International Journal of Medical and Health Research
ISSN: 2454-9142
Received: 23-04-2019; Accepted: 26-05-2019
www.medicalsciencejournal.com
Volume 5; Issue 6; June 2019; Page No. 116-125

Role of diet the improvement in behavior of children with autism spectrum disorders (ASD): A study in Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh

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Abstract

Background: It has been suggested that peptides from gluten and casein may have a role in the origins of autism and that the physiology and phsychology of autism might be explained by excessive opioid activity linked to these peptides. Research has reported abnormal levels of peptides in the urine and cerebrospinal fluid of people with autism.

Objective: To find out the improvement in behavior of children with ASD who have received a specific dietary intervention for 6 months.

Study design: Randomized control trial.

Study setting and period: Department of Paediatrics neuro- science shishu bikash kendro out door of Dhaka Shishu (Children) Hospital, Dhaka, between April, 2012 to September, 2012.

Study population: Children having Autism Spectrum Disorder (ASD) of both sexes who were 6 years old and below.

Methods: Children with ASD were selected from the ongoing Early Intervention (EISCA) clinic and divided into two groups by randomly (using lottery method). In the first group behavior of the children who received a dietary intervention at the beginning of the program and followed up for a period of 6 months (group I). In the second group who didn't not receive a dietary intervention were also followed up for 6 months ((group II). Other treatments were same in both groups. Behavior of the children in both groups were measured by an Autism specific clinical interview questionnaire called PIA-CV at the beginning and at the end of the intervention period.

Main outcome measure (s): Behavioral domains of children with ASD are the main outcome variables.

Result: Autism Spectrum Disorders was more common in male children, where male to female ratio was 5.7:1 in group I and 19:1 in group II and 9:1 in the whole study patients. Most of the patients came from Dhaka district in both groups, which was 16(80.0%) in group I and 18(90.0%) in group II. No schooling patients was found 14(70.0%) in group I and 13(65.0%) in group II. Formal schooling was found 4(20.0%) in group I and 5(25.0%) in group II. Caesarean section was found 16(80.0%) in group I and 15(75.0%) in group II. Majority patient's birth history was uneventful in both groups, which was 18 (90.0%) in group I and 17 (85.0%) in group II. Mild stone of development of the study patients, it was observed that age appropriate was found 19(95.0%) in group I and 20(100.0%) in group II. Patients had previous medical help was found 16(80.0%) in group I and 17(85.0%) in group II. Most of the parents were master degree pass in both groups. Eighty percent (80.0%) in group I and 15(75.0%) in group II patients came from upper (20,000->50,000 TK) income group family. Single family was found 16(80.0%) in group I and 13(65.0%) in group II. About the specific behaviors, the mean pre social relationship was found 2.86±0.37 in group I and 2.76 in group I. The mean post social relationship was 3.67±0.47 in group I and 3.87±0.51 in group II. Mean pre per interaction was found 2.43±0.53 in group I and 2.28±0.48 in group II. The mean post peer interaction was 3.25±0.60 in group I and 3.29±0.53 in group II. Mean pre communication was found 2.58±0.61 in group I and 2.12±0.77 in group II. Mean post communication was found 3.77±0.61 in group I and 3.65±0.68 in group II. Mean pre sensory a response was found 2.82±0.59 in group I and 2.96±0.75 in group II. The mean post sensory responses was found 3.58±0.55 in group I and 3.64±0.48 in group II. All specific behaviors were significantly ($p<0.05$) improved within the groups from pre to post intervention period, but no significant ($p>0.05$) difference were found between the groups during post intervention period.

Conclusion: The improvements in behavior of children with ASD were almost similar between two groups.

Keywords: PIA-CV, Specific clinical interview, ASD Outcome, EISCA

1. Introduction

Autism is a developmental disorder affects children, young people, and adults and describes qualitative difference and impairments in reciprocal social interaction and communication behavior combined with a restricted range of interests and rigid or repetitive behavior. It is diagnosed when features meet the criteria defined in the ICD-10 (international classification of diseases, 10th revision) [1] and the DSM-IV-TR (diagnostic and statistical manual of mental disorders, fourth edition, Text revised) ' for "pervasive developmental disorder" and have a considerable impact on function. Autism is associated with several coexisting conditions including neuro developmental, medical, and mental health problems. Autism was once thought to be an

