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A Formulated Diet-Chart for Patients with Muscular Dystrophy and Its Clinical Significance

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ABSTRACT

Objective: Muscular dystrophy is a genetic disease and associated with progressive muscle wasting and weakness. Impaired muscle strength may be associated with nutritional insufficiency in patients with muscular dystrophy. Morbidity and mortality may be also influenced by poor diet in these patients. Therefore, it is needed to assess nutritional intake as well as formulation of healthy, balanced-diet for dystrophy patients.

Methods: Oxidative-stress based degeneration is the major cause of muscle wasting in patients with muscular dystrophy. Formulation of the diet is based on the protection against oxidative-stress in patients with muscular dystrophy. The diet was given to patients with muscular dystrophy and SOD (Superoxide dismutase), GPx (glutathione peroxidase), CAT (catalase) and LP (lipid peroxidation) was measured in serum. The level of all these parameters was measured in serum of patients with muscular dystrophy vs. control subjects, patients with two months medicinal treatment vs. non-medicinal treatment, and patients with medicinal treatment and formulated diet consumption vs. patients with medicinal treatment after two & four months duration. The muscle power was also determined in patients with medicinal treatment and formulated diet consumption vs. patients with medicinal treatment after two & four month duration.

Results: Level of SOD, GPx, CAT and LP was higher in patients with muscular dystrophy as compared to control subjects. No significant difference was observed in the level of these parameters in patients with two months medicinal treatment vs. non-medicinal treatment. There was a significant difference observed for all these parameters in the serum of patients with medicinal treatment and formulated diet consumption vs. medicinal treatment after two and four month's duration. Grading of muscle power showed the difference in patients with medicinal treatment and formulated diet consumption vs. patients with medicinal treatment after two & four month's duration. But, these differences do not approach the significant.

Discussion: The formulated diet chart might prove helpful for better management of muscular dystrophy. It is a well-known fact that genetic diseases are not curable. Available medications temporary delay the deterioration in patients with muscular dystrophy.

Keywords: Nutrition, Muscular dystrophy, Diet, DMD, BMD, LGMD-2B, Balanced diet, Oxidative-stress

Abbreviations: SOD: Superoxide Dismutase; GPx: Glutathione Peroxidase; CAT: Catalase; LP: Lipid Peroxidation; DMD: Duchenne Muscular Dystrophy; BMD: Becker Muscular Dystrophy; LGMD: Limb-Girdle Muscular Dystrophy; FSHD: Facioscapulohumeral Muscular Dystrophy

INTRODUCTION

Muscular dystrophy results from mutation in genes responsible for the proper working of the muscles rendering the body incapable of maintaining healthy muscles [1]. Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), Limb-girdle muscular dystrophy (LGMD), Facioscapulohumeral muscular dystrophy (FSHD) and congenital muscular dystrophy are the major types. There is an observation of progressive muscle wasting and weakness in all types of muscular dystrophy [1,2]. Several mutated genes have been characterized and linked to different types of muscular dystrophies. The exact mechanism behind the pathophysiology of the muscular dystrophy is still not known. Oxidative-stress may be one of the causes for the pathogenesis of muscular dystrophies [3-5].

The physiological condition of patients with muscular dystrophy may be deteriorated by nutritional insufficiency. Impaired muscle strength may also be linked by nutritional insufficiency. Deprived nutrition has a major influence on morbidity and mortality in patients with muscular dystrophy [6]. There is a need to formulate a diet chart for these patients, which would be helpful for better management of the disease. In the present study, a diet chart was formulated that could protect against oxidative-stress induced muscle degeneration mechanism in a patient with muscular dystrophy [3-5]. So, the nutritional components of the diet could potentially be used to suppress the oxidative-stress. The clinical significance of nutritional supplementation was evaluated by measuring the level of SOD (superoxide dismutase), GPx (glutathione peroxidase), CAT (catalase) and LP (lipid peroxidation) in the serum of patients with muscular dystrophy after consumption of formulated diet.

