Assessment Of Obesity And Its Correlation With Some Gut Microbiota In A Sample Of Egyptian Autistic Children

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Abstract
Background: Autism Spectrum Disorder usually presents in early childhood with qualitative abnormalities in social interactions, noticeably affected communication skills, and restricted repetitive and stereotypic behaviors. Evidence has led to the hypothesis that autistic children may be at increased risk for being overweight or obese.
Aim of the study: This study aims to assess obesity and its correlation with Lactobacillus and Bifidobacteria in stool of autistic children compared to healthy controls.
Methods: This case-control study was conducted in the Learning Disability and Neurological Rehabilitation Clinic, National Research Centre on 30 autistic children, aged from 5 to 8 years compared to 30 healthy children who were age and sex matched. Autistic children were diagnosed according to DSM-5 Diagnostic Criteria for autism, Childhood Autism Rating Scale (CARS) and Gilliam Autism Rating Scale (GARS). Body Weight (Wt), Body Height (Ht), Body circumferences (Head, Neck, Waist, Hip, Mid-upper arm), Skin fold thicknesses (Biceps, Triceps, Subscapular, Suprailiac) were measured. Body Mass Index (BMI Wt/Ht^2) was calculated. Stool Real-time PCR was done to evaluate the Lactobacillus and Bifidobacteria.
Results: There is a statistically significant difference in body weight, BMI, waist circumference, supra-iliac skinfold thickness (<0.05) and neck circumference (<0.001) and Z scores of body weight, BMI, waist, hip, mid upper arm circumferences and sub-scapular and supra-iliac skinfold thicknesses (<0.05) and neck circumference (<0.001) being higher in autistic children than control ones.
The Lactobacillus and Bifidobacteria in stool of autistic children are less abundant than the control group with a statistically significant difference (<0.05). There is no correlation between the Lactobacillus and Bfidobacteria concentrations and the different anthropometric parameters among the autistic children.

Conclusion: The increased incidence of obesity in autistic children isn't related to gut dysbiosis. It could be attributed to their restricted physical activities and serious eating problems.

Key words: *Obesity, Autism, Gut Microbiota.*

INTRODUCTION:

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social-communication deficits and the presence of restricted interests and repetitive behaviors. (1)
The prevalence of autism spectrum disorder in Egypt is estimated at 800,000, according to the Social Solidarity Ministry. One in every 160 children shows signs of having autism and the rate among boys is more than that recorded in girls**.**(2)**.**

Based on epidemiological studies conducted over the past 50 years, the global prevalence of ASD has been increasing which could be explained by improved awareness, expansion of diagnostic criteria, better diagnostic tools and improved reporting. (3).

Obesity is a substantial childhood health problem due to the increased risk of adverse health outcomes including obstructive sleep apnea, metabolic syndrome, impaired glucose tolerance, type 2 diabetes and heart disease. (4).

Autism Spectrum Disorder children have shown an even higher prevalence of overweight and obesity than typically developing children, with estimated prevalence around 30%. (5) This could be explained by various reasons including them having fewer chances to engage in structured exercise, social integration as well as their odd eating habits and autonomic nervous system disorders affecting the intestine**.** (6).

Many autistic subjects exhibit a range of gut disorders, which include constipation, diarrhea, gaseous retention, and abdominal pain and discomfort. (7)They may also exhibit food selectivity, which may further affect GI symptoms. Children with ASD have altered gut flora and multiple nutritional deficiencies compared to children without ASD,which can lead to immunological and neurological problems. (8).

Gut microbiota influence nutrient acquirement and energy regulation in the human body. It is also hypothesized that obesity is characterized by specific alterations in the composition and function of the gut microbiota. These findings raise the likelihood that the gut microbiota plays a significant part in weight regulation and may be partly accountable for the obesity in some people by altering the body‘s specific metabolic efficiency through certain characteristics of the microbiota. (9)**.**

This study aimed to assess and correlate obesity and gut microbiotaof autistic children in comparison to healthy controls.