uncommon developmental disorder, but recent studies have reported increased prevalence and the condition is now thought to occur in at least 1% of children. [3, 5] It is being identified in increasing numbers in Bangladesh. [6] In a study conducted by WHO and National Institute of Mental Health the prevalence of Autism is found to be 8 per 1000 in Bangladesh. [7] No single cause has been established yet, although genetic and environmental factors are implicated. A number of risk factors being investigated include genetic, infectious, metabolic, nutritional and environmental, but less than 10 to 12% of cases specific causes have found. [8] Simultaneously the use of alternative treatment approaches in children with autism has increased, but due to significant methodological flaws, the currently available data are inadequate to guide treatment recommendations. [9] In practice, treatment of ASD usually consists of a comprehensive program of educational intervention, speech therapy, behavioral treatment and developmental therapies. Anecdotal reports and parent surveys and a few research studies have indicated some evidence of diminishing the symptoms of autism by use of diets based on food elimination and rotation, as well as through supplementation and alternative treatments based on intestinal healing [8]. The popularity of these diets indicate a need for more in-depth and rigorous research into their efficacy. There is growing evidence that nutritional therapy can really make a big difference to children with autism. Many have severely disrupted digestion, so a major focus must be restoring balance their blood sugar, check for brain-polluting heavy metals, exclude food additives, identify food allergies and possible nutrient deficiencies, and ensure an optimal intake of essential fats. A popular belief that specific dietary changes can improve the symptoms of children with autism. The effectiveness of elimination diets in improving the behavior of children with autism has only recently been scientifically researched. [10] A gluten-free diet is often used for children with autism in combination with a casein-free diet. Both diets are called elimination diets because a particular type of food is eliminated from the child's meals and snacks. [11] One well-controlled study focused on children with autism who had abnormally high protein by-products in their urine, and therefore were more likely to be sensitive to casein and gluten. One group of these children was fed a strict casein- and gluten-free diet for 12 months. This group had significantly fewer autistic symptoms than the remaining children, who were not fed this diet. [12] Another well-controlled study reported no significant improvements in speech for 13 children who followed a gluten-free casein-free diet for 6 weeks. [13] Sucrose or aspartame also affects behavior and cognitive performance in children. [14] There is some evidence that the ketogenic diet may be used in autistic behavior as an additional or alternative therapy [15]. The popularity of these diets indicates a need for more in-depth and rigorous research into their efficacy.

2. Review of literature

Mulloy *et al*. [16] systematically reviewed research on the effects of gluten-free and/or casein-free (GFCF) diets in the treatment of ASD. Database, hand, and ancestry searches identified 15 articles for review. Each study was analyzed and summarized in terms of (a) participants, (b) specifics of the intervention, (c) dependent variables, (d) results, and (e) certainty of evidence. Critical analysis of each study's methodological rigor and results reveal that the current corpus of research does not support the use of GFCF diets in the treatment of ASD. Given the lack of empirical support, and the adverse consequences often associated with GFCF diets (e.g., stigmatization, diversion of treatment resources, reduced bone cortical thickness), such diets should only be implemented in the event a child with ASD experiences acute behavioral changes, seemingly associated with changes in diet, and/or medical professionals confirm through testing the child has allergies or food intolerances to gluten and/or casein. Rogalski *et al*. [13] retrospectively examined the efficacy of a gluten-free and casein-free (GFCF) diet intervention as a means to improve verbal/nonverbal communication in children with autism spectrum disorders. Data were analyzed retrospectively from a randomized, double-blind, repeated measures cross over design study that included 13 children aged 2-16 years with autism spectrum disorders. Video recordings of at-home parent-child play were analyzed. Recordings were made at baseline, after 6 weeks on one of the diets (GFCF or regular diet), and after 6 weeks on the alternate diet. Findings of their study indicated no statistically significant differences in verbal and nonverbal communication outcomes between GFCF and regular diet conditions. While results of this study demonstrate that double-blind clinical trials of diet intervention are feasible, they are inconclusive regarding the efficacy of diet for improving communication. Perhaps due to the relatively short period of diet intervention used. Directions for future research are discussed as well as Implications for clinical practice. Jyonouchi *et al*. [17] study indicated an association between cellular immune reactivity to common dietary proteins (DPs) and excessive proinflammatory cytokine production with endotoxin (lipopolysaccharide, LPS), a major stimulant of innate immunity in the gut mucosa, in a subset of autism spectrum disorder (ASD) children. However, it is unclear whether such abnormal LPS responses are intrinsic in these ASD children or the results of chronic gastrointestinal (Gl) inflammation secondary to immune reactivity to DPs. Jyonouchi *et al*. [17] study explored possible dysregutated production of proinflammatory and counter-regulatory cytokines with LPS in ASD children and its relationship to Gl symptoms and the effects of dietary intervention measures.

3. Objectives

General objective

1. To find out the improvement in behavior of children with ASD who have received a specific dietary intervention for 6 months.

Specific objectives

1. To find out any difference in behavior of ASD children before and after a dietary intervention.
2. To find out the difference in behavior of study children between the intervention and the nonintervention group.

4. Materials and Methods

Study design: This study was part of an ongoing randomized control trial (RCT) at the Early Intervention Clinic for Socialization and Communication Abilities (EICSCA).

Study period: April, 2012 to September, 2012.

Place of study: Shishu Bikash Kendro, Dhaka Shishu

(Children) Hospital, Dhaka, Bangladesh.

Study population: Shishu Bikash Kendro (SBK) of Dhaka Shishu Hospital has been running a clinic for Early Intervention for Socialization and Communication Abilities (EISCA) since January, 2010. This clinic runs 3 days a week and offers service to 10-12 children and their families per week. As soon as the children attending the SBK are screened positive for Autism Spectrum Disorder (ASD) or socialization and communication difficulties due to any other cause, they are enrolled into the EISCA programme. Their diagnostic workup continues alongside this intervention programme. This programme offers 6-7 subsequent visits to each child at 3-4 weekly intervals. Intervention is offered via educating the parent about their child's strength and difficulties and the need of specific therapies for them. Subsequent sessions continue 1:1 hands on skill transfer to parents about the required therapies and techniques. The specific therapies include speech, language and communication therapy, occupational therapy, sensory integration therapy, behavior modification, structured teaching, play skills development and a dietary intervention. All of these therapies are being taken from evidence based treatment strategies for children with Autism around the world. The specific therapies are offered randomly on every child attending the clinic.

