhibitory neurotransmitter GABA, gamma-aminobutyric acid (C₄H₉NO₂). The proposed final structure for beta-alanine is almost identical to GABA, with the exception of an additional carbon atom present in GABA.

In contrast to our findings, *Adams et al., 2011* reported that yeast was only rarely observed by

culture in the autism or typical groups, and the difference between the two groups was not significant.

Conclusion:

Candida albicans infection may be a part of syndrome related to the immune system and depends on genetic basis of autism, or *Candida albicans* may be etiological factor lead to excessive ammonia in gut which is responsible of autistic behavior in children. More researches are needed to clarify the exact mechanism by which *Candida albicans* affects autistic children.

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