PATIENTS AND METHODS:

This study was a case-control study conducted in the Learning Disability and Neurological Rehabilitation Clinic, Medical Research Centre of Excellence, National Research Centre.Thirty children (26 males and 4 females) aged from 5 to 8 years diagnosed with ASD according to the criteria for ASD as defined in Diagnostic and Statistical Manual of Mental Disorders (DSM-5), Childhood Autism Rating Scale and Gilliam Autism Rating Scale were recruited. Comparable thirty healthy matched for age and sex were included as controls.

Ethical consideration and approval were obtained from the research ethics committee of the Faculty of Postgraduate Childhood studies and National Research Centre.

Written informed consent was obtained from the parents after explanation of the aim of the study and its benefits for their children and other children who have the same condition.
All children were subjected to the following:

1. **Full history taking including**: with emphasis on developmental and dietary history.
2. **Thorough clinical examination including anthropometric measurements**

Body Weight (Wt), Body Height (Ht), Body circumferences (Head, Neck, Waist, Hip, Mid-upper arm), Skin fold thicknesses (Biceps, Triceps, Subscapular, Supra-iliac) were measured using standardized equipment, and following the recommendations of the International Biological Program. Body Mass Index (BMI Wt/Ht^2) was calculated. (10).

3. Detection of Lactobacillus and Bifidobacterium by Real-time PCR:

Fresh fecal specimens were collected and frozen immediately in a portable freezer then transported to the laboratory and stored at _20°C till processing. Specimens were defrosted at room temperature and all processing was performed under anaerobic conditions.

Fecal samples were homogenized, and fecal aliquots were taken for DNA extraction by quantitative real-time PCR**.**(11)

Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA, 2011). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

RESULTS

Table (1): Comparison between autism group and control group regarding anthropometric measurements.

Anthropometric Measurements		*Autism group (n=30)*	*Control group (n=30)*	*t-test*	*p-value*
Body weight (kg)	*Mean±SD*	*26.40±10.57*	*21.23±5.46*	*5.655*	*0.021**
	Range	*11-50*	*13-34*		
Body height (cm)	*Mean±SD*	*117.70±14.67*	*114.40±13.94*	*0.798*	*0.375*
	Range	*91-142*	*80-138*		
Neck C	*Mean±SD*	*28.48±3.17*	*25.67±1.32*	*20.217*	*<0.001***
	Range	*23-36*	*23.5-29*		
Waist C	*Mean±SD*	*60.23±12.52*	*55.50±4.70*	*3.760*	*0.047**
	Range	*40-97*	*48.5-69*		
Hip C	*Mean±SD*	*66.57±11.56*	*62.72±5.02*	*2.798*	*0.100*
	Range	*45-92*	*53-73*		
Mid Upper Arm C	*Mean±SD*	*18.33±3.55*	*17.17±1.69*	*2.641*	*0.110*
	Range	*13-27*	*14-21*		
Biceps SF	*Mean±SD*	*7.95±4.41*	*7.08±3.63*	*0.691*	*0.409*
	Range	*3-19*	*3-17*		
Triceps SF	*Mean±SD*	*11.97±4.77*	*11.68±3.07*	*0.075*	*0.785*
	Range	*5-27*	*6-19*		
Subscapular SF	*Mean±SD*	*10.05±6.08*	*7.97±2.70*	*2.939*	*0.092*
	Range	*4-28*	*4-17*		
Supra-iliac SF	*Mean±SD*	*9.30±7.04*	*6.03±2.70*	*5.633*	*0.021**
	Range	*2.5-28*	*3-15*		
BMI [kg/m2]	*Mean±SD*	*18.41±4.38*	*16.06±1.97*	*7.172*	*0.011**
	Range	*12.45-31.49*	*12.5-23.44*		

t-Independent Sample t-test;
*p-value>0.05 NS; *p-value <0.05 S; **p-value <0.001 HS*
C: Circumference in cm SF: Skin fold thickness

When comparing between the autistic group and control group regarding anthropometric measurements, there was a statistically significant difference in body weight (kg), BMI [Wt / $(Ht)^2$], neck and waist circumferences and supra-iliac skin fold thickness being higher in autistic children than control ones.