Children of both sexes regularly attending this clinic who are 6 years old and below was the study population.

Sampling method: Randomized Controlled Trial

Main outcome variable: Behavioral domains of children with ASD are the main outcome variable.

Sample size: Calculation of sample size

A. Inclusion criteria for cases

1. Children aged 6 years and below.
2. Children enrolled into the EISCA (Early Intervention for Socialization and Communication Abilities) programme.
3. Those who have received an ICD-10 criterion for autism spectrum disorder.

B. Exclusion criteria

1. Children with autism with associated illness such as epilepsy and other neuro developmental disorder.
2. Children who did not meet the ICD-10 criteria for autism.

Procedures of preparing and organizing materials

In this study the effect of a dietary intervention on the behavior of two groups of ASD children was investigated. Children with ASD were randomly selected from the ongoing Early Intervention (EISCA) clinic and were divided into two groups randomly by using lottery method. In the first group behavior of the children who have received a dietary intervention at the beginning of the program was followed up for a period of 6 months. In the second group who were not received a dietary intervention was also followed up for 6 months. Other treatment was same in both groups. Behavior of the children in both groups was measured by an Autism specific clinical interview questionnaire called PIA-CV at the beginning and at the end of the intervention period.

Randomization: Children were divided into two groups randomly by using lottery method.

Procedures of collecting data

Data was collected using a structured questionnaire (Research Instrument) containing all the variables of interest. The questionnaire was finalized following pretesting. Collected data was checked daily and edited (if needed). PIA-CV (Parent's Interview for Autism-clinical version) was administered to every subject at the beginning and at the end of the study period. PIA-CV will measure the behaviors (outcome variable) of interest.

Procedure of data analysis

Statistical analyses were carried out by using the Statistical Package for Social Sciences version 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The mean values were calculated for continuous variables. The quantitative observations were indicated by frequencies and percentages. Chi-Square test with Yates correction was used to analyze the categorical variables, shown with cross tabulation. Student t-test was used for continuous variables. P values <0.05 was considered as statistically significant.

Quality assurance strategy: It is extremely important that data was of good quality.

Ethical implications

Keeping compliance with Helsinki Declaration for Medical Research Involving Human Subjects 1964, the parents/legal guardians was informed verbally about the study design, the purpose of the study, and their right to withdraw their patients from theproject at any time, for any reason, whatsoever. Parents/guardians who was give informed consent to allow their patients to participate in the study was included as study sample (appendix-l)

5. Results

This study was includes ASD children (median age 4.8 years) on the unrestricted (n=100) or elimination (n=77) diet appropriate with their immune reactivity. Controls include children with non-allergic food hypersensitivity (NFH: median age 2.9 years) on the unrestricted (n = 14) or elimination (n = 16) diet, and typically developing children (median age 4.5 years, n = 13). The innate immune responses were assessed by measuring production of proinflammatory (TNF- a, IL-1 P, IL-6, and IL-12) and counter- regulatory (IL-1ra, IL-10, and s TNFRII) cytokines by peripheral blood mononuclear cells (PBMCs) with LPS. The results were also compared to T-cell responses with common DPs and control T-cell mitogens assessed by measuring T-cell cytokine production. ASD and NFH PBMCs produced higher levels of TNF- A with LPS than controls regardless of dietary interventions. However, only in PBMCs from ASD children with positive gastrointestinal (Gl(+) symptoms, did they find a positive association between TNF- a levels produced with LPS and those with cow's milk protein (CMP) and its major components regardless of dietary interventions. In the unrestricted diet group, Gl(+) ASD PBMCs produced higher IL-12 than controls and less IL-10 than Gl(-) ASD PBMCs with LPS. Gl(+) ASD but not Gl(-) ASD or NFH PBMCs produced less counter-regulatory cytokines with LPS in the unrestricted diet group than in the elimination diet group. There was no significant difference among the study groups with regard to cytokine production in responses to T-cell mitogens and other recall antigens. *Their* results revealed that there are findings limited to Gl(+) ASD PBMCs in both the unrestricted and elimination diet groups. Thus their findings indicate intrinsic defects of innate immune

