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Effectiveness of Supplementation of Gluten Free and Casein Free Foods with the Decreasing Symptoms of Autistic Children

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Abstract :
Autism is a disability that profoundly affects the way a child relates and communicates with other people around them. Autism spectrum disorder (ASD) is characterized by varying degrees of impairment of communication skills and social interaction, and unusual repetitive behavior. The present research work was taken up to investigate the effectiveness of gluten and casein free food products on the symptoms of autistic children. Fifteen autistic children aged between 6 to 12 years were purposive selected in this study. Formulation and sensory evaluation of gluten free and casein free food products was conducted and best was selected for supplementation. The study was followed for a period of six months. The assessment of autism symptoms was measured through different domains of Indian scale of autism, Hyderabad (ISSA) in pre and post study. Results about autistic children, who strictly followed dietary exclusion of gluten free casein free diet showed that a positive change in symptoms behavior skills, speech and language and cognitive components significantly.

Keywords – *Autism, Casein free, Gluten free, Psychological, Symptoms.*

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I. Introduction

Autism is a disability that profoundly affects the way a child relates and communicates with other people around them (National Autistic Society,1995). Autism spectrum disorder (ASD) is characterized by varying degrees of impairment of communication skills and social interaction, and unusual repetitive behaviour [1]. ASD can have a significant impact on quality of life since most children remain within the spectrum as adults and continue to experience problems with independent living, employment, relationships, and mental health [2].

Research has reported abnormal levels of peptides in the urine and cerebrospinal fluid of people with autism [3-4]. Intensified opioid activity would result causing the abnormalities of perception, cognition, emotions and behaviours that are observed in autism. This theory is often called 'the Opioid-Excess Theory'.

ASD children may be at an increased risk of nutritional deficiencies and poor diet quality because of picky eating, limited dietary diversity and gastrointestinal (GI) symptoms. Intakes of calories, fat, carbohydrates, calcium and phosphorus in children with ASD were less when compared with the recommended dietary allowance (RDA) by ICMR. Calcium as a molecule plays a significant role in the body, especially in the central nervous system. Calcium homeostasis, especially in the context of the central nervous system, may have crucial implications in many neuropsychiatry conditions. The hypothesis presented will explore the link between the blood-brain barrier (BBB) and calcium homeostasis (CH) as it is a complex, physiological process. Food is no longer valued from a nutritional point of view only, rather it is equally important from a health perspective. We can use food or food products to provide medical or health benefits, including the prevention and treatment of disease [5-6]. Subsequently, appropriate diets would facilitate learning, social behavior, cognitive functioning and communicative skills in individuals with autism.

Gluten-free and casein-free interventions exclude food that contains gluten (e.g. breads, pastas, pizza, crackers, cakes, cookies, oats/ cereals, etc., made from wheat, barley, and rye) and casein which is the phosphor protein present in milk (e.g., milk, cheese, cheese products, yogurt, ice creams, dips, sour cream, dressings, etc.). Seventy to eighty percent of people with autism have elevated levels of opioid peptides in their urine. These

peptides are psychoactive and are originated from improperly digested proteins mainly casein and gluten [7]. The Glutens are proteins found in the plant kingdom subclass of monocotyledon (monocots). These plants are members of the grass family of wheat, oats. The exorphins i.e. casomorphins and gluteomorphins or ghadorphin, which are produced by incomplete break down of casein and gluten are easily transferred across the lumen of the gut into the circulation where they exert opioid-type action on the brain [8-9].

Research demonstrates that a gluten free and casein free diet is somewhat effective in improving the symptoms of autism spectrum disorders. Aims and objectives of this study was:

1.Formulation of gluten free and casein free (GFCF) product according to need of autistic children.

2.Sensory evaluation of gluten and casein free products by semi trained panel and autistic children and best products were selected for supplementation.

3.Autism level assessment during onset of dietary intervention (pre study) and ending of dietary intervention (post study).