MATERIALS AND METHODS

Blood specimens

There were thirty patients (25 male and 5 female) with mean age of 24±8.9 years, were selected for this study from the neurology department of All India Institute of Medical Sciences (AIIMS), New Delhi. Patients were confirmed for muscular dystrophy by clinical, electrophysiological and histopathological diagnostic methods. Seventeen (N=17) male and five (N=5) female with mean age of 25.9±7.8 years, were also selected from AIIMS, New Delhi as a control subjects. Approval of Ethical Committee of All India Institute of Medical Sciences (AIIMS), New Delhi was obtained for conducting this study. Written consent from relatives of the patients was obtained before collecting their blood specimens. Blood samples were drawn from the subjects and collected in sterilized tubes, which was further used for the serum separation. The serum specimens were stored in liquid nitrogen.

Experimental design

The patients were randomly divided into two groups. One group (N=11) received formulated diet along with medicine and the other group (N=11) were with prescribed medicine but no specific diet. Blood samples of patients were collected at 0 month, 2 months and 4 months intervals. The samples of 0 month were at baseline (before start of medication and medication along with the diet). Subsequently, samples were collected after two months and four months of intervention. Activity of antioxidant enzymes (SOD, GPx & CAT) and level of lipid peroxidation (LP) were estimated in the serum from both the groups.

Chemicals

All the chemicals were purchased from Sigma-Aldrich, UK.

Clinical examination

All suspected patients with muscle diseases were examined by a neurologist. Difficulty in walking and standing, difficulty in climbing the stairs, frequent fall and muscular wasting and weakness were complaints by these patients. Gower's and valley sign, along with calf muscle hypertrophy were the basis for the clinical examination of the patients. These are important clinical signs and used to diagnose DMD and BMD [1,7,8]. Calf head on trophy sign was helpful to diagnose the LGMD-2B (dysferlinopathy) [9].

EMG (electromyography) examination

Diagnostic confirmation of myopathy was performed by EMG examination with the use of a concentric bipolar needle. The appearance of myopathic-EMG pattern was helpful in the establishment of empathy [5].

Histopathological and immune-histopathological examination

Histopathological and immunohistochemical examinations were performed on muscle-biopsy specimens for confirming the diagnosis of suspected patients with muscular dystrophy. Immunoblotting was also performed for further confirming the diagnosis, wherever, felt necessary [5].

Biochemical estimations from serum

Blood was collected in plain vials followed by centrifugation at 2000 rpm for 10 min. Clear serum was stored in liquid nitrogen for biochemical studies.

(a) Estimation of lipid peroxidation (LP): An indicator of lipid peroxidation is thiobarbituric acid based reactive substance (TBARS). These substances were estimated by the method of Ohkawa et al. [10]. Amount of TBARS was expressed as nanomoles of malondialdehyde (MDA)/mg protein. Tetramethoxypropane (TMP) was used as a standard.

(b) Determination of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities: The activity of antioxidant enzymes (SOD, CAT and GPx) was determined by the technique of Singh et al. [11]. Enzyme activity was expressed as units/mg protein.

Measurement of muscle power

Muscle power was determined before the medicinal treatment, with the medicinal treatment and with the medicinal treatment & diet therapy after 2 and 4 months duration. Determination of the muscle power was carried out by using the various parameters. These were combined shoulder and hip power, combined elbow and knee power, combined wrist and ankle power, combined finger and toe power, time taken to walk 9 m, time taken to get up from squatting position, time taken to climb 13 stairs with the support of hands on the side railing, amount of weight that could be lifted up to shoulder and arm and amount of weight that could be lifted above the head. Through the combination of above mentioned parameters the grading of muscle power was decided by Brooke and Vignos's scales [12]. Brooke scale is used for the grading of muscle power of upper extremities (the upper limb, including the shoulder, arm, forearm, wrist and hand). Grading of muscle power of lower extremities (the lower limb, including, the hip, thigh, leg, ankle and foot) is performed by Vignos's scale [12].

Statistical analysis

Independent sample t-test for two independent groups was used for the comparison of mean values of antioxidant enzymes (SOD, GPx & CAT) as well as an LP in serum of patients suffering from muscular dystrophy and control subjects. The paired t-test was used for the comparison of the mean values of antioxidant enzymes and LP in serum of patients without and with two months of medication for the same groups. Mean values of antioxidant enzymes and LP in serum of two groups (medication and medication along with the diet) of patients suffering from muscular dystrophy, were compared by independent sample t-test. Statistical significance was considered by the value of p ($p<0.05$).

