Efficacy of a Fish Protein Hydrolysate in Malnourished Children

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Abstract Protein hydrolysates are good nutritional supplements as their bioactive ingredients can be easily absorbed and utilized for various metabolic activities. A fish protein hydrolysate (Amizate), prepared by a unique process of hydrolysis has the advantage of high di/tri peptide content (<10 kDa) along with essential and non essential amino acids, micronutrients and vitamins. The effect of Amizate on malnourished children (6–8 years, a total of 438) of Grade I and II (Gomez's classification) with respect to immunoglobulins, CD4/CD8 ratios and hemoglobin was examined. Measurement of these parameters during the user trial study (at the beginning and the end after 4 months) indicated that the levels of the immunological parameters were not significantly altered by the Amizate treatment. The values of immunoglobulins and CD4/CD8 ratios of malnourished children (India) are in the

normal range and are in accordance with the reported values of various ethnic groups.

Keywords Protein hydrolysates · Immunoglobulin · Malnourishment · Nutrition

Introduction

Nutrition plays a key role directly as well as indirectly in various metabolic and immunological mechanisms [1]. Malnutrition in children can lead to altered immune competence making them susceptible to a broad spectrum of infectious diseases. In view of this, immunoglobulins, the important components of humoral immunity, have been measured under a variety of clinical and diseased conditions in children from different parts of the world [2–4]. T lymphocyte subsets, CD4 and CD8, are markers of viral infections particularly in the subjects with immunodeficiency. These subsets of lymphocytes play an important role in establishing and maximizing the capabilities of the immune system. They are involved in activating and directing B lymphocyte cells. The lymphocyte subpopulation CD4/CD8 ratios and levels of immunoglobulins are considered as diagnostic markers for various disease conditions [5, 6]. Measurement of immunoglobulin levels and CD4/CD8 ratios is of immense value in assessing the immune status of children in relation to a variety of conditions of nutritional imbalance in diseases [7, 8]. Recent advances in the understanding of clinical nutrition in diseases have led to use of effective nutrient supplements based on proteins, carbohydrates, micronutrients and vitamins.

Protein hydrolysates are produced from a variety of protein sources by several processes such as by heating

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with acids or addition of external proteolytic enzymes, followed by protein purification protocols. A protein hydrolysate is more easily digested and absorbed as compared to an intact protein [9, 10]. It enhances the availability of the plasma amino acids, and leads to greater muscle protein synthetic response [11]. Protein hydrolysates have evolved as most promising nutrient supplements, used in clinical and elemental nutrition, weight loss and malnutrition in various clinical conditions [12]. The commercially available protein hydrolysates are complex mixtures of peptides along with free amino acids. A number of protein nutritional supplements, currently available commercially have proved efficient by the clinical trials in different conditions [13]. Whey protein, a byproduct of milk, contains beta-lactoglobulin (~65%), alpha-lactalbumin (~25%), and serum albumin (~8%) along with other essential and non essential amino acids. Soy protein, isolated from dehulled, defatted soybean meal is available as soy flour, concentrates, and isolates. Soy protein also contains essential and non-essential amino acids, legume proteins, leguminins, vicilins, glycinin, beta-conglycinin and prolamines. Another popular nutritional supplement, Spirulina, is a blue green algae with higher amount of protein and widely used as a nutritional supplement over decades.

Fish hydrolysate is claimed to be another beneficial protein nutritional supplement for a variety of clinical conditions. Seacure, a dried fish protein hydrolysate (pacific whiting *Merluccius productus*) contains 75–80% protein constituents, 60% peptides and 40% amino acids. In a recent study, 18 fish protein hydrolysates have been found to have anti-proliferative activity using two human breast cancer cell lines grown in vitro [14]. Tetrapeptides and higher peptides require prior brush border hydrolysis for absorption. Study by Adibi and Morse [15] suggests the absorption of tetraglycine in human jejunum is accomplished through hydrolysis by brush border oligopeptidase. It has been demonstrated that di/tri peptides are absorbed faster than free amino acids [10, 12]. Thus, the proportion of di/tri peptides in protein hydrolysate determines the absorption kinetics of a protein hydrolysate.

Amizate, an Atlantic salmon fish protein hydrolysate used in the current study, comprises more than 60% of di/tri peptides (<10 kDa), 30–40% of all essential and non essential amino acids, micro nutrients and vitamins. Amizate was prepared without any external hydrolyzing agents to optimally retain the nutrients [16, 17]. This hydrolysate by virtue of its balanced composition can be anticipated to have multifaceted metabolic activity. Further, with these qualitative advantages, Amizate is as cost effective as other commercial products available for malnutrition.