responses in Gl(+) ASD children but not in NFH or Gl(-) ASD children, suggesting a possible link between Gl and behavioral symptoms mediated by innate immune abnormalities. It has been suggested that peptides from gluten and casein may have a role in the origins of autism and that the physiology and psychology of autism might be explained by excessive opioid activity linked to these peptides. Millward *et al*. [18] determined the efficacy of gluten and/or casein free diets as an intervention to improve behavior, cognitive and social functioning in individuals with autism. Two small randomized controlled trials (RCTs) were identified (n = 35). No meta-analysis was possible. There were only three significant treatment effects in favor of the intervention: overall autistic traits, mean difference (MD) = -5.60 (95% Cl -9.02 to -2.18), z = 3.21, p=0.001 [12]; social isolation, MD = -3.20 (95% Cl -5.20 to 1.20). *z* = 3.14, p = 0.002) and overall ability to communicate and interact, MD = 1.70 (95% Cl 0.50 to 2.90), z = 2.77, p = 0.006) [19]. In addition three outcomes showed no significant difference between the treatment and control group and they were unable to calculate mean differences for ten outcomes because the data were skewed. No outcomes were reported for disbenefits including harms. The authors concluded that research has shown of high rates of use of complementary and alternative therapies (CAM) for children with autism including gluten and/or casein exclusion diets. Current evidence for efficacy of these diets is poor. Experts disagree about the causes and significance of the recent increases in the prevalence of autism spectrum disorders (ASDs) reported by Kim *et al*. [20]. Limited data on population base rates contribute to this uncertainty. Using a population-based sample, the authors sought to estimate the prevalence and describe the clinical characteristics of ASDs in school-age children. The target population was all 7- to 12-year-old children in a South Korean community; the study used a high- probability group from special education schools and a disability registry and a low-probability, general-population sample from regular schools. To identify cases, the authors used the Autism Spectrum Screening Questionnaire for systematic, multi-informant screening. Parents of children who screened positive were offered comprehensive assessments using standardized diagnostic procedures. The prevalence of ASDs was estimated to be 2.64% (95% 01=1.91-3.37), with 1.89% (95% 01=1.43-2.36) in the general-population sample and 0.75% (95% 01=0.58-0.93) in the high-probability group. ASD characteristics differed between the two groups: the male-to-female ratios were 2.5:1 and 5.1:1 in the general population sample and high-probability group, respectively, and the ratios of autistic disorders to other ASD subtypes were 1:2.6 and 2.6:1, respectively; 12% in the general-population sample had superior IQs, compared with 7% in the high-probability group; and 16% in the general-population sample had intellectual disability, compared with 59% in the high-probability group. Two-thirds of ASD cases in the overall sample were in the mainstream school population, undiagnosed and untreated. These findings suggest that rigorous screening and comprehensive population coverage are necessary to produce more accurate ASD prevalence estimates and underscore the need for better detection, assessment, and services. There is increasing interest in the use of gluten- and casein-free diets for children with autism spectrum disorders (ASDs). Whitely *et al*. [21] reported results from a two-stage, 24-month, randomized, controlled trial incorporating an adaptive 'catch-up' design and interim analysis. Stage 1 of the trial saw 72 Danish children (aged 4 years to 10 years 11 months) assigned to diet (A) or non-diet (B) groups by stratified randomization. Autism Diagnostic Observation Schedule (ADOS) and the Gilliam Autism Rating Scale (GARS) were used to assess core autism behaviors, Vineland Adaptive Behavior Scales (VABS) to ascertain developmental level, and Attention-Deficit Hyperactivity Disorder - IV scale (ADHD-IV) to determine inattention and hyperactivity. Participants were tested at baseline, 8, and 12 months. Based on per protocol repeated measures analysis, data for 26 diet children and 29 controls were available at 12 months. At this point, there was a significant improvement to mean diet group scores (time*treatment interaction) on sub-domains of ADOS, GARS and ADHD-IV measures. Surpassing of predefined statistical thresholds as evidence of improvement in group A at 12 months sanctioned the re-assignment of group B participants to active dietary treatment. Stage 2 data for 18 group A and 17 group B participants were available at 24 months. Multiple scenario analysis based on inter-and intra-group comparisons showed some evidence of sustained clinical group improvements although possibly indicative of a plateau effect for intervention. Their results suggested that dietary intervention may positively affect developmental outcome for some children diagnosed with ASD. In the absence of a placebo condition to the current investigation, they are, however, unable to disqualify potential effects derived from intervention outside of dietary changes. Further studies are required to ascertain potential best- and non-responders to intervention.

Table 1: Distribution of the study patients by sex (n=40)

Sex	Group I (n=20)			Group II (n=20)	P value
	n	%	n	%	
Male Female	17 3	85.0 15.0	19 1	95.0 5.0	0.302^{ns}

Group l: Children who have received a dietary intervention Group Il-Children who have not received a dietary intervention
Ns=not significant
P value reached from fisher's exact test

Table. 1 shows sex distribution of the study patients, male were predominant in both groups, which was 17(85.0%) in group I and 19(95.0%) in group II. The difference was not statistically significant (p>0.05) between two groups.

Table 2: Distribution of the study patients by residence (n=40)

Residence	Group 1 (n=20)		Group II (n=20)	*P* value
	N	%	n %	
Dhaka	16	80.0	18 90.0	
Chittagong	2	10.0	2 10.0	0.548[ns]
Rajshahi	1	5.0	0 0.0	
Sylhet	1	5.0	0 0.0	

Ns=not significant
P value reached from fisher's exact test

Table 2: shows residence of the study patients, it was observed that majority patients were came from Dhaka district in both groups, which was 16(80.0%) in group I and 18(90.0%) in group II. The difference was not statistically significant (p>0.05) between two groups.

Table 3: Distribution of the study patients by schooling (n=40)

Schooling	Group 1 (n=20)	Group II (n=20)	*P* value
	N %	n %	
Formal	4 20.0	5 25.0	
Special	1 5.0	1 5.0	
Daycare Centre	1 5.0	0 0.0	0.708[ns]
Dropout	0 0.0	1 5.0	
No schooling	14 70.0	13 65.0	

Ns=not significant
P value reached from fisher's exact test

Table 3: shows schooling of the study patients, it was observed that no schooling patients was found 14(70.0%) in group I and 13(65.0%) in group II. Formal schooling was found 4(20.0%) in group I and 5(25.0%) in group II. The difference was not statistically significant (p>0.05) between two groups.

