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Research Article
Effect of Casein Gluten Free Snack, Nutrition Education and Level of Pb and Zn Metal on Clinical Manifestation of Autism

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Abstract

Background and Objective: People with autism in Medan are quite high due to the high Casein and Gluten intake, low Zn metal and high Pb metal. The aim of this study was giving Casein Gluten Free (CGF) Snacks snacks and nutritional education to reduce the symptoms of clinical manifestations of people with autism. **Materials and Methods:** The study was Quasi experimental with the design of post test only control group design with 4 autistic schools location in Medan. The sample was divided into 3 groups namely controls group, CGF Snack (group 1) and CGF Snack and Nutrition Education (group 2). Analysis was carried out with Pearson correlation tests and one way ANOVA. **Results:** Metal Pb was significantly associated with the occurrence of clinical manifestations ($p<0.05$) and had a very strong closeness ($r = 0.825$) and Zn metal was not significantly associated ($p = 0.141$). The group 2 made the most significant contribution ($p<0.05$) to the decrease of clinical manifestation. **Conclusion:** The results of the study showed that snack CGF and nutritional education were the most influential Pb metal had an effect, while Zn did not affect the clinical manifestations.

Key words: Autistism, snack casein and gluten free (CGF), nutrition education, heavy metal, clinical manifestations

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Autism syndrome is a developmental disorder in some parts of the brain such as; the cerebrum and hypothalamus which are indicated by qualitative disorders such as; the ability to interact socially, communication disorders and habits of doing a mindset, repetitive behavior and activity caused by the dysfunction of the dipeptidyl peptidase IV (DPP-IV) enzyme[1,2]. Heavy metals pro-oxidants such as; Pb in hair show chronic poisoning over the past 6 months that has an effect on the brain[3]. Low Zn (zinc) metal also influences metallothionein by suppressing inflammatory cytokines in the body with IL-6 and TNF-α production significantly[4]. Danuatmaja[5] stated that 80% of autistic people in Indonesia experience Pb poisoning due to air, water, soil and food pollution. In 2008, the ratio of autistic spectrum disorders was 1: 100. In 2012, the ratio was increased to 1:88 and predicted to increase by 500 people every year. In 2013, it was predicted that the number of people with autism spectrum disorders reached 3,000,000 people with a ratio of 6 out of 10,000 births[6]. In April, 2012 the number of autistic in north Sumatra ranged from 10,000 people[7] and in May, 2017 in Medan there were 13 autistic schools with the number of each of 15-35 people indicating high prevalence of autism in Medan.

The result of National Economy Social Survey/SUSENAS in 2002 showed that snack can contribute energy about 25% from nutritional adequacy rate/AKG. Research in 2012, involved treatment of egg addition to snack type of Garut Blondies to increase nutrient content and nutritional status of people with autism[8]. Other researches had involved the manufacturing of gluten and casein free biscuits[9].

Provision of CGF snacks are useful when accompanied by health promotion by providing nutrition education to mothers/ therapists who are competent to care for autism. Nutrition education can be performed with persuasive, appeal, invitations to provide information, awareness and training[10]. Forms of treatment performed on children with autism revolve around educational therapy or diet modification of CGF snacks and heavy metals. A previous study stated that a lot of autism's mothers feel it difficult to adhere to dietary rules and even do not implement diet of CGF[11]. Giving CGF snack and nutrition education like counselling and training can help the autism and their therapist to obey the casein and gluten free diet. Hence, the purpose of this research was providing CGF snacks and nutritional education to minimize the symptoms of clinical manifestations of people having autism.

MATERIALS AND METHODS

Population and ethical approval: The study between heavy metal (Pb and Zn) and clinical manifestation was a cross sectional study and the study with treatment of giving CGF snack was a Quasi experimental with post test only. Three groups namely control group, snacking group (group 1) and snacking and nutrition education group (group 2) were designed[12]. Each group consisted of 20 respondens. There were 4 autism schools for this study namely Yayasan Pondok Peduli Autis, Rumah Sarah Terapi, Yayasan Tali Kasih dan Bina Ananda Mandiri. Data collecting was carried out on September 1st-October 15th, 2017. This study had fulfilled the research code of ethics as evidenced by letter number 044/KEPK/POLTEKKES KEMENKES MEDAN/2017.

