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Effect of Nutri-Gluten Free Flour on Intervening Anaemia in Celiac Children

Aditi Gupta[1], Vimla Dunkwal[2], Madhu Goyal[3]

[1]Subject Matter Specialist (Food and Nutrition) KVK Chandgothi, Churu., Department of Food and Nutrition, College of Home Science. SKRAU, Bikaner, Rajasthan, India

[2]Associate Professor, Department of Food and Nutrition, College of Home Science. SKRAU, Bikaner, Rajasthan, India

[3]Associate Professor, Department of Food and Nutrition, College of Home Science. SKRAU, Bikaner, Rajasthan, India

Abstract: ***The only effective treatment is a life-long gluten free diet (Kupper, 2005). Strict adherence to the diet allows the intestine to heal, leading to resolution of all the symptoms in most cases (Akonberg and Thomas, 2008). Zamani et al. (2008) in a cross-sectional study of gluten sensitive enteropathy in 4120 patients with iron deficiency anaemia (IDA) also found that the severity of anaemia was in parallel with the severity of duodenal lesions. There was high prevalence (14.6%) of gluten sensitive enteropathy in patients with IDA of obscure origin. Acknowledging the need for flour and other non gluten containing food stuffs, here is an attempt designed to serve celiac patients. In present study the grains used to making flour for celiac patients are totally gluten free and nutritious such as finger millet, sorghum, soyabean, rice, maize, pearl millet, black gram. Keeping in view the nutritional and health benefits of millets, in the present study efforts were made to study functional and nutritional characteristics of composite flour and to explore its effect on blood hemoglobin level of school going children age of 6-12 years suffering from celiac disease. In the study nutri-celiac flour had been served to improving health status focusing iron deficiency of the celiac subjects. Two study groups were framed i. e. Control group (n=60) and experimental group (n=60). In experimental group each subject given 100 g gluten free flour on daily basis for the period of 4 months.***

1. Review of Literature

Among people who have a first-degree relative-a parent, sibling, or child-diagnosed with celiac disease, as many as 1 in 22 people may have the disease (Fasano *et al.*, 2003). Unexplained iron deficiency anaemia is a common symptom of undiagnosed celiac disease. Haapalahti *et al.* (2005) reported that serum tests in 26 children with celiac disease had lower folic acid and iron status among 30% of children compared to 14% of controls. Harper *et al.* (2007) studied 405 celiac patients and found that they were suffering with iron deficiency anaemia occurring in 33% of men and 19 % of women. A study conducted Zarkadas *et al.* (2006) to evaluate the impact of gluten free diet on 2,681 adults (≥ 16 years) biopsy proven celiac patients. Among them 90 % of the respondents were following gluten free diet strictly having relief in symptoms.

While Harper *et al.* (2007) in a study of the impact of gluten free diet on iron status on 405 celiac patients revealed that a gluten free diet increased serum ferritin in iron deficient patients and decreased ferritin level in those with high ferritin ($r2=0.46, p<0.01$). Similar to this study by Zamani *et al.* (2008) on 14 celiac patients found improve anaemia in patients who had mild duodenal lesions without villous atrophy and found increased mean haemoglobin level from 9.9 ± 1.6 to 12.8 ± 1.0 g/dl($p<0.01$) in patients adhered to gluten free diet. Composite flour technology is initially referred to process of mixing flour with cereals and legumes for making bread and biscuits. However, the term can also be used with regard to mixing of non-wheat flours, roots and tubers and other raw materials (Dendy, 1992).

2. Methodology

2.1 Procurement of Materials

The present study was carried out on the celiac patient‘s dietary management. For the purpose of standardizing gluten free flour eight grains i.e. rice, sorghum, finger millet, soyabean, black gram, pearl millet, oat and corn of variety Ratna, CSV10, VL-149, Pusa-16, T-27, H-77, Kent and Shakti respectively were procured from the National Seed Project (NSP), SKRAU, Bikaner.

2.2 Development and Standardization of Gluten Free Flour

2.2.1 Selection of gluten free grains- The main grains chosen for the purpose of making gluten free flour were finger millet, pearl millet, sorghum, rice, black gram, maize and soyabean as per having high nutritive values.