II. Materials and Methods

Fifteen children with autism were randomly selected from the Gwalior city, in which thirteen were boys and two were girls of age between 6-12 years. Out of which 7 children were in 6-7 years age group, 5 in 8-9 years group and 3 were in 10-11 years age group. Personal information, anthropometric measurements, dietary habits and clinical symptoms were examined of individual children. The subjects selected had mild and moderate autism. Subjects were on medicine for decreasing hyper activeness (Resperidon doze). Out of 15 children, 09 were under weight, 3 were overweight, 1 was obese. The parents were told to record the day to day food intake of the subject in the food diary.

Methods

I. AUTISM LEVEL ASSESSMENT

Assessment of autism symptoms were done by the Indian Scale for Assessment of Autism (ISAA) developed for the diagnosing and measuring severity of autism in 2009 by the National Institute for Mentally Handicapped (NIMH) Hyderabad. This scale was based on CARS scale and has 40 items divided under six domains as social relationship and reciprocity, emotional responsiveness, speech, language and communication, behavior patterns; sensory aspects and cognitive component. The items are rated from 1 to 5, increasing score indicating increasing severity of the problem. A score of <70 indicates no autism, 70-106 (mild autism), 107-153 (moderate autism), and >153 (severe autism). It takes about 15 to 20 minutes for administration of ISAA. The ISAA was devised with the aim of quantifying the severity of autistic symptoms so as to enable measurement of associated disability [10]. In this pilot study, we observe efficacy of gluten free casein free product on symptoms of autistic children. They are as follows:

1. Speech language and communication: Here 9 sub domains were present which are acquired speech and lost, has difficulty in non verbal language or gestures to communicate, repetitive use of language, echolalic speech, produce unusual noises, unable to initiate conversation to others, uses of meaning less words, uses pronoun reversals, unable to pragmatics of communication. As 9 sub domains, if these symptoms were had rare score was 1 and, sometimes for 2, frequently 3 mostly for 4 and always b 5. If these symptoms were rare then minimum score 9x1=9 and these were always then maximum score 9x5=45. This was same for other symptoms.
2. Behavior patterns: Here seven sub domains were present which are engaged in sterotyped and repetitive motor mechanisms, show attachment to inanimate objects, shows hyperactivity, shows aggressive behavior, throws temper tantrums, engages in self injurious behavior and insists on sameness. If these were rare then minimum score 7x1=7 and if always then maximum score were 7x5=35.
3. Cognitive component: Here four sub domains were present which are inconsistent attention and concentration, shows delay in responding, has unusual memory of some kind and has savant ability. If these symptoms were rare then minimum score was 4x1=4 and if always then maximum were 4x5=20 , as increasing score detects the severity of autism [11].This scale was applying to autistic children in pre and post study.

II. GLUTEN AND CASEIN FREE PRODUCTS FORMULATION

Adopting diets and tailored to individual disease symptoms, is linked to the nutritional requirements and food preferences of the ASD patient [12]. GF-CF diet consists in completely removing those products which are the main source of gluten as well as casein. Several studies were focused on the investigation of the use of cereal grains, pseudo cereals and legumes as alternative- wheat ingredients to produce high quality gluten- free baked products [13-15]. The Pauling theory stated that mental disorders can arise from various disruptions in the body such as from biochemical reactions, genetic factors, diet and deficiencies of vitamins

and/or minerals [16]. In turn, many studies demonstrate the need to supplement the nutritional deficiencies of autistic patients with vitamins and minerals in combination with medical and psychological interventions [17].

i. Gluten Free Casein Free Composite Flour Blends For Chapati

Flour is one of the components in food preparation and pharmaceutical mixes. The usage of flour in our daily diet is far more comparable than rice. Preparation of each experimental treatment of gluten-free (GF) composite flour blend was weighed, individually blended to be homogenized, packed in polyethylene bags, tightly closed and kept at chilled condition until ready for laboratory evaluation. In this study Rice flour (*Oryza sativa*), Sorghum flour (*Sorghum bicolor*) and Soyabean (*Glycine max*) flour were used as blends for the formulation of GF composite flour treatments.T1, T2, T3, T4 and T5 had varying percentage of rice flour, sorghum flour and soyabean flour respectively. Each GF treatment is equal to 100%. Table 1 shows the nutritional composition of these flours and table 2 shows the different formulation of composite flour blends with 0.5% salt and 2% binding agent.