Diet formulation

A specific diet-chart was formulated for patients with muscular dystrophy. The basis of this formulation is the dietary components, which prevent the oxidative-stress induced muscle degeneration and promote muscle regeneration in dystrophy patients [1-4,13]. Antioxidants components have the capability to neutralize the effect of oxidative-stress and muscle regeneration process requires the amino acids or proteins [14-17]. Diet is also supplemented with essential minerals and vitamins in a well-balanced quantity including the carbohydrate, lipid and protein [18]. This formulated diet chart is described below:

Mung beans and black gram (100 g each) consumed in the form of sprout (Once day preferably during breakfast)

Mung bean (*Vigna radiate* L. Wilczek) contains 24% protein, 63% carbohydrate and 1% fat. The total dietary fiber content is 16%. Mung bean also contains the calcium, magnesium, phosphorous, manganese, potassium, iron, zinc, copper and selenium. This is also supplied the water-soluble as well as fat-soluble vitamins. The 100 g mung bean provides 347 Cal [18,19] as shown in Table 1.

Table 1: The sprouts mung bean has following nutritive values (100 g)

S. No.	Composition	Units	Nutritional content that provide benefit to the patients
1	Water	90.4 g	They provide essential minerals, vitamins and proteins as well as antioxidants to muscular dystrophy patients
2	Energy	30 Kcal	
3	Proteins	3.04 g	
4	Total lipid (fat)	0.18 g	
5	Carbohydrates	5.94 g	
6	Fibers	1.8 g	
7	Sugar	4.13 g	
8	Calcium	13 mg	
9	Iron	0.91 mg	
10	Magnesium	21 mg	
11	Phosphorous	54 mg	
12	Potassium	149 mg	
13	Sodium	6 mg	
14	Zinc	0.41 mg	
15	Vitamin C	13.2 mg	
16	Thiamine	0.084 mg	
17	Riboflavin	0.124 mg	
18	Niacin	0.749 mg	
19	Vitamin B6	0.088 mg	
20	Folic acid	61 µg	
21	Vitamin A	21 IU	
22	Vitamin E	0.1 mg	
23	Vitamin K	33 µg	

Black gram (*Vigna mungo* L.) is a good source of vitamins and minerals. It is also a rich source of calcium, potassium, iron, magnesium, copper and manganese and dietary fibers. This contains a huge amount of potassium and supports to balance the sodium-potassium levels in our system. This is necessary for the growth and development of the human body. The iron content of black gram maintains to improve the human memory. The fats and calories of black gram are required for appropriate growth of the human body. Black gram is a relaxing mediator. This is an aphrodisiac and nerve stimulant. The 100 g black gram provides 341 Cal [20,21] as shown in Table 2.

Table 2: The sprouts black gram (*Vigna mungo*) has following nutritive values (100 g)

S. No.	Composition	Percentage (%)	Nutritional content that provide benefit to the patients
1	Moisture	72.70%	They provide essential minerals, vitamins and proteins as well as antioxidants to muscular dystrophy patients
2	pH	6.78%	
3	Protein	8.50%	
4	Lipid	0.24%	
5	Total sugar	1.13%	

6	Reducing sugar	0.44%	
7	Fructose	0.27%	
8	Glucose	0.17%	
9	Sucrose	0.69%	
10	ß-carotene (µg/100 g)	57.05 + 3.46	
11	Ascorbic acid (mg/100 g)	31.41 + 0.72	
12	Total antioxidants (% DPPH inhibition per 100 g)	36.64 + 0.10	
13	Total phenol (mg/100 g)	40.96 + 1.23	
14	Flavonoids (mg/100 g)	178.6 + 2.83	

100 ml pomegranate juice consumed in daily diet (one time in a day)

Pomegranate (*Punica granatum*) juice is a huge resource of the antioxidant system because it contains the largest amount of phytochemicals. The majority of phytochemicals in pomegranate juice are polyphenols and ellagitannins. These ellagitannins are produced from the binding of carbohydrate with ellagic acid and/or gallic acid and also known as punicalagins [22]. Delphinidin, cyanidin and pelargonidin glycosides are responsible for the red color of juice [22]. Pigmentation in juice increases during fruit ripening. The juice is a huge source of vitamin C and also contains all the vitamins. Minerals are also present in the juice and potassium is the chief content. The 100 g pomegranate provides 65 Kcal [23,24] as shown in Table 3.