Clinical manifestation: Interview techniques with 35 questions were used to obtain clinical manifestation data. If the answer was less convincing, an in-depth interview had been conducted. Every "yes" answer for symptoms of clinical manifestation was given 1 point.

Level of pre-oxidant (Pb) and antioxidant (Zn) on hair: Atomic Absorbance Spectrophotometer (AAS)[13] method was used to examine pre-oxidant (Pb) and anti-oxidant (Zn) metals in hair samples. The hair weighed 50 mg with analytical scales was inserted in a clean plastic tube and labeled with numbers and dates. Then triton X 1% solution and 15 mL Triton X 1% were shaked with a mechanical mixer for 30 min and poured on a plastic filter. At rewards, it was rinsed with spray ±100cc deionized. Then, it was dried in an oven 1100°C for 12 h, initial weight was measured and 10 mL of concentrated HNO_3 was poured. It was then dried with sand bath until it was close to dry, 2% HNO_3 was poured with a measuring pipette. The original bottle was moved according to the label and number. The bottle was then covered with parafilm and it was got ready to be prepared on the Atomic Absorbance Spectrophotometer (AAS) with the brand Shimadzu / AA-6200.

CGF snack: The CGF snack consisted of 20 types and was based on local habits. Snack processing was carried out in a diet laboratory in the field of nutrition at the Medan Health Polytechnic. The CGF Snacks were given directly by researchers assisted with enumerators for 21 days each 2 times a day, namely at 10:00 WIB and 16:00 WIB. Supervision of snack consumption was carried out by the closest people who had been briefed and got paid by researchers called the Treatment Consumption Supervisor (PPT).

Nutrition education: Nutrition education in the form of counseling on the autistic diet was carried out for 21 days as much as 6 temes (2x a week) and every meeting consisted of 3 h. Snack-making training was conducted twice during the study period with the help of booklets.

Statistical analysis: The normality test with Kolmogorov Smirnov was carried out before carrying out bivariate analysis, because the data were normally distributed to see the relationship between Pb and Zn metal to clinical manifestations using Pearson correlation and to see differences in clinical manifestations between treatment groups (control group, treatment group 1 and treatment group 2) using One Way ANOVA.

RESULTS

Sample characteristics: Characteristics of the sample included age and sex, which amounted to 20 peoples from each treatment. The percentage of sample age was pre-dominantly 5-12 years old in treatment group 1 (60%), treatment group 2 (100%) and control group (85%). The sex (%) of most of the sample of male sex in each treatment was in the treatment of group 1 as much as 95% (19 people), the treatment of group 2 was 95% (19 people) and the control group was 80% (16 people) (Table 1).

Characteristics of respondents: Characteristics of respondents included education and activities. Education of respondents mostly dominated by high school education 31 people (51.67%). The data in Table 2 also showed that some respondents did not work or only as housewife of 41 peoples (68.3%).

Pro-oxidant (Pb) and antioxidants (Zn): The mean of Pb was 24.068 ppm and the mean of Zn was 226.8 ppm (Table 3).

Clinical manifestations: Collection of signs or symptoms that arise in people with autism is called clinical manifestations. Each symptoms that arises is given a score of 1. The results in Table 4 showed that the average score of the largest reduction in clinical manifestations occurred in group 2 with a difference of 6.65 compared to group 1 and controls of 2.65 and 0.65, respectively.

Metal Pb was significantly associated with the occurrence of clinical manifestations ($p<0.05$) and had a very strong closeness ($r = 0.825$) and Zn metal was not significantly associated ($p = 0.141$) (Table 5).

