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Gluten Sensitivity among Egyptian Infants with Congenital Heart Disease

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Abstract

BACKGROUND: Gastrointestinal symptoms are a common feature in infants with congenital heart disease.

AIM: This study was designed to evaluate age-dependent serum levels of antigliadin antibodies among malnourished Egyptian infants with congenital heart disease (CHD) and gastrointestinal symptoms.

SUBJECTS AND METHODS: This case-control study conducted on 60 infants with established congenital heart disease. They were subdivided into cyanotic and acyanotic groups, and each group includes 30 patients compared with thirty apparently healthy infants of matched age, sex, and social class. Serum antigliadin antibodies levels were measured using ELISA.

RESULTS: The mean age of introduction of cereals in the diet and appearance of gastrointestinal symptoms were six months. On comparison with controls, patients showed highly significant higher serum levels of antigliadin antibodies ($P < 0.000$). On analysing risk factors using odds ratio, the age at onset of GIT symptoms, diarrhoea, abdominal pain, and distension had been found to be significantly associated with high serum antigliadin antibodies among malnourished CHD infants with a prediction of 95%.

CONCLUSION: Serum IgA, IgM, and IgG class antibodies to gliadin play a significant role in the pathogenesis of malnutrition in infants with CHD. Gluten containing foods should never be introduced before the end of the six months.

Introduction

Congenital heart diseases (CHD) are documented in 0.8 % of all live birth infants. They are characterised by gross structural abnormalities of the heart or the great vessels that interfere with normal cardiac function [1]. Malnourished infants with CHD showed growth retardation, frequent hospitalisation, poor surgical outcomes and higher mortality rate [2]. They also showed poor food intake, malabsorption, increased requirements, and higher metabolic rate in the first year of life [3]. Retarded growth was accompanied by frequent diarrhoea attacks and infectious diseases [4].

Gliadin is part of the gluten protein found in the grains wheat, barley, rye, and oats. It is a unique protein based on its structure that lends a doughy, elastic consistency to flours derived from these grains. Some children have gluten protein intolerance, which may be attributed to enhanced T-cell-mediated immune reaction in the proximal small bowel that damages the villi of the small intestine and leads to nutrients malabsorption [5]. The inflammatory response continues as long as patients continue to ingest protein [6]. The Gluten sensitivity usually manifests in childhood, and symptoms include failure to thrive, diarrhoea, and abdominal pain. Subclinical cases may have no overt gastrointestinal symptoms but suffer osteopenia, anaemia, and irritability [7, 8].

Studies to date regarding the immune response to gluten in infants with CHD and its association with gluten sensitivity have been

inconsistent. Therefore, this study was planned to evaluate age-dependent serum levels of IgA, IgG, and IgM antigliadin antibodies among malnourished Egyptian infants with CHD and gastrointestinal symptoms and to investigate if these antibodies have any relation to growth, nutritional status, and gastrointestinal symptoms.

Subjects and Methods

Design and Setting of the study

This case-control study was conducted on 60 infants with congenital heart disease (CHD) and recurrent gastrointestinal symptoms (40 % girls and 60% boys) who were attending the Nutrition Clinic of the Center of Excellence, National Research Center (NRC) for nutritional management of malnourished patients with CHD over a period of one year according to inclusion criteria. They were referred from the outpatient Pediatric Cardiology Clinics of the National Cardiac Institute, Egypt, during their regular follow up. The sample size was calculated to detect the mean differences in the scores of the factors probably affecting growth, nutritional status of infants with congenital heart disease (CHD).

Subjects

Congenital cardiac defects were diagnosed by two-dimensional echocardiography. They were classified into two subgroups according to the presence or absence of the cyanosis into two subgroups; thirty cyanotic patients in subgroup I, and thirty acyanotic patients in subgroup II compared with thirty apparently healthy infants of matched age, sex, and social class. The inclusion criteria for selection included malnourished infants with uncorrected symptomatic congenital cardiac defects and history of gastrointestinal symptoms. The exclusion criteria included infants with palliated or corrected CHD, confirmed or suspected genetic syndromes, hospitalised, and infants with asymptomatic CHD. Written informed consent was obtained from the parents of the participating infants.

Methods

Information on age, parental consanguinity of CHD, duration of illness and treatment modalities were collected via a questionnaire from parents. All the studied patients were subjected to through history taking, including onset of the cyanosis, hypercyanotic spells, tachypnea, feeding difficulty, poor weight gain, repeated chest infections, gastroenteritis, and congestive heart failure. Patients and controls were subjected to a complete physical examination, nutritional assessment, anthropometric measures, and laboratory investigations that were done at the National Research Center.