Table 1: Composition of different flour

Flour	**Protein (gm)**	**Fat (gm)**	**Carbohydrate (gm)**	**Calcium (mg)**	**Phosphorus (mg)**
Rice flour	6.8	0.5	78.2	10	190
Sorghum flour	10.4	1.9	72.6	25	222
Soyabean flour	43.2	19.5	20.9	240	690
Wheat flour	12.1	1.7	69.4	48	335

Reference by Gopalan et al [18], Nutritive value of Indian foods, National Institute of Nutrition ,ICMR Hyderabad, 2012.

Table 2: Formulation of composite flour blends of chapati

TREATMENT	**Rice Flour**	**Soya Flour**	**Sorghum Flour**
T1	40	10	50
T2	**40**	**20**	**40**
T3	**40**	**30**	**30**
T4	40	40	20
T5	40	50	10

In the study of Hegazy et al [19], revealed that blend with 50% rice flour, 35% cornstarch, 7.5% defatted soy flour and 7.5% chickpea flour exhibited good sensory properties compared with wheat flour when used in the preparation of bread. Gluten-free yeast breads were produced based on rice flour (80%) and potato starch (20%) in the study of Ylimaki et al [20].

ii. Gluten And Casein Free Cookies

Cookies, among the bakery products, are most significant product in the world. These are an important food product used as snacks by children and adults. Cookies differ from other baked products like bread and cakes because of their low moisture content which ensures that they are free from microbial spoilage and confer a long shelf life on the product [21]. The ingredients used to produce the cookies in this study were: malted ragi flour, horse gram flour, coconut which were brought from Rajmata Vijayaraje Scindia Krishi Vishiwa Vidhyalaya. Rice bran oil and sugar were bought from local stores from the city of Gwalior. The base formulation to develop the cookies was proposed by Dhankhar P [22], with some modifications. Malted ragi are prepared by method Desai et al [23]. Nutritional composition of these ingredients given in a Table 3. Five trails were developed, listed on Table 4.

Table 3: Composition of Raw Material For Cookies

Flour	Protein (gm)	Fat (gm)	Carbohydrate (gm)	Calcium (mg)	Phosphorus (mg)	Iron (mg)
Malted Ragi flour	7.60	1.14	76.18	429.8	305.3	12.4
Horse gram flour	22.60	0.5	57.2	287	311	6.77
Dry Coconut powder	6.8	62.3	18.4	400	210	7.8

Reference malted ragi by Desai et al and Horse gram flour and coconut flour by Gopalan et al [18]

Table 4: Preparation of trials of cookies

Cookie Code	Ragi flour(gm)	Horse gram(gm)	Dry Coconut (gm)
Co1	50	50	-
Co2	-	50	50
Co3	45	45	10
Co4	40	40	20
Co5	50	25	25

Methodology: Before starting the study, a gluten free and casein free diet module was distributed to all mothers and guardians as a single pamphlet to stick in kitchens or near dining area, in which information was provided as to which foods are restricted and which foods are to be taken as substitute. A 24 dietary recall and three days food dairy were noticed continuously throughout the study. A screening questionnaire and Indian scale of Autism assessment were used for efficacy of GFCF food products on symptoms of autistic children. After sensory evaluation, best trial in overall acceptability was supplemented during the study. Supplementation of flour and cookies were started in the experiment and noted the results.