Table (2): Comparison between autism group and control group regarding anthropometric measurements z-scores.

Anthropometric Measurements	*Autism group (n=30)*	*Control group (n=30)*	*t-test*	*p-value*
zBody weight	*0.34±0.62*	*-0.30±0.39*	*4.637*	*0.015**
zBody height	*0.14±0.53*	*-0.12±0.61*	*0.654*	*0.270*
zNeck C	*0.57±0.58*	*-0.50±0.30*	*16.578*	*<0.001***
zWaist C	*0.27±0.67*	*-0.24±0.31*	*3.084*	*0.041**
zHip C	*0.24±0.66*	*-0.21±0.35*	*2.294*	*0.037**
zMid Upper Arm C	*0.24±0.65*	*-0.21±0.38*	*3.166*	*0.028**
zBiceps SF	*0.12±0.57*	*-0.11±0.57*	*0.567*	*0.295*
zTriceps SF	*0.05±0.62*	*-0.04±0.49*	*0.061*	*0.565*
zSub-scapular SF	*0.25±0.66*	*-0.22±0.36*	*3.410*	*0.036**
zSupra-iliac SF	*0.34±0.66*	*-0.30±0.31*	*4.619*	*0.015**
zBMI	*0.37±0.63*	*-0.33±0.35*	*5.881*	*0.007**

t-Independent Sample t-test; Z: Z score
*p-value>0.05 NS; *p-value <0.05 S; **p-value <0.001 HS*

When comparing between the autistic group and control group regarding the anthropometric measurements Z-scores, there was a statistically significant difference between groups regarding body weight (kg), BMI [wt/(ht)^2] neck , waist, Hip and Mid Upper Arm circumferences, Sub-scapular and Supra-iliac skin fold thickness Z scores, being higher in Autistic children than the control ones.