Table 3: The major antioxidant activity of pomegranate juice is due to phenolic components. The phenolic composition is described below

S. No.	Phenolic compounds	mg/L	Nutritional content that provide benefit to the patients
1	First group: Anthocyanins		Antioxidants of pomegranate juice help to reduce oxidative-stress induced and prevent oxidative stress induced degeneration of muscle in muscular dystrophy
	Delphinidin 3,5-diglucoside	42.9	
	Cyanidin 3,5-diglucoside	53	
	Delphinidin 3-glucoside	76	
	Cyanidin 3-glucoside	128.3	
	Pelargonidin-3-glucoside	5.9	
	Total anthocyanins	306	
2	Second group: gallagy-l-type-tannins		
	Punicalagin B	12.7	
	Punicalagin D	10.1	
	Other	45.1	
	Total gallagyl-type tannins	67.9	
3	Third group: Ellagic acid derivatives		
	Ellagic acid glucoside	17.9	
	Ellagic acid	15.3	
	Total ellagic derivatives	33.2	
4	Fourth group: Other hydrolyzable tannins		
	Galloyl glucose	51.1	

	Compound C	224.5	
	Other compounds	204.1	
	Total hydrolyzable tannins	539.2	

Prepare 1 kg mixed flour with each of 100 g wheat, maize, barley, bajra and 600 g soybeans. Everyday 300 g of this mixed flour used for the preparation of bread, which should be consumed by the patients (two times in a day)

The wheat (*Triticum* spp.) flour (100 g) gives 327 Cal energy. Protein, dietary fiber, manganese, phosphorus and niacin are found in a significant amount in it. Vitamins B complex and minerals are also present in it [25,26] as shown in Table 4.

Table 4: The wheat flour (100 g) has the following nutritive value described below

S. No.	Components	Amount	Nutritional content that provide benefit to the patients
1	Protein	11%	This is a rich source of carbohydrates and proteins, which is required for muscle regeneration process
2	Fat	0.90%	
3	Carbohydrates	73.90%	
4	Minerals	0.60%	
5	Calcium	23 mg	
6	Magnesium	42 mg	
7	Total Iron	2.5 mg	
8	Total Phosphorous	121 mg	
9	Vitamin A	43 IU	
10	Thiamine	0.12 mg	
11	Riboflavin	0.07 mg	
12	Nicotinic acid	0.9 mg	

The 100 g maize (*Zea mays* subsp. *mays*) kernel gives 86 Cal energy. Vitamin B complex, thiamin, niacin, pantothenic acid (B5) and foliate are found in a higher amount in it. Moderate amounts of dietary-fiber and the essential-minerals (magnesium and phosphorus) are also obtained from this. Little amounts of other nutrients also occur in it [25,26] as shown in Table 5.

Table 5: The maize flour (100 g) has the following nutritive value described below

S. No.	Components	Amount	Nutritional content that provide benefit to the patients
1	Protein	11.10%	This is a rich source of carbohydrates and proteins, which is required for muscle regeneration process
2	Fat	3.60%	
3	Carbohydrates	66.20%	
4	Minerals	1.50%	
5	Calcium	10 mg	
6	Magnesium	144 mg	
7	Total Iron	2.0 mg	
8	Total Phosphorous	348 mg	

9	Vitamin A	1502 IU	
10	Thiamine	0.42 mg	
11	Riboflavin	0.10 mg	
12	Nicotinic acid	1.4 mg	

In a 100 g of raw barley (*Hordeum vulgare* L), gives 352 Cal and it is a notable source of protein, dietary fiber, vitamin B, niacin and vitamin B6, and several dietary minerals. Manganese and phosphorus are also present in a huge amount. Raw barley contains 78% carbohydrates, 1% fat, 10% protein and 10% water [25,26] as shown in Table 6.