III. Results And Discussion

A. NUTRITIONAL COMPOSITION

From, Table 5, it is clear understood, as concentration of soya flour was increases, there was increase in protein, fat, calcium and Phosphorus content which was a dietary requirement for autistic children. But due to soya flour, its carbohydrate decreases vice versa with sorghum flour. According to nutritional composition, best nutritional value was of treatment T4 and T5 respectively. As soya flour is have high protein and fat, that's why a less fat content, and cheap flour as sorghum flour was chosen in this study.

Table 5: Nutritional composition of composite flour blends

Composite flour blends	Protein (gm)	Fat (gm)	Carbohydrate (gm)	Calcium (mg)	Phosphorus (mg)
T1	**15.52**	**3.1**	**69.67**	**40.5**	**256.0**
T2	18.32	4.86	64.50	62.0	302.8
T3	21.60	6.59	59.33	83.5	349.0
T4	24.88	8.38	54.16	105.0	396.44
T5	**28.16**	**10.84**	**48.99**	**126.50**	**444.22**

From Table 6, it is clear that highest protein was found in Co 1 because it were contain only high protein source malted ragi and horse gram flour, whereas highest fat was in Co2 as, it was combination of horse gram and coconut(1:1) and coconut was contained high amount of fat. Highest calcium content was found in Co 5, whereas highest phosphorus content in Co3. So, no cookies was provided marginal amount of all nutrients**.**
Data in above table also indicated that, these foods were rich in calcium although they are free of gluten/ casein and can supplemented to autistic children to fulfill their needs of calcium; the calcium has a good effect on brain function according to Atmaram et al [24].

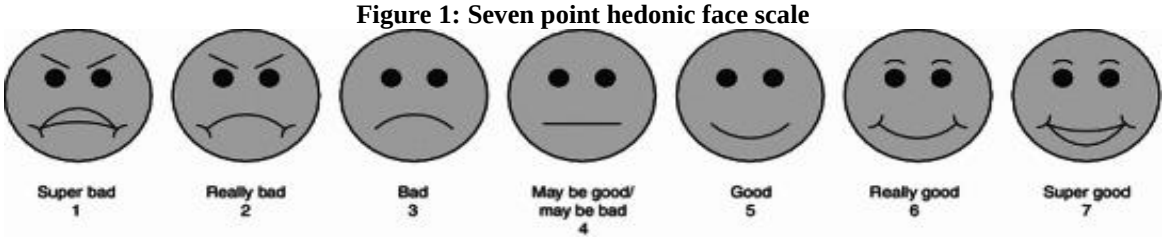
Table 6: Nutritional composition of cookies

Cookies	Protein (gm)	Fat (gm)	Carbohydrate (gm)	Calcium (mg)	Phosphorus (mg)
Co1	**14.80**	41.14	92.96	291.45	230.13
Co2	14.44	**71.27**	63.26	271.75	183.13
Co3	14.0	46.96	100.88	362.56	**298.42**
Co4	13.20	53.47	97.56	365.6	288.40
Co5	11.00	56.49	97.56	**386.65**	282.2

B. SENSORY EVALUATION OF THE GFCF FOOD PRODUCTS:

Sensory evaluation is considered to be a valuable tool in solving problems involving food acceptability, product improvement, quality maintenance and in a new product development . The sensory evaluations of products were made by semi trained panel of 15 judges as described by Amerine et al [25].
Seven point hedonic face scale was used to measure ASD children liking of the food product that exhibited the best nutritional and technological qualities according to Guinard [26].

Figure 1: Seven point hedonic face scale



The scale, shown in Figure 1, was graded from "really bad" to "really good". After the children had tasted the GFCF food product, they also answered if they would consider to eat the same sample again (yes, maybe, no). The best two trials were given to autistic children for sensory evaluation.