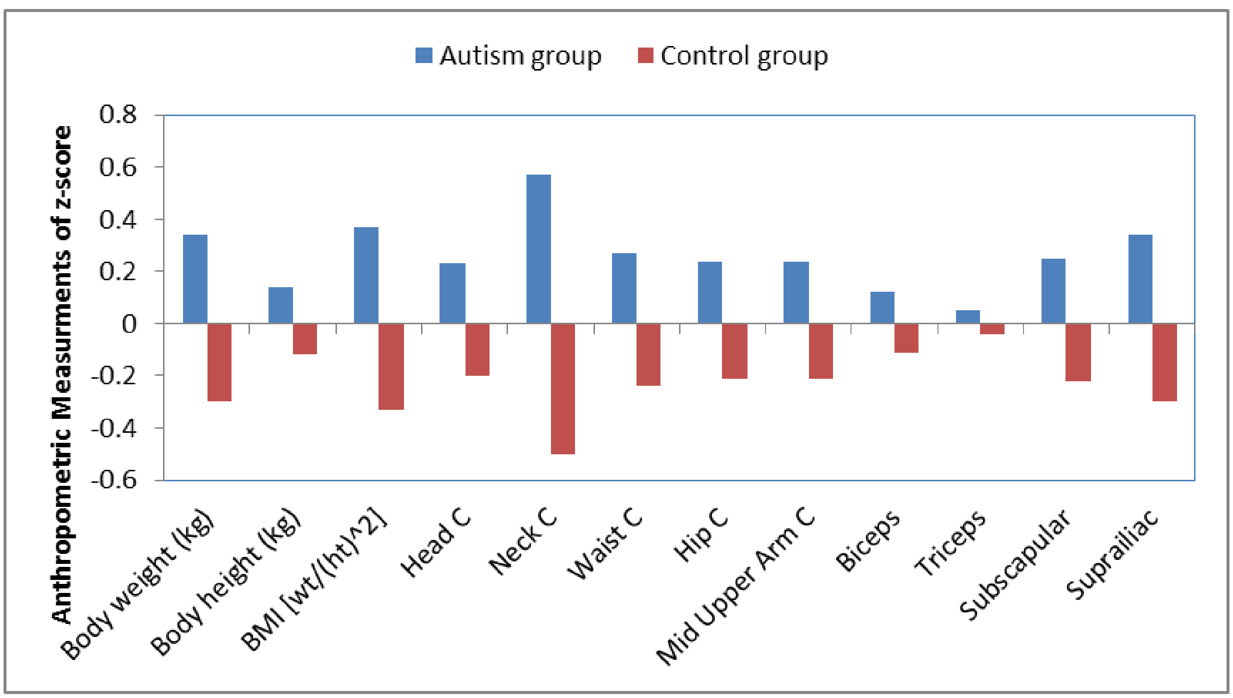

Fig.(4): Comparison between autism group and control group regarding anthropometric measurements z-score.

Table (3): Lactobacillus and Bifidobateria concentrations in stool among autistic cases and controls.

Stool	*Autism group (n=30)*	*Control group (n=30)*	*t-test*	*p-value*
Lactobacillus				
Mean±SD	*130,998.67 ± 62,960.64*	*4,366,311.33 ± 12,099,387.99*	*3.676*	*0.032**
Range	*36,001 - 299,000*	*17000 - 60,000,000*		
Bifidobacteria				
Mean±SD	*20,172.80 ± 14,289.14*	*233,726.13 ± 511,277.95*	*5.230*	*0.026**
Range	*4,154 – 68,000*	*17,000 – 2,654,154*		

*t-Independent Sample t-test; *p-value <0.05 S*

Measuring the concentrations of Lactobacillus and Bifidobacteria in stool, lower abundance of these bacteria is observed in stool of autistic children with a statistical significant difference.

Table (4): Correlation between Lactobacillus, Bifidobacteria with anthropometric measurements, in autism group.

Anthropometric Measurements	*Lactobacillus*		*Bifidobacteria*	
	r	*p-value*	*r*	*p-value*
Body weight (kg)	-0.197	0.132	-0.171	0.190
Body height (cm)	-0.215	0.099	-0.165	0.209
Neck C	-0.148	0.257	-0.068	0.604
Waist C	-0.120	0.360	-0.152	0.247
Hip C	-0.144	0.272	-0.121	0.358
Mid Upper Arm C	-0.163	0.212	-0.153	0.243
Biceps	-0.050	0.706	-0.130	0.323
Triceps	-0.044	0.738	-0.059	0.653
Subscapular	-0.065	0.621	-0.120	0.363
Suprailiac	-0.141	0.282	-0.147	0.262
BMI [wt/(ht)^2]	-0.113	0.388	-0.103	0.435

r-Pearson Correlation Coefficient; p-value>0.05 NS

Investigating the correlation between Lactobacillus, Bifidobacteria with anthropometric measurements by z-score, there is no statistically significant correlation found

Table (5): Correlation between Lactobacillus, Bifidobacteria with anthropometric measurements by z-score in autism group.

Anthropometric Measurements of z-score	*Lactobacillus*		*Bifidobacteria*	
	r	*p-value*	*r*	*p-value*
zBody weight (kg)	-0.192	0.141	-0.168	0.199
zBody height (cm)	-0.210	0.108	-0.161	0.220
zBMI [wt/(ht)^2]	-0.113	0.391	-0.103	0.434
zNeck C	-0.148	0.260	-0.071	0.588
zWaist C	-0.119	0.364	-0.150	0.251
zHip C	-0.143	0.277	-0.121	0.358
zMid Upper Arm C	-0.161	0.218	-0.152	0.246
zBiceps	-0.050	0.704	-0.130	0.323
zTriceps	-0.044	0.737	-0.060	0.651
zSubscapular	-0.066	0.615	-0.120	0.361
zSuprailiac	-0.140	0.286	-0.146	0.266
zBMI [wt/(ht)^2]	-0.113	0.391	-0.103	0.434

r-Pearson Correlation Coefficient; p-value>0.05 NS

There was no statistically significant correlation between Lactobacillus, Bifidobacteria with anthropometric measurements by z-score.