Table 4: Distribution of the study patients by mode of delivery (n=40)

Mode of delivery	Group 1 (n=20) n %	Group II (n=20) n %	*P* value
Normal C/S	4 20.0 16 80.0	5 25.0 15 75.0	0.500[ns]

Ns=not significant
P value reached from fisher's exact test

Table 4: shows mode of delivery of the study patients, it was observed that majority patients had caesarean section in both groups, which was 16(80.0%) in group I and 15(75.0%) in group II. The difference was not statistically significant (p>0.05) between two groups.

Table 5: Distribution of the study patients by birth history (n=40)

birth history	Group 1 (n=20) n %	Group II (n=20) n %	*P* value
Eventful Uneventful	2 10.0 18 90.0	3 15.0 17 85.0	0.632[ns]

Table 5: shows birth history of the study patient's, it was observed that majority patient's birth history was uneventful in both groups, which was 18 (90.0%) in group I and 17 (85.0%) in group II. The difference was not statistically significant (p>0.05) between two groups.

Table 6: Distribution of the study patients by mile stone of development (n=40)

Mile stone of development	Group 1 (n=20) N %	Group II (n=20) n %	*P* value
Age appropriate Delay	19 95.0 1 5.0	20 100.0 0 0.0	0.500[ns]

Ns=not significant
P value reached from fisher's exact test

Table. 6 shows mild stone of development of the study patients, it was observed that age appropriate was found 19(95.0%) in group I and 20(100.0%) in group II. The difference was not statistically significant (p>0.05) between two groups.

Table 7: Distribution of the study patients by previous medical help (n=40)

Previous medical help	Group 1 (n=20) n %	Group II (n=20) n %	*P* value
Yes No	16 80.0 4 20.0	17 85.0 3 15.0	0.500[ns]

Ns=not significant
P value reached from fisher's exact test

Table 7: shows previous medical help of the study patients, it was observed that patients had previous medical help was found 16(80.0%) in group I and 17(85.0%) in group.

Table 8: Distribution of the study patients by personal information of patients (n=40)

Personal information of patients		Group 1 (n=20)		Group II (n=20)
	N	%	n	%
Fathers educations				
Post Graduate	3	15.0	0	0.0
Masters	13	65.0	17	85.0
Secondary	4	20.0	1	5.0
Primary	0	0.0	2	10.0
Fathers occupations				
Service	11	55.0	5	25.0
Professional	1	5.0	5	25.0
Business	6	30.0	5	25.0
Skilled worker	2	10.0	4	20.0
Unemployed	0	0.0	1	5.0
Mothers educations				W
Masters	14	70.0	15	75.0
Graduate	0	0.0	1	5.0
Secondary	5	25.0	3	15.0
Primary	1	5.0	1	5.0
Mothers occupations				
Professional	14	70.0	14	70.0
Business	1	5.0	0	0.0
Skilled worked	4	20.0	4	20.0
Housewife	1	5.0	2	10.0
Socio economic Status				
Poor (3000-<5000)	3	15.0	0	0.0
Lower Middle (5000-10000)	1	5.0	3	15.0
Middle (10000-20000)	0	0.0	2	10.0
Upper (20000->50000)	16	80.0	15	75.0
Family type				
Single	16	80.0	13	65.0
Joint	4	20.0	7	35.0

Table 8 shows Most of the fathers were masters pass education in both groups, which was 13(65.0%) in group I and 17(85.0%) in group II. Majority 11(55.0%) fathers were service holder in group I and 5(25.0%) in group II. Most 14(70.0%) mothers were masters pass education level in group I and 15(75.0%) in group II. Majority mothers were professional occupations. Most (80.0%) in group I and 15(75.0%) in group II patients came from upper (20,000->50,000 tk) income group family. Single family was found 16(80.0%) in group I and 13(65.0%) in group 2.

Table 9: Distribution of the study respondents by dietary question (n=40)

Group 1 (n=20)			Group II (n=20)		P value
Bottle feeding status	N	%	n	%	
Yes No	4 16	20.0 80.0	6 14	30.0 70.0	0.465^{ns}
Does your child actively/ voluntarily take food to mouth by himself? Yes No	7 13	35.0 65.0	5 15	25.0 75.0	0.490^{ns}
Do you passively feed your child while he is unmindful during watching the television or playing his favorite game Yes No	6 14	30.0 70.0	7 13	35.0 65.0	0.735^{ns}
Does he have an excessive affinity for junk foods such as, chocolates, chips, chanachur, aachar (prickles) etc. Please describe the items (names and amount) Yes No	19 1	95.0 5.0	17 3	•^-' 85.0 15.0	0.302^{ns}
Does your child distress trying new foods Yes No	18 2	90.0 10.0	15 5	75.0 25.0	0.203^{ns}
Obsession with familiarity, such as one particular make and flavor Yes No	5 15	25.0 75.0	9 11	45.0 55.0	0.184^{ns}
Ammonia Pre Normal Increased	6 14	30.0 70.0	8 12	40.0 60.0	0.507^{ns}
Lactate Pre Normal Increased	7 13	35.0 65.0	9 11	45.0 55.0	0.490^{ns}
ns=not significant P value reached from fisher's exact	test				