The anthropometric measures included measurement of body weight, recumbent length or height, body mass index (BMI), occipitofrontal, mid-arm, and mid chest circumferences. The body weight was determined to the nearest 0.1 kg on a sea scale balance with the subject dressed minimum clothes and no shoes. Heights or recumbent length (for infants <2 years of age) were measured using Seca mechanical infantometer. The mid-upper arms, mid-chest and occipitofrontal circumferences were measured with a measuring tape using standard procedures. Each measurement was taken as the mean of three consecutive readings as recommended by the International Biological program [9].

The anthropometric analysis for all infants was accomplished through the calculation of Z-scores, based on the WHO growth standards [10], and Anthro 2007© software [11]. Z-scores were calculated for the following rates: weight/age, weight/length, length /age. The following were adopted as cut-off points for the z-values: normal values between two units of a standard deviation below and above the average value. In all cases, a Z-score of less than -2 was considered as the cut-off point for malnutrition. Values between ± 1 and ± 2 SD units of standard deviation constituted the zone of risk.

Biochemical measurements

From all cases, and controls five cc venous blood samples were obtained for laboratory assays, which were performed in the National Research Center. Serum antigliadin IgA, IgG, and IgM antibodies levels were measured by enzyme-linked immunosorbent assay (ELISA) commercial kit according to the method of Trocone and Ferguson [12]. The cutoff value was calculated from healthy control samples.

Serum calcium concentration was assayed by a colorimetric method according to the method described by Endres and Rude [13]. Samples for assaying serum alkaline phosphatase activity (ALP) were kept at room temperature and assayed according to the manufacturer's guidelines [14]. Haemoglobin level was measured using Hemoglobin Photometer [15]. Serum iron and total iron binding capacity were measured according to the method described by Perrotta and Kaplan [16].

Statistical analysis

Statistical analysis was performed using the SPSS statistical package software for Windows version 21 (SSPS Inc, Chicago, USA) and the results were presented as tables and figures. Quantitative

variables are expressed as the mean ± SD.

Categorical data were expressed as frequencies and percentages and were analysed with the two-tailed chi-square test. Correlations between continuous variables were done using Pearson correlation. The comparison between groups was performed with one-way analysis of variance (ANOVA). Univariate analysis of each covariate (item by item) was performed to identify significant of high serum levels of antigliadin antibodies in malnourished patients with CHD and gastrointestinal symptoms. A P value < 0.05 was considered significant and p < 0.005 was considered highly significant.

Results

This study comprised 60 patients with established CHD. Their ages ranged from 4-12 months (mean 8.72 ± 6.68 months) were enrolled in the present study. They were 36 boys (60 %) and 24 girls (40%) with a male to female ratio 1.5:1. Cyanosis was detected in 30 patients (50%). About 60% of the studied patients were on bottle feeding, and 40% patients were breastfed.

Cereals were introduced at a mean age of 6 months (ranging from 4 to 8 months), and the mean age of onset of gastrointestinal symptoms was six months. Such symptoms entailed chronic diarrhoea in 58 (96.7%), vomiting in 26 (43.3%), abdominal pain in 38 (63.3%), and abdominal distension in 14 (23.3%). Growth failure was seen in 48 patients (80%), pallor in 44 patients (73.3%), and rickets in 34 patients (56.7%). Thirty patients (50%) received anti-failure medications. All these clinical findings concerning the patient's group are shown in Table 1.

Table 1: Clinical findings of the studied patients

Variables	No (%)	Variables	No (%)
Male	26 (43.3%)	Pallor	44 (73.3%)
Female	34 (56.7%)		
Positive consanguinity	24 (40 %)	Rickets	34 (56.7%)
Diarrhea	58 (96.7%)	Repeated chest infections	12 (20%)
Vomiting	26 (43.3%)	Anti-heart failure medications	30 (50%)
Abdominal pain	38 (63.3%)	Abdominal distension	14 (23.3%)

The mean measurements of z scores of weight for age, weight for height, height for age, and the circumferences of occipitofrontal, mid arm, and mid chest of the studied patients were statistically highly significant lower compared to controls (P < 0.001). The mean weight for age, weight for height Z-scores, circumferences of occipitofrontal, mid arm of the cyanotic group were statistically significant lower about the acyanotic group (P < 0.05). Table 2 demonstrates the anthropometric measures of patients versus control.