DISCUSSION:

Autism Spectrum Disorder (ASD) is group of complex disorders that affect brain development. ASD is typically associated with difficulties in social communication, social interaction and restricted, repetitive patterns of behavior, interests or activities. (12)

The global prevalence of ASD has increased vastly probably explained by improved awareness, better diagnostic tools and improved reporting. (3)

Despite the rising rate of ASD diagnoses, with nearly 1–2% of children are currently diagnosed around the world, the causes of this disease are still poorly understood. Complex genetic and environmental factors seem to be implicated in such disease, of which the intestinal flora is an environmental factor that is partially acquired from the mother. (13)

In the current study, concerning anthropometric measurements, autistic children had higher body weight, BMI, waist circumference, supra-iliac skinfold thickness (<0.05) and neck circumference (<0.001) compared to the control group with a statistical significant difference. Also, a statistically significant difference was found between the 2 groups in Z score of Body weight, BMI, waist, hip, mid-upper arm circumferences, sub-scapular and supra-iliac skinfold thicknesses (<0.05) and neck circumference (<0.001) being higher in autistic children. These results could be possibly attributable to the preference of sedentary life style, bizarre eating habits or previous intake of antipsychotics. Since there was no statistical difference between the 2 groups regarding the biceps and triceps skinfold thicknesses, then the obesity observed in autistic children is central and should be taken into consideration while planning for special diet for these children.

The results come in agreement with ***Meguid et al.*** (14), whose results showed a tendency towards the increase of Z body weight indicating an increase in total body mass as well as weight per height. Also, their study showed the skinfold thickness on the triceps, sub-scapular and supra-iliac skinfolds are significantly higher in autistic children than in normal children.

Another study found obesity was significantly prevalent among children below five years but not among ages 6–11 years. (15)

Another study (16) reported a statistically significant difference between two groups according to z Weight, being higher in autistic children. However, they found no statistical significant difference between the 2 groups as regard skinfold thickness on the biceps, triceps, subscapular and suprailiac areas.

In contrast to our results, a study that compared the anthropometry of autistic children to typically developing children ,found no statistical significant variations in Z height and Z weight between the two groups while the mean BMI and ZBMI in autistic children were significantly higher ($P < 0{\cdot}05$). (17)

Furthermore,(18)'s results showed high risk of being underweight in all children with ASD and boys with ASD. In addition, children with ASD overall and boys (this didn't not occur with girls) were shorter than Typically Developing children. In another study done later,they (19)compared weight, height and BMI in children with autism spectrum disorder (ASD) and typically developing (TD) children. They found that the differences between children with ASD and TD children were not significant.

Another study done by ***Emond et al.*** (20) did not find significant differences in body mass index between cases and controls, and the estimates suggested that energy intake was similar in controls of a similar age, despite parents reported that the children with autism spectrum disorders were difficult, selective, and demanding eaters, and started eating solids relatively late and resisted trying new food.

While, (21) measured the basic anthropometric parameters such as weight, height, head circumference and body mass index. The incidence of stunting, underweight and wasting was higher in ASD children. The prevalence of malnutrition was significant ($p<0.05$). These results could be explained by the different demographic characteristics of the studied children and nutritional traditions.

According to this research, Lactobacillus and Bifidobacteria concentrations were lower in the stool of autistic children compared to control ones, with a statistical significant difference (0.032) and (0.026) respectively. When correlating the Lactobacillus and Bifidobacteria with the anthropometric parameters in autistic children, there was no statistically significant correlation between Lactobacillus and Bifidobacteria neither with anthropometric measurements nor with anthropometric measurements with Z-score.

In agreement with our study,(22)found no relationship was evident between the levels of any of the bacterial populations examined and anthropometric data.

However in contrast to this study, (23) found alteration in faecalmicrobiota composition seems to cause overweight in children, early in life. Children maintaining normal weight showed an increased number of Bifidobacterium.

Furthermore, (16) compared the six species of bacteria with anthropometric parameters in autistic children and found a statistically significant correlation between *clostridioforme*with Biceps skinfold and Waist/Hip.

CONCLUSION:

The obesity observed among ASD children isn't related to gut dysbiosis. It may be related to other various risk factors including sedentary life style, food selectivity and previous exposure to antipsychotic medications.

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