Table 9 shows bottle feeding status was found 4(20.0%) in group I and 6(30.0%) in group II. Seven 7(35.0%) in group I and 5(25.0%) in group II respondents mentioned that does her child actively/voluntarily take food to mouth by himself. Six (30.0%) in group I and 7(35.0%) in group II respondents mentioned that they passively feed her child while he is unmindful during watching the television or playing his favorite game. Nineteen (95.0%) in group I and 17(85.0%)

respondents mentioned that he have an excessive affinity for junk foods such as, chocolates, chips, chanachur. aachar (prickles) etc. Eighteen (90.0%) in group I and 15(75.0%) in group II respondents mentioned that child distress trying new foods. Five (25.0%) in group I and 9(45.0%) in group II respondents mentioned that obsession with familiarity, such as one particular make and flavor. Increase ammonia pre was 14(70.0%) in group I and 12(60.0%) in group II. Increase lactate pre was found 13(65.0%) in group I and 11(55.0%) in group II. The difference was not statistically significant (p>0.005) between two groups.

Table 10: Distribution of the study patients by specific behaviors (n=40)

Specific behaviors	Group I (n=20)	Group II (n=20)	*"p* value
Social relationship	Meant SD	Meani SD	
Pre Range (min-max)	2.86±0.37 2.24-3.40	2.76±0.51 1.76-3.88	0.318"[5]
Post Range (min-max)	3.67±0.47 2.57-4.81	3.87±0.51 2.88-4.80	0.072[ns]
[b]P *value*	*0.001*[s]	*0.001*[s]	
Peer interaction			
Pre Range (min-max)	2.43±0.53 1.40-3.40	2.28±0.48 1.00-2.84	0.188[ns]
Post Range (min-max)	3.25±0.60 2.20-4.26	3.29±0.53 2.42-4.40	0.752[ns]
[b]*P value*	*0.001*[s]	*0.001*[s]	
Communication			
Pre Range (min-max)	2.58±0.61 1.38-3.54	2.12±0.77 1.30-4.23	0.004[s]
Post Range (min-max)	3.77±0.61 2.54-4.80	3.65±0.68 2.00-4.60	0.408[ns]
[a]*P value*	*0.001*[s]	*0.001*[s]	
Sensory responses			
Pre Range (min-max)	2.82±0.59 1.65-3.75	2.96±0.75 1.80-4.86	0.356[rs]
Post Range (min-max) [b]*P value*	3.58±0.55 2.37-4.80 *0.001*[s]	3.64±0.48 2.75-4.47 *0.001*[s]	0.604[rs]

s=significant; ns=not significant
P value reached from unpaired t-test

Table 10 shows specific behaviors of the study patients, it was observed that mean pre social relationship was found 2.86±0.37 in group I and 2.76 in group I. Mean post social relationship was 3.67±0.47 in group I and 3.87±0.51 in group II. Mean pre per interaction was found 2.43±0.53 in group I and 2.28±0.48 in group II. Mean post peer interaction was 3.25±0.60 in group I and 3.29±0.53 in group II. Mean pre communication was found 2.58±0.61 in group I and 2.12±0.77 in group II. Mean post communication was found 3.77+0.61 in group I and 3.65±0.68 in group II. Mean pre sensory responses were found 2.82±0.59 in group I and 2.96±0.75 in group II. Mean post sensory responses was found 3.58±0.55 in group I and 3.64±0.48 in group II. Only mean pre communication difference was statistically significant (p<0.05) but olhevs were not statistically significant (p>0.05) between two groups. All specific behaviors between pre *aaa'-ecB was* statistically significant (pO.OS) within the groups.

6. Discussion

This randomized control trial (RCT) was carried out with an aim to find out any difference in behavior of Autism Spectrum Disorders (ASD) children before and after a dietary intervention and to find out the difference in behavior of study children between specific dietary intervention received and children not received this specific intervention as well as to find out the improvement in behavior of children having ASD between the two groups. A total of 40 children (6 years or under 6 years) having Autism Spectrum Disorders (ASD), that of randomly selected (lottery method) 20 children received a specific dietary intervention was considered as group I and 20 children who not received a specific dietary intervention was considered as group II, who came in the MTW (More than word) clinic during April, 2012 to September, 2012, were included in this study. Children having autism with associated illness such as epilepsy and other neuro developmental disorder and children who did not meet the ICD-10 criteria for autism were excluded from the study. The present study findings were discussed and compared with previously published relevant studies. In this current study it was observed that Autism Spectrum Disorders was more common in male children, which was 85.0% and 95.0% in group I and group II respectively. Male to female ratio was 5.7:1 in group I and 19:1 in group II and 9:1 in the whole study patients. The difference was not statistically significant (p>0.05) between two groups. Kim *et al.* [20] mentioned in their study that the male-to-female ratios were 2.5:1 and 5.1:1 in the general population sample and high-probability group, respectively. Parker [24] found the sex ratio in the total sample was five boys to one female, similar to the average 4:1 given in the literature observed by Poustka *et al.* [25]. Similarly, Rahman *et al.* [6] showed male to female ratio was 1.7:1 having autism spectrum disorders (ASD). Baron-Cohen *et al.* [26] noted that predominance of ASD and other neuro-develop mental disorders in boys may be an "extreme expression of the male brain". This concept states that boys and girls brains begin to differ early in the uterus. These differences result in different strengths and weaknesses. ASC may be an example of the male brain development gone too far. In this present study it was observed that majority patients were came from Dhaka district in both groups (80.0% in group I and 90.0% in group II). The difference was not statistically significant (p>0.05) between two groups. Rahman *et al.* [6] mentioned that the children diagnosed with autism had parents from 96.0% urban and 4.0% from rural area. In this current series it was observed that most (70.0% group I vs. 65.0% group II) of the children had no schooling in both groups. Formal schooling was found only 20.0% and 25.0% in group I and group II respectively. The difference was not statistically significant (p>0.05) between two groups. Kim *et al.* [20] found in their study that birth cohort distributions for children in the disability registry/special education schools