2.2.2 The malting of finger millet- Finger millet provides highest level of calcium, antioxidants properties and phytochemicals, which make it easily and slowly digestible. In the germination process, both starch and protein are partially degraded, important for better digestibility and some of the flatus factors are also degraded. There is overall improvement in the flavour profile also (Nirmala *et al.*, 2000).

The malting of finger millet was carried out with slight modification in the procedure suggested by Nirmala *et al.*, 2000. In brief the finger millet seeds were washed with water for 5 times and soaked in water for 5 h. Excess water was drained, seeds were tied in a muslin cloth and 5 kg weight was kept on it. These seeds were germinated at 27± 3ºC for 24h. and dried in shade for 2 days. The malted finger millet seeds were grounded into flour by using the electric

grinder. The detailed flow chart for the preparation of malted finger millet flour is given in Fig. 1

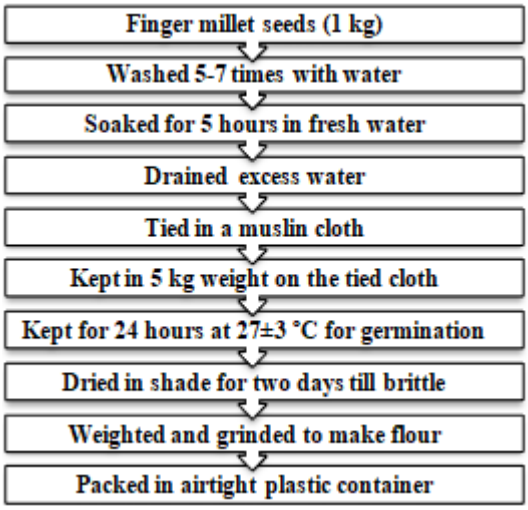

Figure 1: Flow diagram for the preparation of malted finger millet flour

2.2.3 Development of gluten free flours

In Indian context in every household, the chapatti is considered as the basic part of the main course in daily diet, So the formulation of gluten free flours were designed to kept in mind that to make ease of making process such as dough making and rolling for the celiac patients as necessary part of diet. For making gluten free flour above mentioned grains were mixed in different proportions and ratios as per cereal pulse combination provides better biological values than cereals alone. A double mix of a staple and a pulse in the ratio of 4:1, which can be gradually improved to 3:1, is nutritionally reinforcing; the deficiency of lysine in the staple and the deficiency of methionine in the pulse are offset mutually (Dhaar and Robbani, 2008). Different combinations of cereal-pulse were made and depicted in Table-1

Table 1: Different composite gluten free flour (GFF) combinations

Composite gluten free flour combination	Grain Percentage (%)			
	30	20	25	25
1.	Rice	Sorghum	Pearl millet	Black Gram
2.	Rice	Sorghum	Pearl millet	Soyabean
3.	Rice	Sorghum	Finger millet	Black Gram
4.	Rice	Sorghum	Finger millet	Soyabean
5.	Rice	Corn	Pearl millet	Black Gram
6.	Rice	Corn	Pearl millet	Soyabean
7.	Rice	Corn	Finger millet	Black Gram
8.	Rice	Corn	Finger millet	Soyabean

2.2.4 Selection of gluten free flour

From above prepared gluten free flours combinations most organolepticaly acceptable gluten free flour selected for intervention. The selected GFF_4 named as 'nutri-celiac flour'.The process for preparation of gluten free flour was standardized using simple processing techniques i.e. washing, cleaning, drying, grinding and mixing, except finger millet as these grains were malted before use for better nutritional availability.

2.3 Selection of Subjects

The proposed study was carried out in the Department of Food & Nutrition, College of Home Science, Swami Keshwanand Rajasthan Agricultural University (SKRAU), Bikaner in collaboration with the Department of Pediatrics, Gastroenterology and Pathology S.P. Medical College and Associated Group of Hospitals, Bikaner. Subjects (n=142) were selected purposively for the present study on the basis of patients already registered with consultation of gastroenterologist and available reports of serological testing regarding biopsy, anti-tTG and haemoglobin from the age group of 6-12 yrs. i.e. school going age available at PBM (Prince Bijoy Singh Memorial) hospital, Bikaner. From the study group 12 subjects were excluded because presence of other disease like diabetes or neurological dysfunction to make it more homogeneous nature. Among rest selected subjects (n=130), 7 subjects from control and 3 subject from experimental drop out due to irregular attendance. Finally two groups were having 60 subjects in each group. The intervention was done on experimental group who were ready to participate and cooperate for present investigation under supervision of researcher. Whereas no intervention was done on control group.