Table 7: Sensory evaluation of Chapati

COMOSITE FLOUR BLEND	Color	Odour	Texture	Taste	Over all acceptability
T1	8.2	7.4	6.7	7.2	7.37
T2	**8.4**	**7.3**	**6.9**	**7.4**	7.50
T3	**8.2**	**6.9**	**7.1**	**7.2**	7.35
T4	7.5	6.5	6.8	6.9	7.07
T5	7.1	5.9	6.4	6.9	6.57

From above Table 7, it is clearly understood that on increasing the soya flour composition and decreasing sorghum flour composition, the color and taste of chapati on hedonic decreases, because soya flour attribute to yellow color to chapati, which is not inanimate like wheat like chapati and also gives characteristic beany flavor which was not acceptable. Treatment T2 and T3, composite flour blends were gave mock chapati as of wheat chapati, which have same color, odor, texture and taste from over all acceptability. These two treatment T3 and T4 was given to sensory for autistic children and they selected T2 treatment as a somewhat chapati, similar to wheat flour chapati for as supplementation during the study.

Table 8: Sensory evaluation of cookies

Cookie code	Color	Odour	Crispiness	Taste	Over all acceptability
Co1	6.4	6.2	5.8	5.9	6.07
Co2	6.1	6.5	5.4	6.2	6.05
Co3	7.2	6.9	6.6	6.9	6.90
Co4	**7.5**	**7.4**	**7.6**	**7.7**	7.55
Co5	**7.4**	**7.6**	**7.7**	**7.6**	7.57

From Table 8, cookies Co1 and Co 2 were not at all acceptable at all in taste, as Co1 gave harsh flavor due to high malted ragi and horse gram without any flavor and Co 2 was too soft because it contain high amount fat content due to coconut powder, Whereas Co3, Co4 and Co 5 were acceptable in all hedonic parameters, as it contains all the ingredients in marginal amounts, and gave a soft, light crispiness cookie flavor. Co 3, Co 4 and Co5 have good overall acceptability. On the basis of overall acceptability, Co4 and Co 5 was given for sensory to autistic children and they selected Co 4 as more acceptable. That's why Co 4 was selected as supplements during dietary intervention. So, autistic children after sensory evaluation selected two supplements, which they liked which were Co 4 and treatment T2.

Table 9: Nutritional composition of supplemented gluten free casein free products

Daily supplementation	Protein (gm)	Fat (gm)	Carbohydrate (gm)	Calcium (mg)	Phosphorus (mg)
Chapati (100gm)	18.32	4.86	64.50	62.0	302.8
Cookies (4cookies)	6.60	26.73	48.78	182.5	144.20
Total supplementation	**24.92**	**31.59**	**113.28**	**244.50**	**447.00**
Required RDA for 6-12 children	**30-40**	**32-35**	-	**600-800**	-

Reference: Nutrient requirements and Recommended Dietary allowances for Indians (2010) [27]

Hence, Table 9, are the nutritional composition of supplemented GFCF products during dietary intervention with the total supplementation of nutrients and also the percent of nutrients provided according to RDA. Average requirement of flour of autistic children was 100 gm per days, as from 100 gm four chapati were formed, in which according to diet plan two chapati in morning and two chapati in dinner were given in a diet, whereas average monthly requirement was approx 3 kg of flour. Average requirement of cookies, as two cookies daily, so average 14 cookies per week. In 100 gm of composition, eight cookies were formed.

C. ASSESSMENT OF AUTISTIC SYMPTOMS:

Assessment of autism symptoms as three domains of ISSA[11] which are speech, language and communication, behavior patterns, cognitive components are given in Table 10, from where it is clearly understood that the three major symptoms of autistic children which were behavior patterns, speech, language and communication and cognitive components were found to improve. This result agrees with that of Whiteley and Knivesberg et al studies [28-29]. Out of fifteen children, seven children dropped out the gluten and casein free dietary intervention and only eight children successfully followed the intervention with supplementation. Out of eight children, only five children showed improvement in autistic symptoms [**GF1B, GF2B, GF6B, GF11B** and **GF15B]**. These children mainly belonged to 7-9 yrs age group. This indicates that nutrient requirement of younger children was fulfilled rather than that of elder ones. Secondly, it was also seen that BMI of these children also improved towards normalcy.