Table 6: The barley flour has the following nutritive value described below

S. No.	Components	Amount	Nutritional content that provide benefit to the patients
1	Protein	11.50%	This is a rich source of carbohydrates and proteins, which is required for muscle regeneration process
2	Fat	1.30%	
3	Carbohydrates	69.60%	
4	Minerals	1.20%	
5	Calcium	26 mg	
6	Magnesium	127 mg	
7	Total Iron	3.0 mg	
8	Total Phosphorous	215 mg	
9	Vitamin A	79 IU	
10	Thiamine	0.37 mg	
11	Riboflavin	0.28 mg	
12	Nicotinic acid	1.8 mg	

The 100 g bajra (*Pennisetum glaucum*) gives 378 Cal. Protein (11 g), carbohydrate (73 g), dietary fibers (8.5 g), fat (4.2 g), vitamins and minerals are also present in it [24,25] as shown in Table 7.

Table 7: The bajra flour has the following nutritive value described below

S. No.	Components	Amount	Nutritional content that provide benefit to the patients
1	Protein	11.60%	This is a rich source of carbohydrates and proteins, which is required for muscle regeneration process
2	Fat	5.00%	
3	Carbohydrates	67.50%	
4	Minerals	2.30%	
5	Calcium	42 mg	
6	Magnesium	125 mg	
7	Total Iron	14.3 mg	
8	Total Phosphorous	269 mg	
9	Vitamin A	220 IU	
10	Thiamine	0.33 mg	
11	Riboflavin	0.16 mg	
12	Nicotinic acid	3.2 mg	

Soyabean (*Glycine max*) flour (100 g) contains 40% protein, 23% lipid and 32% carbohydrates and gives 456 Kcal energy [27]. Soybean proteins are composed of eighteen amino acids. All of these nine of the essential amino acids and rest are semi-essential and non-essential amino acids. The essential amino acids are histidine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, threonine, tryptophan and non-essential amino acid are alanine, arginine, aspartic acid, glutamic acid, glycine, proline and serine. Sulfur-containing amino acids and aromatic amino acids are also present in soya-proteins. Phospholipids are the major component of soyabean [25-27] as shown in Table 8.

Table 8: The soybean flour has the following nutritive value described below

S. No.	Components	Amount	Nutritional content that provide benefit to the patients
1	Protein	43.20%	This is the richest source of protein and phospholipids. High protein diet provides amino acids for muscle regeneration and the phospholipids are required to construct the cell membranes
2	Fat	19.50%	
3	Carbohydrates	20.90%	
4	Minerals	4.60%	
5	Calcium	240 mg	
6	Total Iron	11.5 mg	
7	Total Phosphorous	690 mg	
8	Vitamin A	710 IU	
9	Thiamine	0.73 mg	
10	Riboflavin	0.76 mg	
11	Nicotinic acid	2.4 mg	

100 g cheese and two boiled hen's eggs should be included in the diet (one time in a day)

A significant source of high-quality proteins, vitamins and minerals are a cheese. The chief protein of cheese is casein and little amount of lactalbumin and lactoglobulin may also be present. The presence of protein depends on the amount of whey entrapped in the cheese. The low-fat content cheese has a higher protein-to-fat ratio. Polyunsaturated fatty acids (C 18:2 and C 18:3) are present in a small amount in cheese. It gives 348 Kcal energy (100 g) [28,29] as shown in Table 9.

Table 9: The cheese has the following nutritive value described below

S. No.	Components	Amount	Nutritional content that provide benefit to the patients
1	Protein	14.60%	This contains protein, vitamins and minerals required for, proper and regulated growth and development
2	Fat	31.20%	
3	Carbohydrates	20.50%	
4	Minerals	3.10%	
5	Calcium	650 mg	
6	Total Iron	5.8 mg	
7	Total Phosphorous	420 mg	
8	Vitamin A	273 IU	

Hen's eggs contain the 74.57% water. This contains proteins (12.14%), lipids (11.15%), all necessary vitamins (except vitamin C) and minerals (calcium, iron, magnesium, zinc, copper, sodium, potassium, sulfur and iodine) [30]. The majority of egg carbohydrates are composed by albumin. The content of carbohydrate in the hen's egg is only about 1% of the whole egg. Fat soluble vitamins are present in the yolk [30]. One egg may deliver 12% vitamin A,

more than 6% of vitamin D, 9% riboflavin and 8% pantothenic acid. This also gives the saturated fatty acids (14:0; 16:0; 8:0), mono-unsaturated fatty acids (14:1; 16:1; 18:1), polyunsaturated fatty acids (18:2; 18:3; 20:4), cholesterol, phosphatidylcholine and phosphatidylethanolamine [30]. This also provides alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, Isoleucine, leucine, methionine, phenyalanine, proline, serine, threonine, tryptophan, tyrosine and valine. It contains 173 Kcal energy per 100 g [25,26] as shown in Table 10.