Table 1: Sample characteristics including age and sex

Sample characteristics	Control		Group 1		Group 2	
	Number	Percentage	Number	Percentage	Number	Percentage
Age (years)						
5-12	17	85	12	60	20	100
>12	3	15	8	40	0	0
Sex						
Male	16	80	19	95	19	95
Woman	4	20	1	5	1	5

Table 2: Distribution of characteristics of respondents

Characteristics of respondents	Number	Percentage
Education		
Elementary school	1	1.67
Junior high school	2	3.33
Senior high school	31	51.67
University	26	43.33
Activity		
Employee	19	31.70
Unemployee	41	68.30

Table 3: Levels of pro-oxidant metals (Pb) and antioxidants (Zn)

Indicators	n	Minimum	Maximum	Mean	Deviation
Pb level	60	0.86	127.69	24.068	21.84095
Zn level	60	66.46	708.32	226.800	129.38195

Table 4: Distribution of clinical manifestations

Groups	Clinical manifestations	N	Minimum	Maximum	Mean	Deviation
Control	Before	20	5	27	15.00	7.427
	After	20	5	26	14.35	7.140
	Difference	20	-2	3	0.65	1.182
Group 1	Before	20	2	27	13.45	6.048
	After	20	2	24	10.80	5.625
	Difference	20	0	4	2.65	1.040
Group 2	Before	20	3	26	12.75	5.946
	After	20	2	12	6.10	2.594
	Difference	20	1	14	6.65	3.689

Table 5: Pb and Zn levels with clinical manifestations

Indicators	N	r-value	p-value
Pb levels with clinical manifestations	60	0.825	0.001*
Zn levels with clinical manifestations	60	0.192	0.141

*Significant

Table 6: Analysis of clinical manifestations with various treatment

Parameters	Time	Control	Group 1	Group 2	Total
Clinical manifestations	Before	15.00±7.427[a]	13.45±6.048[a]	12.75±5.946[a]	13.73±6.467
	After	14.35±7.14[a]	10.80±5.625[a]	6.10±2.594[b]	10.42±6.355
	Difference	0.65±1.182[a]	2.65±1.04[b]	6.65±3.689[c]	3.32±3.392

[a,b,c]Different notations show significant differences (p<0.05)

Relationship between giving CGF snacks and nutrition education with clinical manifestations: The results of the one-way ANOVA analysis showed that there was a decrease in the symptoms of clinical manifestations at CGF snacks (group 1). However, the CGF snack treatment and nutrition education (group 2) made the most significant contribution (p<0.05). The decrease in difference due to group 2 was the largest, namely 6.65±3.689 (Table 6).

DISCUSSION

The results of the study found that most autistic people are male in each treatment, namely in the treatment group 1 as much as 80% (16 people), treatment group 2 as much as 95% (19 people) and in the control group as much as 70% (14 person). The results of this study are in line with several previous researchers who stated that prevalence of autistic patients were more commonly found in men compared to women, then Pratiwi continued in 2013 with research found from 30 research subjects found 25 subjects with male[14]. Generally people with autism are 2-4 times more common in men than women[15]. This is thought to be due to the presence of genes or several genes on the X chromosome involved with autistic persons. Men only have one X chromosome, so they don't have a backup when another X chromosome is abnormal. A number of studies suggested that genes on the X chromosome are not the main cause of autism, but one gene on the X chromosome that influences social interactions can have a share of behaviors related to people with autism[16].

Communication, psychosocial and psychomotor disorders in people with autism usually begin to be detected at the age of >3 years[17]. This study also found that the age ranged between 5-19 years old, where this was also found in a study in Bandung which stated that persons with autism from a special school of autism having age >3 years, the same thing was also found by research in Yogyakarta, in which a sample of autistic people from special schools ranging in age from 5-12 years was involved[18]. Previous study stated that autistic people were also required to get proper education such as; other normal persons and samples obtained were aged 7-18 years[19].