Therapeutic effect of Amizate on BMI (physical and anthropometric parameters) of malnourished children

(grade I and II malnutrition categories as per Gomez's classification [18]) was evaluated in a user trial study in India (user trial Protocol ID No.: 2008LOT001). The user trial showed that Body Mass Index (BMI) values have increased significantly in Amizate treated groups of children suggesting that Amizate could be a useful nutritional supplement for malnourished children (User trial Protocol ID No.: 2008LOT001; Nesse et al. 2011, communicated). The present study was undertaken as a part of user trial to evaluate the safety of Amizate with respect to any adverse alteration in immune response as indicated by change in immunoglobulin levels, CD4/CD8 ratios and hemoglobin levels of these malnourished children before and after the administration of Amizate.

Materials and Methods

Materials

Amizate is a fish protein hydrolysate uniquely prepared by endogenous hydrolyzing agents containing 60–70% di/tri peptides of 10 kDa, all the essential and non essential free amino acids (30–40%), micronutrients and vitamins in a balanced composition (Table 1).

Study Design

A total of 438 malnourished Indian school children aged between 6 and 8 years (Ghaziabad, Uttar Pradesh) with mild and moderate malnutrition as per Gomez's classification were randomly grouped into three arms. Arm A was administered 3 gm of Amizate in chocolate drink of 120 ml, while Arm B was given 6 gm of Amizate in chocolate drink of 120 ml. The children grouped as Arm C were given plain chocolate drink of 120 ml without Amizate (Table 2). Each group had a total of 146 participants. This was a randomized, double blind, parallel, 3-arm, multicentric, User Trial. The purpose was to identify changes in immunological parameters of the subjects in response to administration of Amizate 3 and 6 g in the chocolate drink as a total daily dose (single dose per day) versus control group. Blood samples were collected twice from the study participants [at the beginning of the User Trial (Visit-1) and at the end (Visit-10)] and were analyzed for immunological parameters.

Assay for Immunological Parameters

Levels of serum immunoglobulins (IgG, IgM and IgA), CD4/CD8 ratios and hemoglobin levels were measured at the beginning of the study period (Visit-1) and after 120th day (Visit-10) of administration of Amizate in the three

Table 1 Composition of amino acids and micronutrients of amizate

Essential amino acids	Average amino acid profile per 15 gm
Isoleucine	60 mg
Leucine	119.8 mg
Lysine	119 mg
Methionine, cysteine	46.2 mg
Phenyl alaline + Tyrosine	122.4 mg
Threonine	75.4 mg
Tryptophan	16.2 mg
Valine	79.6 mg
Histidine	46.2 mg
Non essential amino acids	Average amino acid profile per 15 gm
Glycine	107 mg
Proline	98 mg
Serine	66 mg
Aspartate + Asparagine	126.8 mg
Alanine	85 mg
Arginine	83.6 mg
Cysteine	12.4 mg
Glutamate + Glutamine	216.4 mg
Micronutrient	Average amino acid profile per 15 gm
Iron	0.129 mg
Zinc	1.485 mg
Iodine	3 µg
Calcium	0.225 mg
Chloride	30 mg
Magnesium	0.17 mg
Phosphorus	13.95 mg
Potassium	21 mg
Selenium	9.9 µg
Sodium	66 mg
Copper	174 µg
Chromium	1.95 µg
Vitamin C	0.855 mg
B1-Thiamin	< 3.6 µg
B2-Riboflavin	3.15 µg
B3-Niacin	63 µg
B6-Pyridoxine	10.05 µg
B9-Folate	2.85 µg
B-12-Cyanocobalamin	2.4 µg

Arms. The levels of immunoglobulins (IgG, IgM and IgA), the CD4/CD8 ratios and the hemoglobin were analyzed by M/s Piramal Diagnostics, as per their Standard Operating Procedures. The levels of immunoglobulins were measured by immunoturbidometry assays, and the CD4/CD8 ratios

Table 2 Study design

Groups	Randomized to	Age (years)	Male	Female	Total
Arm A	3 gm Amizate	6–8	77	69	146
Arm B	6 gm Amizate	6–8	76	70	146
Arm C	Plain chocolate drinks	6–8	74	72	146

were measured by flow cytometric analysis of the whole blood. Amount of hemoglobin (Hb) was determined by cyanomethemoglobin method.

Statistical Analysis

Descriptive statistics were calculated in terms of Mean and SD. Paired-t-test was performed to evaluate the significant differences between the values of samples collected at the beginning and end of the user trial period.

Ethical Committee Clearance

Ethical committee approval of the User Trial was obtained (Protocol ID: 2008LOT001) and appropriate information of consent and assent were received from the children's parents.