Table 10: The hen's egg has the following nutritive value described below

S. No.	Components	Amount	Nutritional content that provide benefit to the patients
1	Carbohydrate	0 g	This is the rich source of phospholipids and cholesterol required for formation of the muscle membrane
2	Fat	81 g	
3	Saturated fat	51 g	
4	Mono unsaturated fatty acid	21 g	
5	Poly unsaturated fatty acid	3 g	
6	Protein	1 g	
7	Vitamin A	684 µg	
8	Vitamin D	60 IU	
9	Vitamin E	2.32 mg	
10	Cholesterol	215 mg	

Include 20 g of butter in daily diet (one time in a day)

Butter contains the 80% fat, 16% water and 2% of non-fat milk solids. It provides the 729 kcal energy per 100 g [25,26] as shown in Table 11.

Table 11: The butter (100 g) has the following nutritive value described below

S. No.	Components	Amount	Nutritional content that provide benefit to the patients
1	Protein	13.30%	This contains higher dioleo-monounsaturated fatty acids which are required for development of strong immune system in muscular dystrophy patients
2	Fat	13.30%	
4	Minerals	1.00%	
5	Calcium	60 mg	
6	Total Iron	21 mg	
7	Total Phosphorous	220 mg	
8	Vitamin A	1200 IU	
9	Thiamine	0.30 mg	
10	Riboflavin	0.18 mg	
11	Nicotinic acid	0.1 mg	

Drink the warm cow's milk of 100 ml with 5 g turmeric added to it, before going to sleep (one time at night)

Cow's milk contains all the essential nutrients. This is a chief source of calcium, iodine, potassium phosphorus, good quality protein and the vitamins B complex. It gives 67 Kcal energy per 100 g [25,26] as shown in Table 12.

Table 12: The cow's milk has the following nutritive value described below

S. No.	Components	Amount	Nutritional content that provide benefit to the patients
1	Protein	3.20%	The cow's milk contain essential dietary component (protein, fat and minerals) for growth and development of muscular dystrophy patients
2	Fat	4.10%	
3	Carbohydrate	4.40%	
4	Minerals	0.80%	
5	Calcium	149 mg	
6	Total Iron	2.3 mg	
7	Total phosphorous	96 mg	
8	Vitamin A	150 IU	
9	Thiamine	0.05 mg	
10	Riboflavin	0.18 mg	
11	Nicotinic acid	0.10 mg	
12	Vitamin C	2 mg	

Turmeric (*Curcuma longa* L.) is used as dried rhizome powder or whole. The rhizome possesses the moisture (11-13%), protein (6-9%), fat (5-10%), carbohydrate (60-70%; mainly starch), fiber (2-7%), ash (3-7%), minerals (potassium, calcium, iron, sodium and phosphorous), ascorbic acid, vitamin C, sugars (glucose, fructose, arabinose), curcuminoids and essential oil (2-10%). The essential oil contains ar-turmerone (60%), curlone, ar-curcumene, zingiberene, α-phellandrene and sabinene. The yellow color is appearing due to presence of curcumin. It gives 349 Kcal energy per 100 g [31].

Take 50 g of groundnut soaked overnight in 200 ml cow's milk. In morning, grind it properly and boil for a few minutes. Drink after adding sugar to it

Groundnut or peanut (*Arachis hypogaea*) of 100 g provides 570 Cal energy and a chief source of several vitamin B complex, vitamin E, manganese, magnesium and phosphorus and dietary fiber. 100 g of groundnuts contain about 25 g protein and 567 Kcal energy [25,26] as shown in Table 13.