2.4 Assessment of Nutritional Status

Haemoglobin estimation -Haemoglobin level of all the subjects (n=120) was estimated by using Sahli's haemoglobin meter in the interval of one month in the intervention period of four months.

Technique for the estimation of haemoglobin- The finger tip of the subject was cleaned with a spirit swab and 0.02ml blood was drawn into the pipette of Sahli's haemoglobin meter and blown in 0.2ml of dilute hydrochloric acid (N/10) to the haemoglobin meter tube to develop the brown acid hematin pigment. Further dilution was made with distilled water till the color exactly matched with the standard brown glass tube in a comparator. The reading thus obtained from caliberated haemoglobin meter was considered as haemoglobin concentration of the subjects. The haemoglobin concentration was expressed in g/dl (WHO, 1989). The haemoglobin level of > 12g/dl was considered as normal.

2.5 Intervention of Nutri-Celiac Flour to the Patients

During the intervention programme experimental group (n=60) of the study received the nutria-celiac flour for four months. The experimental subjects were receiving daily 100 g of nutri-celiac flour by the researcher at the hospital per month. Whereas the control group (n=60) did not receive any supplementation.

3. Results and Discussion

3.1 Organoleptic Evaluation of Developed Gluten Free Flours

As considering the need for alteration in food source as a substitute of wheat flour to celiac patients, different cereal and pulses combination based gluten free flours (GFF) were

developed and evaluated organolepticaly. Mainly two cereals based combinations i.e. sorghum and corn based flours were developed after making chapatti out of all flours and their acceptability was assessed on 9-point hedonic scale. Among all the combinations made of gluten free flour rice, sorghum, finger millet, soybean combination in the ratio of 30:20:25:25 respectively was found to be more organoleptically acceptable than rest of combinations developed.

3.2 Impact Analysis of the Intervention Programme

Prevalence of Anemia

Haemoglobin estimation is regarded as a screening index useful in defining various degrees of iron deficiency anemia. It is evident from the Table 2 that mean haemoglobin level of the subjects was 7.56 g/dl, which is lower than the normal (>12g/dl) levels. Mean haemoglobin level observed 7.29 and 7.83 g/dl in control and experimental group, respectively.

The percent distribution of different degrees of anemia among the subjects has been displayed in Table 2 and Fig.2 The overall prevalence of anaemia among school going subjects was about 100 percent, having the haemoglobin level below the cut-off level recommended by the WHO (1993). It was observed that out of 120 subjects examined for iron deficiency anaemia all subjects were suffering from severe to moderate range of anaemia, not only single subjects reported to have normal haemoglobin levels

Table 2 also indicates that 38 percent of the subject was found to be severely anaemic and they were more likely to be mild anaemic as their diets are significantly deficient and dietary adequacy is lacking, at times due to unawareness and irresponsibility towards health issues. In control group percent of severe anaemic subjects (37 %) was comparable to severe anaemic subjects of experimental group (40%). Similar to this study Zamani *et al*. (2008) in a cross-sectional study of gluten sensitive enteropathy on 4120 patients with iron deficiency anaemia (IDA) also found that the severity of anaemia was in parallel with the severity of duodenal lesions. There was high prevalence (14.6%) of gluten sensitive enteropathy in patients with IDA of obscure origin.