Table 10: Score of Autistic symptoms: Speech, language and communication; Behavior Patterns and Cognitive Components before and after dietary intervention

Children 's code	Speech, language and communication		Behavior Patterns		Cognitive Components	
	Before dietary intervention	After dietary intervention	Before dietary intervention	After dietary intervention	Before dietary intervention	After dietary intervention
GF1B	32	30	26	19	14	13
GF2B	25	23	13	12	09	08
GF3B	**21**	**0**	**20**	**0**	**12**	**00**
GF4B	36	36	22	20	15	15
GF5G	26	25	24	24	12	12
GF6B	19	15	12	9	10	09
GF7B	**29**	**0**	**21**	**0**	**12**	**00**
GF8B	**29**	**0**	**24**	**0**	**15**	**00**
GF9G	**28**	**0**	**17**	**0**	**09**	**00**
GF10B	**22**	**0**	**20**	**0**	**10**	**00**
GF11B	26	20	21	18	12	11
GF12B	22	18	22	17	11	11
GF13B	**28**	**0**	**23**	**0**	**14**	**00**
GF14B	31	30	26	25	17	15
GF15B	**24**	**0**	**22**	**0**	**00**	**00**

***0 = Drop out the diet**

Shattock and Whiteley state that putting a patient on a gluten free diet coincided with the improvements of the behaviors connected to schizophrenia [28]. A review of fourteen different experiments done on the gluten/ casein free diet assessed that "seven studies reported positive results (47%), four reported negative results (27%), and two reported mixed results (13%) [29]. Inevitably, the adoption of the GFCF diets may have some impact on families, including higher cost of special/alternative food products, extra time in food purchase/preparation, greater risk of nutrient deficiencies, and possible needs for additional supplements (e.g., calcium, vitamins, multivitamin with minerals). Atmaram et al., describes absence of organic deficits associated with conditions, such as pervasive developmental disorder (PDD), autism spectrum disorders (ASD), mental retardation (MR), and attention deficit hyperactivity disorder (ADHD) [24].

Mageshwari and Minitha [30] reported that 80% of intervention subgroup had behavioral improvements with majority improving in hyperactivity and digestion. Some authors found no group differences on behavioral/developmental measures for dietary intervention group [31-33]. Seung, found no significant group differences in verbal communication variables measured pre and post 6 week intervention [34].

Some authors found statistically significant differences in pre- and post-intervention behavioral ratings ie. reduction of ASD behaviors, and physiological and social symptoms [35-37]. Patel and Curtis [38], found improved behavior, social, motor, and GI symptoms; statistically significant reduction of urinary lead levels and 4 participants able to attend mainstream classes. Pedersen et al [39], found statistically significant regression analysis indicate children, ages 7 – 9 years, who have clinically significant ADHD-IV scores at baseline have strongest probability of benefiting from GFCF diet.

IV. Conclusion

It could be concluded that the results of this study clearly demonstrate that the gluten and casein free dietary intervention were successful. Out of fifteen children, improvements were seen in only five children as due to drop out of dietary intervention of seven children. Hence, a healthy substitute is very important for this dietary intervention to reduce the drop out the dietary intervention. Different formulations as a valuable food addition to enhance the nutritional qualities with organoleptic qualities of gluten and casein free products. They need a good source of dietary protein, crude fibers, calcium and phosphorus. Hence from effective dietary intervention with supplementation, found improvement in the autistic symptoms. Hence, implementation of a strict casein and gluten free diet showed symptomatic improvements in children with autism and lays the foundation for a diet that can markedly benefit the condition. All these notes led the necessity of training the mothers and teachers to be aware of suitable nutrition for autistic children.

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