Table 2: Comparison of anthropometric measures of the studied patients, and control groups

Variables	Total Patients N=60	Cyanotic subgpI n=30	Acyanotic subgpII n=30	Control Group N=30	subgpI versus subgpII	Total patients versus controls
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	P value	P value
Occipitofrontal circumference(cm)	42.93±3.59	41.53±3.86	44.33±2.76	46.4±3.55	0.03*	0.002**
Mid-arm circumference(cm)	12.2±1.66	11.43±1.69	12.97±1.27	13.49±0.9	0.002**	0.003**
Mid-chest circumference(cm)	44.0±4.38	43.27±5.2	44.73±3.42	49.85±3.31	0.32	0.000**
Weight for age z-score	-2.55±1.27	-3.18±0.9	-2.02±1.29	-0.25±0.42	0.003**	0.000**
Height for age z-score	-2.62±1.4	-3.07±1.28	-2.38±1.35	0.25±0.55	0.107	0.000**
Wt for Ht z score	-0.75±1.0	-1.34±0.88	-0.28±1.07	-0.24±0.65	0.001**	0.03*

*Significant difference at p<0.05, **highly significant difference at p<0.005.

The patients' group demonstrated a statistically highly significant increase in serum levels of IgA, IgM, and IgG class antibodies to gliadin on healthy controls (P < 0.000). ANOVA test revealed statistically highly significant rise in the serum levels of IgA, IgM, and IgG class antibodies to gliadin, alkaline phosphatase activity, total iron binding capacity, and statistically highly significant reduction in blood hemoglobin, serum calcium and iron levels between the patients' subgroups and controls with the lowest value in the cyanotic group (P < 0.001) as shown in Table 3.

Table 3: Comparison of laboratory findings of the studied patients, and control groups

Variables	Cyanotic subgroup I n=30	Acyanotic subgroup II n=30	Control Group N=30	ANOVA	
	Mean ±SD	Mean ±SD	Mean ±SD	F	P
Serum antigliadin IgA (IU/ml)	151.95±59.4	150.81±27.84	92.07±2.67	104.17	0.000**
Serum antigliadin IgG (IU/ml)	111.95±109.83	142.16±138.57	70.77±17.51	1.4	0.251
Serum antigliadin IgM (IU/ml)	3.9±2.63	2.8±1.52	1.1± 0.33	431.96	0.00**
Serum calcium (mg/dl)	8.35±0.23	8.46± 0.52	9.38±0.29	31.35	0.00**
Serum alkaline phosphatase (IU/l)	336.53±84.1	380.71±110.56	106.4±14.38	66.702	0.00**
HB (gm/dl)	13.78 ± 1.8	11.54 ± 1.7	13.05 ± 0.77	5.495	0.007**
Serum iron (mcg/dl)	40.56±8.03	33.40±6.10	73.5±8.75	138.22	0.00**
Serum TIBC (mcg/dl)	432.0±14.52	388.53±37.44	285.4±27.4	109.28	0.00**

*Significant difference at p<0.05, **highly significant difference at p<0.005.

In the patient's group, serum IgM levels showed significantly negative correlation with serum calcium levels, and height for age z- score (P < 0.05). Serum antigliadin IgG levels were significantly positively correlated with serum alkaline phosphatase activity, and negatively correlated with z- score of weight for age. Correlations between anthropometric measures and serum antigliadin antibodies of the studied patients are shown in Table 4.

Table 4: Correlations between weight and height for age z-score, some laboratory measures and serum antigliadin antibodies of the studied patients

Variables	Weight/age z-score	Height / age z-score	Serum calcium	Serum alkaline phosphatase
Serum antigliadin IgM (IU/ml)	-0.107	-0.265*	-0.331*	0.117
Serum antigliadin IgG (IU/ml)	-0.261*	0.111	0.189	0.292*
Serum antigliadin IgA (IU/ml)	0.105	0.162	0.031	0.036

*Significant difference at p<0.05, **highly significant difference at p<0.005.