Table 2: Mean haemoglobin levels of the subjects

Particulars	Groups	Haemoglobin Values (g/dl)
Mean Hb level (g/dl)	Total (N=120)	7.56 ± 2.09
	Control group (n=60)	7.29 ± 2.07
	Experimental group(n=60)	7.83 ± 2.10
Normal range		> 12g/dl

WHO (1993)

Table 3: Percent distribution of degree of anaemia in subjects

Hb Levels (g/dl)	Degree of anaemia	Groups		
		Total (N=120)	Control (n=60)	Experimental (n=60)
> 12.0	Normal	Nil	Nil	Nil
10.0-11.9	Moderate	18(21)	15(9)	20(12)
7.0-10.0	Mild	44(53)	48(29)	40(24)
< 7.0	Severe	38(46)	37(22)	40(24)

Value in parenthesis indicates percent of the subjects

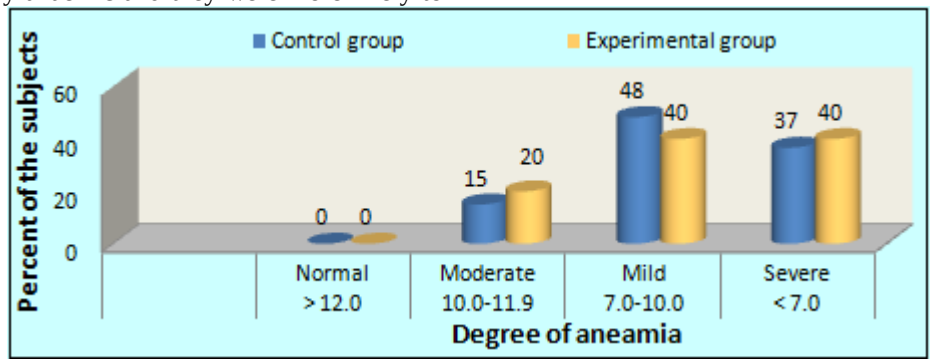

Figure 2: Percent distribution of degree of anaemia among subjects

Table 4: Mean haemoglobin levels of the subjects during intervention

Group	Mean haemoglobin of the subjects during intervention				
	0 Days	30 Days	60 Days	90 Days	120 Days
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control group	7.29 ± 2.07	7.30 ± 2.02	7.30 ± 1.97	7.31 ± 1.95	7.34 ± 1.95
Experimental group	7.83 ± 2.10	8.18 ± 2.01	8.35 ± 1.91	8.48 ± 1.86	8.56 ± 1.89
CD	1.14^{NS}	1.08^{NS}	1.05*	1.02**	1.05**

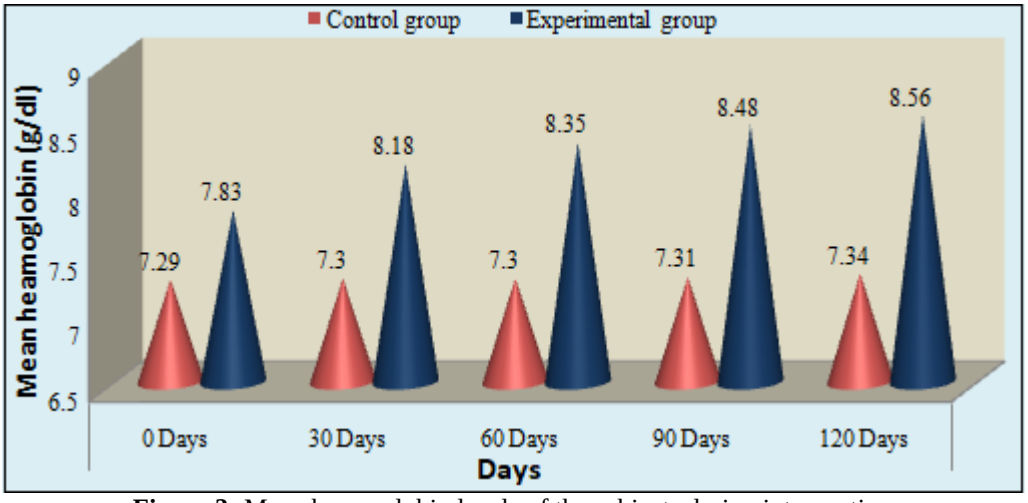

Figure 3: Mean haemoglobin levels of the subjects during intervention

Prevalence of anaemia

Mean haemoglobin level of the subjects was observed to be 7.94 g/dl after intervention. In control group mean haemoglobin was 7.34 g/dl and experimental group it was 8.56 g/dl [Table 4].