and regular-schools groups were similar, with significantly more boys in the disability registry/special education schools 82.0% compared with 48.0% (p<0.001). In this present series it was observed that majority of the mother underwent caesarean section in both groups, which was 80.0% in group I and 75.0% in group II. The difference was not statistically significant (p>0.05) between two groups. On the other hand, it was observed that majority patient's had birth history uneventful in both groups, which was 90.0% in group I and 85.0% in group II. Eventful birth history was found 10.0% and 15.0% in group I and group II respectively. The difference was not statistically significant (p>0.05) between two groups. In this series it was observed that most of the fathers were master degree pass in both groups that was 65.0% in group I and 85.0% in group II. More than a half (55.0%) of the fathers was service holder in group I and 25.0% in group II. Most (70.0%) of the mothers were master's degree pass in group I and 75.0% in group II. Majority mothers had professional occupations. Eighty percent in group I and 75.0% in group II patients came from upper (20,000->50,000 tk) income family group. Single family was found 80.0% in group I and 65.0% in group II. Similarly, Rahman *et al*. [6] showed both of the parents (97.0% father and 79.0% mother) were highly educated. Most (62.0%) of the fathers were service holder, businessman 28.0% and doctor 11.0%. Majority (62.0%) of the patients came from upper income class, 33.0% middle class and 4.0% came from poor family. Typical symptoms of ASD, including repetitive mannerisms, impulsive acts, emotional outbursts, restricted interests, inflexible adherence to specific routines, and social communication deficits, were found to be correlated with executive dysfunctions in response selection, alteration, and inhibition [22]. Intervention that can reduce the executive dysfunctions of children with ASD may reduce their behavioral problems in daily life. Chan *et al*. [22] study showed that a specific diet modification based on the Chinese Chan medical approach had some positive effects on improving executive functions and typical behavioral symptoms of children with ASD. Their results are encouraging since commonly used behavioral interventions for ASD children are very time consuming and not cost-effective, while the present diet modification is less time consuming and more economical. In this current study it was observed that bottle feeding was found 20.0% in group I and 30.0% in group II. More than one third (35.0%) in group I and 25.0% in group II respondents mentioned that her child actively/voluntarily take food to mouth by himself. Thirty percent (30.0%) in group I and 35.0% in group II respondents mentioned that they passively feed her child while he is unmindful during watching the television or playing his favorite game. Ninety five percent (95.0%) in group I and 85.0% in group II respondents mentioned that he have an excessive affinity for junk foods such as, chocolates, chips, chanachur, aachar (prickles) etc. Ninety percent (90.0%) in group I and 75.0% in group II respondents mentioned that child distress trying new foods. One fourth (25.0%) in group I and 45.0% in group II respondents mentioned that obsession with familiarity, such as one particular make and flavor. Increase ammonia pre was found 70.0% in group I and 60.0% in group II. Increase lactate pre was observed 65.0% in group I and 55.0% in group II. The above findings about dietary question were almost similar between two groups regarding the. Many dietitians still remain uncertain on the effectiveness of the GF/CF diet in children with ASD [24]. With a growing number of referrals to the dietetic service regarding this area obtained by Bowers [27], more research is needed mentioned by Parker [24] to provide practicing dietitians with up to date information. Forty dietitians responded to the straw poll with the majority of practicing dietitians (62.5%) believing the GF/CF diet had a role in the management of ASD, however 17.5% did not answer this question indicating the uncertainty in this area reported by Parker[24]. The idea of food as medicine has also drawn increasing attention in western scientific research. A number of studies done by Craig [28]; Fraser [29]; Beezhold, Johnston, and Daigle [30] have supported the beneficial effects of a vegetarian or vegan diet in promoting the health of cardiovascular and digestive systems, reducing cancers and degenerative diseases and improving mood. Isaacs *et al*. [31] and Gale *et al*. [32] studies have also revealed a significant linkage between a balanced nutritional diet and level of cognitive functions and cognitive development in early life. However, many of them are observational studies and there is also counterevidence against the positive dietary effects obtained by Benton [33]. Therefore, it remains inconclusive in terms of the actual outcomes and the choice of type of diet. In this current study about the specific behaviors, it was observed that mean pre social relationship was found 2.86±0.37 in group I and 2.76 in group I. The mean post social relationship was 3.67±0.47 in group I and 3.87±0.51 in group II, which was significantly (P<0.05) improved. Mean pre per interaction was found 2.43+0.53 in group I and 2.28±0.48 in group II. The mean post peer interaction was 3.25±0.60 in group I and 3.29±0.53 in group II, that was also significantly (P<0.05) improved. Mean pre communication was found 2.58±0.61 in group I and 2.12±0.77 in group II. Mean post communication was found 3.77±0.61 in group I and 3.65±0.68 in group II, which was significantly (P<0.05) improved. Mean pre sensory a response was found 2.82+0.59 in group I and 2.96±0.75 in group II. The mean post sensory responses was found 3.58±0.55 in group I and 3.64±0.48 in group II which was significantly (PO.05) improved. All specific behaviors were significantly (p<0.05) improved within the groups from pre to post intervention period, but no significant (p>0.05) difference were found between the groups during post intervention period. Chan *et al*. [22] finding may suggest an alternative or complementary intervention for the executive control of behaviors among ASD children. It should be noted that the positive effect of diet modification was also applicable to the low-functioning children with IQ at or below 70 in their experiment. Specifically, there were 8 low-functioning children in the experimental group and 9 in the control group. Those in the experimental group showed improvement in tests of mental flexibility and inhibitory control with a large effect size (although some pre-post differences did not reach significance due to their small sample size). However, the low functioning children in the control group did not show such improvement in Chan *et al*. [22] study. Given that behavioral training is particularly difficult and time and labor intensive when applied on children with limited intelligence, diet modification that can be monitored by parents may therefore be considered as an alternative [22]. The underlying mechanism that may explain the change in behaviors was evaluated with an electrophysiological. method [22]. LORETA (low-resolution electromagnetic tomography) localization of theta activity