Results and Discussion

Serum immunoglobulin G, A and M levels were measured in Arms A, B and C of malnourished children at the beginning and end of the User Trial. The values of immunoglobulins among the three groups of children administered with 3, 6 and 0 gm of Amizate were not significantly different. Mean values of immunoglobulins G, A and M in Arms A, B, C groups of participants were in the normal range (Table 3). The scattered diagram for other immunoglobulins, CD4, CD8 and hemoglobin are also within the normal range and are of similar pattern, with and without the administration of Amizate (Fig. 1; Table 3). The lymphocyte subset percentage is observed to be comparable to that of healthy population from various ethnic groups (Table 4) [5, 19, 20].

Munson et al. [21] reported that fortification with protein as food supplement in children aged 1–6 years with 3–4 gm/kg body wt resulted in unaltered humoral immunocompetence before and after treatment. Interestingly, we have observed similar results in the current user trial study in malnourished children of the higher age group of 6–8 years. In a recent study, it was reported that intestinal immunoglobulin levels in the undernourished children among various ethnic populations such as Caucasian

Table 3 Immunoglobulin levels IgG, IgM, IgA, CD4/CD8 ratio, Hb and in malnourished children

	Arm A-(3g treated) Mean $\pm$ SD		Arm B-(6g treated) Mean $\pm$ SD		Arm C-(untreated) Mean $\pm$ SD	
	Screening	End visit	Screening	End visit	Screening	End visit
IgG	1240.14 $\pm$ 234.63	1287.72 $\pm$ 277.58	1290.66 $\pm$ 366.49	1238.27 $\pm$ 269.40	1298.70 $\pm$ 284.75	1321.00 $\pm$ 242.02
IgM	153.62 $\pm$ 64.53	148.69 $\pm$ 71.37	162.53 $\pm$ 74.15	141.68 $\pm$ 69.83	162.26 $\pm$ 67.79	142.59 $\pm$ 60.96
IgA	156.32 $\pm$ 58.79	147.32 $\pm$ 48.82	129.73 $\pm$ 48.65	144.95 $\pm$ 58.76	145.55 $\pm$ 52.13	160.40 $\pm$ 65.51
CD4/CD8	1.41 $\pm$ 0.51	1.50 $\pm$ 0.55	1.49 $\pm$ 0.45	1.44 $\pm$ 0.47	1.48 $\pm$ 0.60	1.67 $\pm$ 0.65
Hb	12.51 $\pm$ 1.353	12.08 $\pm$ 1.02	12.3 $\pm$ 0.83	12.08 $\pm$ 0.91	12.23 $\pm$ 1.13	11.75 $\pm$ 1.15

* P value for IgG = 0.271, IgM = 0.443, IgA = 0.118, Hb = 0.652

Fig. 1 Change in mean IgG levels in mg/dl [Reported normal range 519–1484 mgs/dl]. Mean values of serum Immunoglobulin G (IgG) of the three groups; Arm A, B and C at screening and end visits

Table 4 Values of ratios of CD4 and CD8 in different populations

Populations	Age group (years)	CD4/CD8 ratio	References
India (2000)	18–45	1.9	[20]
India (2003)	18–74	1.7	[19]
India (2004)	25–45	1.1	[5]
USA (1991)	18–70	1.4	[25]
UK (1992)	1–71	1.5	[7]
Normal range	18–74	1.0–2.0	Coulter reference range

Table 5 Immunoglobulin levels in normal and malnourished children

Conditions	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)	References
Serum immunoglobulin level with healthy children				
Netherland	519–1484	31–128	39–243	[23]
Bangladesh	1755 ± 377	229 ± 131	161 ± 60	[24]
Serum immunoglobulin level with malnourished children				
Australian aboriginal population	1300 ± 196	172 ± 11	67 ± 09	[22]
Indonesian population	1443 ± 220	218 ± 19	143 ± 20	[22]
Indian population (present study)	1298 ± 284	162 ± 67	145 ± 52	

population, Indonesian and Australian Aboriginal populations were within the normal range (Table 5) [22]. The study showed no statistically significant differences among various groups of undernourished children. Similar observations were made in the present study.

In conclusion, use of the Amizate, a protein hydrolysate (a salmon fish protein) prepared by a process based on endogenous enzymes [16, 17] provided a desirable composition of di/tri peptides and vitamins showed significant improvements in malnourished children. Significant improvement of BMI values in response to administration of Amizate clearly established improvement of malnourished children (Nesse et al. 2011, communicated). The present study confirmed that administration of Amizate has not resulted in any adverse alteration of immune status in the mild malnourished children of the present study. Collectively, the two studies infer that Amizate could be useful as a safe therapeutic nutritional supplement for malnourished children. The present study also provides baseline data for malnourished children in India (age group 6–8 years). The data is useful for comparison of immune

status of children from different geographical regions of the country and elsewhere.

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