Table 13: The groundnut or peanut has the following nutritive value described below

S. No.	Components	Amount	Nutritional content that provide benefit to the patients
1	Protein	26.70%	This is the richest source of mono-unsaturated fatty acids and this is required for the development of the immune system. This also contains protein and minerals which is more important for growth and development
2	Fat	40.10%	
4	Carbohydrate	20.30%	
5	Minerals	1.90%	
6	Calcium	50 mg	
7	Total Iron	1.6 mg	
8	Total phosphorous	390 mg	
9	Thiamine	0.90 mg	
10	Riboflavin	0.30 mg	
11	Nicotinic acid	14.1 mg	

RESULTS

Clinical examination

All 22 cases of muscle diseases could be suspected of having DMD, BMD and LGMD, on the basis of clinical examination, including symptoms, signs and family history.

EMG (electromyography) examination

All the 22 cases of muscle diseases showed a myopathic EMG pattern in the form of the almost complete interference pattern and low amplitude (<500 μv) MUAs (multiple unit activity) was of short duration (5-8 ms) and where most of the polyphasia.

Histopathological and immunohistopathological examination

Based on histopathological and immunohistochemical examination of the muscle biopsy samples, following types of muscle diseases were confirmed, the eight cases (N=8) of DMD, four cases (N=4) of BMD and ten cases of (N=10) LGMD-2B shown in Figure 1.

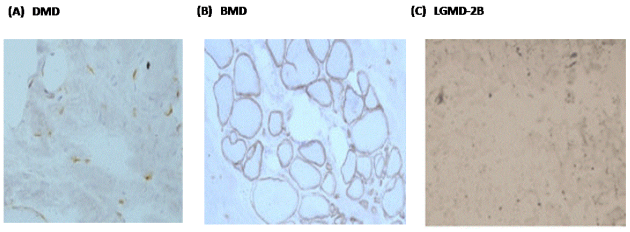

Figure 1: Histopathological and immunohistopathological examination of muscle samples confirmation the (A) DMD (complete loss of dystrophin in dystrophin staining), (B) BMD (reduce and discontinuous dystrophin staining) and (C) LGMD-2B (negative dysferlin staining)

Biochemical estimations of serum

Antioxidant enzymes (SOD, GPx & CAT) activity and LP content in serum of patients with muscular dystrophy as compared to control subjects: Activity of antioxidant enzymes SOD, GPx and CAT are significantly higher ($p<0.05$) in the serum of patients with muscular dystrophy (n=22) as compared to control/healthy (n=22) subjects. The level of LP is also significantly higher ($p<0.005$) in the serum of patients with muscular dystrophy (n=22) as compared to control/healthy (n=22) subjects as shown in Figure 2.

Level of antioxidant enzymes (SOD, GPx & CAT) and LP in serum of patients with muscular dystrophy (before medication) as compared to two months medication: SOD, GPx & CAT activity is not significantly different ($p>0.05$) in the serum of patients with muscular dystrophy (n=11) with two months medication as compared to without medication. The level of LP is not significantly different ($p>0.05$) in the serum of patients with muscular dystrophy (n=22) with two months medication as compared to without medication shown in Figure 3.

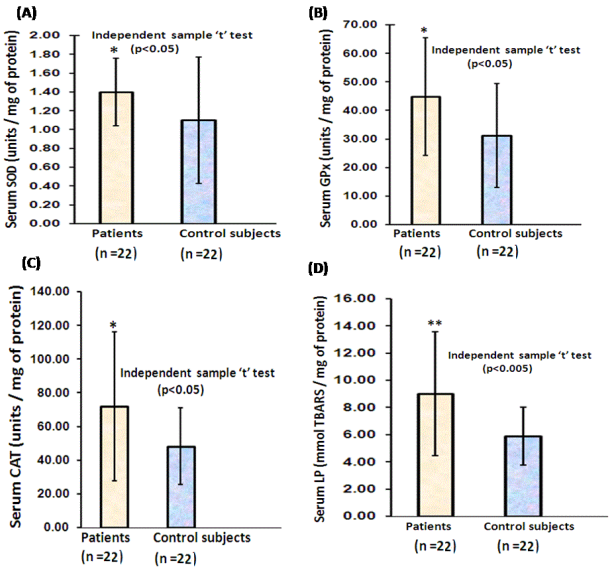

Figure 2: The level of enzyme activity (SOD, GPx & CAT) is significantly higher ($p<0.05$) in the serum of patients with muscular dystrophy (n=22) as compared to control/healthy (n=22) subjects. The level of LP is also significantly higher ($p<0.005$) in the serum of patients with muscular dystrophy (n=22) as compared to control/healthy (n=22) subjects (Level of enzyme activity expressed as mean±SD)