Table 5: Univariate analysis between GIT symptoms and serum antigliadin IgA antibodies levels in the studied patients

Covariates		Serum antigliadin antibodies (IgA) High 26 (60%) No %	Normal 34 (40%) No %	P value	Odd ratio (95% CI)
Age at onset of symptoms	Before 6 month	8 (30.8%)	22 (64.7%)	0.04*	4.13 (0.88,19.27)
Diarrhea	Negative	4 (15.4%)	26 (76.5%)	0.02*	0.06 (0.01, 0.37)
Abdominal pain	Positive	20 (76.9%)	22 (64.7%)	0.01*	0.55 (0.11, 2.80)
Abdominal distension	Positive	20 (76.9%)	10 (29.4%)	0.01*	0.13 (0.02, 0.66)

*Significant difference at $p < 0.05$, **highly significant difference at $p < 0.005$. Gastrointestinal symptoms (GIT).

On analysing risk factors using odds ratio, the age at onset of gastrointestinal symptoms in the form of diarrhoea, abdominal pain and distension were documented as a significant association of raised serum levels of antigliadin antibodies in the patients with a prediction of 95% as shown in Tables 5, 6, 7.

Table 6: Univariate analysis between GIT symptoms and serum antigliadin IgG antibodies levels in the studied patients

Covariates		Serum antigliadin antibodies (IgG) High 12 (20%) No %	Normal 48 (80%) No %	P value	Odd ratio (95% CI)
Age at onset of symptoms	Before 6 month	16 (38.1%)	14 (77.8%)	0.04*	5.69 (0.94, 34.46)
Diarrhea	Negative	0 (0%)	30 (62.5%)	0.02*	0.06 (0.01, 0.37)
Abdominal pain	Positive	10 (83.3%)	32 (66.7%)	0.01*	0.55 (0.11, 2.80)
Abdominal distension	Positive	12 (100%)	18 (37.5%)	0.01*	0.13 (0.02, 0.66)

*Significant difference at $p < 0.05$, **highly significant difference at $p < 0.005$. Gastrointestinal symptoms (GIT).

Discussion

The poor growth seen in infants born with complex heart defects may result from factors beyond deficient nutrition [17]. The cause is not yet identified. However, it may be a consequence of a disordered immune response to gliadin proteins in genetically predisposed infants or may be attributed to the early introduction of cereals in the infant's diet before the age of 6 months, yielding higher levels of antibodies against such proteins [6]. There are three types of antigliadin antibodies, IgA, IgM, and IgG. IgA antibody is specific, and the IgG antibody is a sensitive marker of gluten sensitivity.

Table 7: Univariate analysis between GIT symptoms and serum antigliadin IgM antibodies levels in the studied patients

Covariates		Serum antigliadin antibodies (IgM) High 42 (70%) No %	Normal 18 (30%) No %	P value	Odd ratio (95% CI)
Age at onset of symptoms	Before 6 month	20 (38.1%)	14 (77.8%)	0.04*	5.69 (0.49, 4.46)
Diarrhea	Negative	20 (47.6%)	10 (55.6%)	0.60	0.73 (0.15, 3.49)
Abdominal pain	Positive	10 (83.3%)	2 (11.1%)	0.00**	0.01 (0.00, 0.11)
Abdominal distension	Positive	26 (61.9%)	4 (22.2%)	0.04*	0.18 (0.03, 1.07)

Measurement of the combined antibodies provides a specificity of 84% and a sensitivity of 94% for the diagnosis of gluten sensitivity disease [18].

Up till now, there is no available information in the literature regarding the presence of serum antigliadin antibodies among malnourished infants with congenital heart diseases.

Our study comprised sixty patients suffering from CHD. They were diagnosed by clinical examination, echocardiography, and other routine tests. Such patients were further subdivided into acyanotic and cyanotic subgroups. The commonest cyanotic lesions were tetralogy of Fallot (TOF) and transposition of the great arteries (20% in each). Almost 5 % of our patients suffered from pulmonary atresia with ventricular septal defect (VSD), and 5 % presented with double outlet right ventricle (DORV) with malposed great vessels.

Ventricular septal defect (VSD) was the most frequent acyanotic lesions (20%), 15% of our patients were diagnosed as patent ductus arteriosus (PDA), 10% as atrial septal defect (ASD), and 5% as an atrioventricular canal (A-V canal).

About 60% of our patients were on mixed feeding, and 40% patients were breastfed. Cereals were introduced at a mean age of 6 months (ranging from 4 to 8 months) and mean age of onset of gastrointestinal symptoms was six months. Such symptoms included chronic diarrhoea in (96.7%), vomiting in (43.3%), abdominal pain in (63.3%), and abdominal distension in (23.3%). Growth failure was seen in (70%), pallor in (73.3%), and rickets in (56.7%) of patients. The earlier onset of gastrointestinal symptoms in our studied patients was not in agreement with Assiri et al., [19] who found that, gastrointestinal symptoms started at a mean age of 57.2 months (ranging from 4 to 156 months) and manifested in 54% as chronic diarrhea, in 22.2% as vomiting, in 17.5% as abdominal pain, and in 3.2% patients as abdominal distension. Growth failure was detected in 74.6% patients. The early introduction of cereals in our patients may be responsible for the early appearance of gastrointestinal symptoms.