showed that after one month of diet modification, the ASD children demonstrated increased activation of the ACC (anterior cingulate cortex) specific to the rostral and subgenual subdivisions. The control group did not show such improvement in Chan *et al*. [22] study. A previous study done by Chan *et al*. [34] found that ASD children demonstrated hypoactivity in the ACC when performing a response-monitoring and inhibitory task (i.e., Go/No-go task) as compared to normally developed children. Chan *et al*. [22] study showed that a one-month diet modification was able to enhance the activity of the ACC while the child was performing the same task. The above finding suggests that the behavioral improvement of children with ASD may be associated with increased activity in the ACC; however, how diet can change and improve activity in the neural system warrants further investigation in future studies. Another case study done by Chan *et al*. [34] on a low-functioning autistic child demonstrated significantly improved inhibitory control and cognitive flexibility and increased EEG cordance (an index associated with cerebral perfusion) of the whole brain after Dejian mindbody intervention. Chan *et al*. [22] study has shed some light on the potential application of the Chinese Chanbased diet modification as a complementary intervention for rehabilitation of individuals with ASD and other individuals with emotional and cognitive problems. Chan *et al*. [34] study had shown the positive effects of a one month dietary modification on the executive functions and autistic symptoms of children with autism; however, its long term effect is still unknown which is worth further investigation. In addition, the sample size of the low-functioning subgroup is relatively small in their study; therefore, future studies with larger sample sizes will be helpful to verify the effect of diet modification obtained by the authors. Given the preliminary evidence on the effects of diet change in the low-functioning subgroups, it will also be worth investigating if this can benefit patients with severe brain disorders or physical disabilities (e.g., demented or stroke patients) in a well-controlled study. Last but not least, given the increasing research interest in food as medicine to improve health in Western countries, the applicability and effectiveness of this Chinese Chan-based dietary modification to the Caucasian population can also be investigated in future studies.

7. Conclusion

The improvements in behavior of children with ASD were almost similar between two groups. This randomized control trial (RCT) was carried out with an aim to find out any difference in behavior of Autism Spectrum Disorders (ASD) children before and after a dietary intervention and to find out the difference in behavior of study children between specific dietary intervention received and children not received this specific intervention as well as to find out the improvement in behavior of children having ASD between the two groups. This concept states that boys and girls brains begin to differ early in the uterus. These differences result in different strengths and weaknesses. ASC may be an example of the male brain development gone too far. In this present study it was observed that majority patients were came from Dhaka district in both groups (80.0% in group I and 90.0% in group II). The difference was not statistically significant ($p>0.05$) between two groups. Rahman *et al*. [6] mentioned that the children diagnosed with autism had parents from 96.0% urban and 4.0% from rural area. In this current series it was observed that most (70.0% group I vs. 65.0% group II) of the children had no schooling in both groups. Formal schooling was found only 20.0% and 25.0% in group I and group II respectively.

8. Limitations of the study

The study population was selected from one selected hospital in Dhaka city, so that the results of the study may not be reflect the exact picture of the country. The present study was conducted at a very short period of time. Limitations of this study include the relatively small proportion of children in the whole sample who received a full diagnostic assessment. Therefore, in future further study may be under taken with large sample size. The conservative approach of considering non participants as non-cases may have resulted in an underestimation of ASD prevalence in the high-probability Regroup. This study found a significant group of children with ASDs who were functioning at various levels in the general population while not receiving services, the general-population sample may vary qualitatively and quantitatively in different cultures and communities.

Acknowledgement: We acknowledged the Dhaka Shishu (Children) Hospital authority, and related personnel who was engaged in the Crosse sectional**.**

Conflict of interest: The Author has no conflict of interest of the study.

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