The results showed that Pb metal was significantly associated with the occurrence of clinical manifestations (p<0.05) and had a very strong closeness (r = 0.825), but Zn levels were not significantly associated (p = 0.141). Significant relationship between Pb and clinical manifestations is in line with research in Makassar[20]. As many as 75% of the samples experienced neurotransmitter disorders due to disruption of the central nerve so that samples became more reactive such as crying, tantrums, screaming, repeating, not concentrating/focusing and not feeling afraid. This is in line with Reissmann's[18] research, who said poisoning due to heavy metals caused a disturbance in the normality of neurotransmitters and amygdala in parts of the brain[18]. Heavy metal Pb is toxic and should not be present in the human body including autistic. The Pb comes from pollution caused by motorized vehicles, factory smoke, ceramic flooring or water[21]. The Pb is an inorganic substance absorbed through

the digestive tract and lungs. Measurement of Pb levels in hair shows chronic poisoning over the past 6 months[5]. The non-significant relationship between Zn levels and clinical manifestations in this study is in line with the results of a study in Malang who found high levels of Zn in hair in autistic patients, but it is contrary to the results of previous research which stated that Zn levels of autistic are lower than normal people[22,23]. Zn metal functions as an antioxidant that protected the body from attack by lipid peroxidation. Low levels of Zn caused the body not to bind free radicals so that the immune system decreases. Changes in intestinal flora and abnormalities of absorption functions that interfere with myelin formation in the brain, which can aggravate hyperactivity in autistic patients can also be caused by low levels of Zn[24-26]. The results of one way ANOVA analysis showed that there was a decrease in the symptoms of clinical manifestations at CGF snacks (group 1), where the notation shown was in the notation b, but the CGF snack treatment and nutrition education (group 2) made the most significant contribution ($p<0.05$). This is in line with previous research, which provided a gluten-free and casein-free diet for 3 months, showing 81% of autistic experienced significant behavioral changes. Previous research also stated that gluten and casein free diets can reduce hyperactive behaviour and improve digestion in autistic[27-29].

Giving CGF snacks in stages aimed to reduce the symptoms of autism and avoid the addictive effect[9]. Giving snacks CGF of 50-60 g for 21 consecutive days except Sunday also serves as an exercise for autistic to get used to consume gluten and casein free foods. Autistic cannot digest either gluten and casein types of peptide, so that the peptide will circulate in the blood and bind to opioid receptors in the brain that cause behavioral abnormalities. Provision of gluten-free diets and casein have been shown to reduce behavioral abnormalities in people with autism[30,31]. Giving CGF snacks accompanied by providing nutrition education to parents/therapists showed positive results for clinical manifestations. Nutrition education in this study was carried out 6 times, once at the beginning, 4 times in the middle and once at the end of study. The form of nutrition education is counselling in class and counselling per individual with giving leaflets. Training on making CGF snacks was given twice during the study. Nutrition education is expected to change the behavior of parents/therapists in the provision of diet in autistic. This study is in line with previous research, which stated that training on diet can change the behavior of parents with autism[32]. Sarwono[33] research, in which training to mothers was provided, showed amplified role of mothers in monitoring and supporting autistic to behave better. Hidayati[34] research provided material related to autism and has an impact on changes in the behavior of people with autism[34]. The findings of this study can be applied to people with autism in various parts of the region in Indonesia by prioritizing snacks based on local food wisdom.

CONCLUSION

This study found that treatment of giving CGF snacks accompanied with nutritional education was very effective in reducing symptoms/signs of clinical manifestations. Metal Pb had a significant influence on symptoms/signs of clinical manifestations while Zn metal had no effect on the clinical manifestations of persons with autism.

SIGNIFICANCE STATEMENT

The result of this study can be an alternative in reducing the clinical manifestations of autistics by giving CGF snacks with local ingredients. The effectiveness of giving CGF snacks increased when accompanied by the provision of nutrition education in the form of counselling in class, individual counseling and CGF snack making training. This research can be an inspiration for the world to create CGF snacks made from local ingredients.

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