In view of our data, the mean measurements of z-scores of weight for age and height for age, weight for height, as well as the circumferences of occipitofrontal, mid arm, and mid chest of the patients group showed statistically highly significantly decrease when compared to controls ($P < 0.001$), and statistically significantly lower in cyanotic group than acyanotic group ($P < 0.05$). Severe malnutrition was found in thirty-three (55%) of the studied patients, while moderate malnutrition was shown in twenty-seven (45%). Thirty-six (60%) of our patients manifested a decreased WHZ (wasting), which was proportionately more documented in the cyanotic group ($P < 0.001$). Our results are in agreement with WHO reports, which demonstrated that malnutrition manifests mainly as wasting rather than underweight and stunting [20].

Studies concerning malnutrition patterns amongst patients with CHD yielded incontinent results

(21-23). In South India, Vaidyanathan et al., [21] recorded underweight in (59.0%) with wasting being more evident than stunting in infants suffering from CHD. These results came from our data. El-Alameey et al., [22], and Varen et al., [23], stated that wasting was more common in cyanotic CHD than in acyanotic CHD.

Anaemia is an important risk factor for morbidity and mortality among infants suffering from CHD (cyanotic and acyanotic) in the absence of vitamin or mineral deficiency, or hemolytic causes [24]. More than 30% of the patients with CHD had iron deficiency anaemia [25]. It may co-exist and worsen acyanotic CHD heart failure [26].

Iron deficiency anaemia was evidenced in 73.3 % of our patients with a statistically highly significant increase in serum levels of total iron binding capacity compared to control group ($P < 0.000$).

By our study, Assiri et al. [19] found. Also, rickets was present in 6 patients (10%), it may be secondary to calcium deficiency, or intestinal malabsorption.

Our patients demonstrated statistically highly significant increased serum alkaline phosphatase activity and decreased serum levels of calcium than the control group ($P < 0.000$).

Gluten sensitivity leads to raised serum level of anti-gliadin IgA and IgG antibodies. Antigliadin IgA antibodies are more specific markers for disease than antigliadin IgG antibodies serving for initial screening, assessing diseases activity, and judging management with a gluten-free diet [6].

To our knowledge, the present study is the first to document raised serum levels of antigliadin antibodies in malnourished infants with CHD and gastrointestinal symptoms. Forty-two patients with CHD had statistically significant higher levels of IgM antibody to gliadin, twenty-six patients exhibited a significant elevation of the serum levels of IgA anti-gliadin antibodies and twelve patients demonstrated significantly increased serum levels of IgG antigliadin antibodies compared to control group ($P < 0.000$). A statistically highly significant elevation of the serum levels of IgA, IgM, and IgG antigliadin antibodies was evidenced in our studied patients compared to control group ($P < 0.000$). On analysing risk factors using odds ratio, the age at onset of gastrointestinal symptoms in the form of diarrhoea, abdominal pain and distension were documented as a significant strong association of raised serum levels of antigliadin antibodies in the infants with CHD with a prediction of 95%.

In our patients, serum antigliadin IgM levels were significantly negatively correlated with serum calcium levels and height for age z- score ($P < 0.05$). Serum antigliadin IgG levels were significantly positively correlated with serum alkaline phosphatase activity, and negatively correlated with z- score of weight for age. These data indicated that when serum levels of antigliadin IgM, and IgG increased, more stunting and underweight was found.

Interestingly, our study showed clinical improvement of some patients on the exclusion of gluten from the diet and continuing of breastfeeding. Rapid recovery was reported concerning weight gain. Breastfeeding protects against repeated episodes of acute gastroenteritis which have been linked to increased risk of gluten sensitivity. This reduction could be mediated via immunoglobulins present in human milk [27, 28].

From the current findings, it could be concluded that serum IgA, IgM, and IgG class antibodies to gliadin play a significant role in the pathogenesis of malnutrition.

Breastfeeding is protective, may be beneficial in delaying or preventing gluten sensitivity. Babies born with CHD must be breastfed for at least one ear. Gluten containing foods should be avoided for the first 8th months of life.

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