Table 5 showing the comparison of haemoglobin level to the standard value(12 g/dl) of the subjects before and after intervention. In subjects mean haemoglobin per cent raised 63 to 66.16 per cent. In experimental group observed 5.19 per cent gain in mean haemoglobin level whereas in control group recorded 7.34 g/dl haemoglobin represents 0.41 reduction in mean haemoglobin value. Mean haemoglobin gain was found to be highly significant ($p<0.1$) in experimental group. Table 6 represents mean gain in haemoglobin during intervention was 0.001 to o.15 g/dl in control group and 0.34 to 0.86 g/dl in experimental group.

Table 7, Fig.2 depicts after intervention of subjects to nutri-celiac flour in experimental group 4 per cent subjects attained the normal haemoglobin level and only 13 subjects were severe aneamic whereas in control group severe aneamic subjects were increased up to 42 per cent. It was observed 7.0 per cent increase in the normal hemoglobin and 18 per cent reduction of severe aneamic subjects in experimental group. Whereas in control group severe aneamic subjects increased up to 5 per cent compared to hemoglobin level at 0 day [Table 5]. Zamani *et al* (2008) on 14 celiac patients found improve anaemia in patients who have mild duodenal lesions without villous atrophy and found increased mean haemoglobin level from 9.9 ± 1.6 to 12.8 ± 1.0 g/dl ($p<0.01$) in patients adhered to gluten free diet similar results obtained by Annibale *et al.* (2001).

Table 4: Mean hemoglobin levels of the subjects after intervention

Particulars		Values
Mean Hb level (g/dl)	Total (N=120)	7.94 ± 2.01
	Control group (n=60)	7.34 ± 1.95
	Experimental group(n=60)	8.56 ± 1.89
Normal range		> 12g/dl

Source: WHO (1993)

Table 5: Comparison of the haemoglobin in subjects before and after intervention

Parameters	Standard Value *(WHO)	Pre experimental phase (% standard value)			Post experimental phase (% standard value)			% Gain in haemoglobin		
		Total subjects (N=120)	Control group (n=60)	Experimental group (n=60)	Total subjects (N=120)	Control group (n=60)	Experimental group (n=60)	Total subjects (N=120)	Control group (n=60)	Experimental group (n=60)
Hb	12g/dl	63	60.75	65.25	66.16	61.16	71.34	3.16	-0.41	5.19

***WHO (1993)**

Table 6: Mean gain in heamoglobin of the subjects

Group	Mean heamoglobin gain of the subjects			
	30 Days	60 Days	90 Days	120 Days
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control group	0.001 ± 0.10	0.01 ± 0.26	0.03 ± 0.22	0.15 ± 0.22
Experimental group	0.34 ± 0.32	0.52 ± 0.61	0.64 ± 0.69	0.86 ± 0.59
SEm	0.04	0.08	0.09	0.08
't' values	7.83**	6.06**	6.81**	8.73**

** ($p < 0.01$) Highly-significant *($p < 0.05$) significant NS- Non –significant

Table 7: Percent distribution of degree of anaemia among subjects after intervention

Hb Levels (g/dl)	Degree of anaemia	Groups		
		Total subjects (N=120)	Control group (n=60)	Experimental group (n=60)
> 12.0	Normal	3 (4)	Nil	7(4)
10.0-11.9	Moderate	20(24)	15(9)	25(15)
7.0-10.0	Mild	45(54)	43(26)	47(28)
< 7.0	Severe	32(38)	42(25)	22(13)

Source: WHO (1993) Figures in the parenthesis indicates the number of the subjects

4. Summary

As considering the need for alteration in food source as a substitute of wheat flour to celiac patient's different cereal and pulses combination based developed nutria- gluten free flours can consider to recover nutritional deficiencies, intestinal healing and normalizing haemoglobin level via supply of nutrients in daily diet. The daily requirement of protein, fat, calcium and iron for the age of 6-12 is about 1 gm/kg body weight, 30-35g, 600-800 mg and 16-27 g/day respectively. Daily use of 100 g developed nutri- celiac free flour thus supplemented nutrient almost 1/3rd of RDA.

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