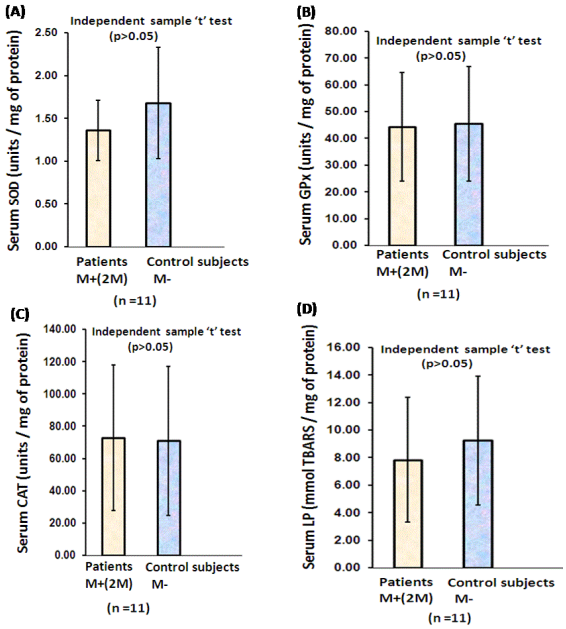

Figure 3: The level of enzyme activity (SOD, GPx & CAT) is significantly higher ($p<0.05$) in the serum of patients with muscular dystrophy (n=22) as compared to control/healthy (n=22) subjects. The level of LP is also significantly higher ($p<0.005$) in the serum of patients with muscular dystrophy (n=22) as compared to control/healthy (n=22) subjects (Level of enzyme activity expressed as mean±SD)

Level of antioxidant enzymes (SOD, GPx & CAT) and LP in serum of patients with muscular dystrophy with medication and diet consumption as compared to patients with muscular dystrophy only on medication (after 2 & 4 month's duration)

The antioxidant enzyme activity (SOD, GPx & CAT) is significantly reduced ($p<0.05$ & $p<0.005$) in serum of muscular dystrophy patients (n=11) treated with medication and diet after two & four months of treatment as compared to patients (n=11) treated only with medication. Level of LP is also significantly reduced ($p<0.05$ &

p<0.005) in serum of medicine and diet treated muscular dystrophy patients as compared to only medicine treats patients after two & four months treatment duration shown in Figures 4 and 5.

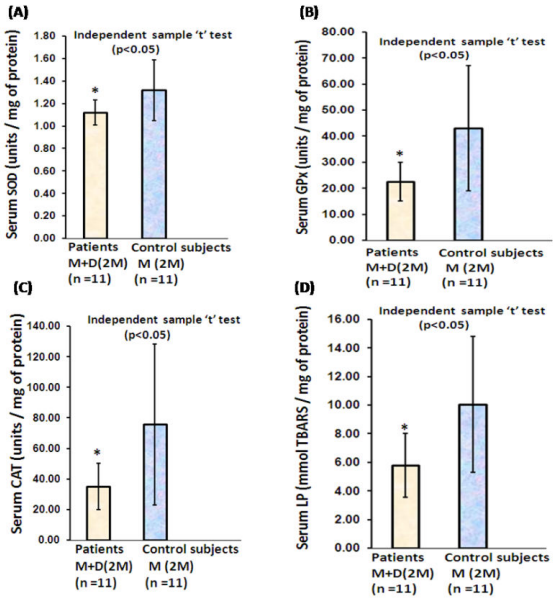

Figure 4: The level of enzyme activity (SOD, GPx & CAT) is not significantly different (p>0.05) in the serum of patients with muscular dystrophy (n=11) with two months medication as compared to without medication. The level of LP is not significantly different (p>0.05) in the serum of patients with muscular dystrophy (n=11) with two months medication as compared to without medication (Level of enzyme activity expressed as mean±SD)

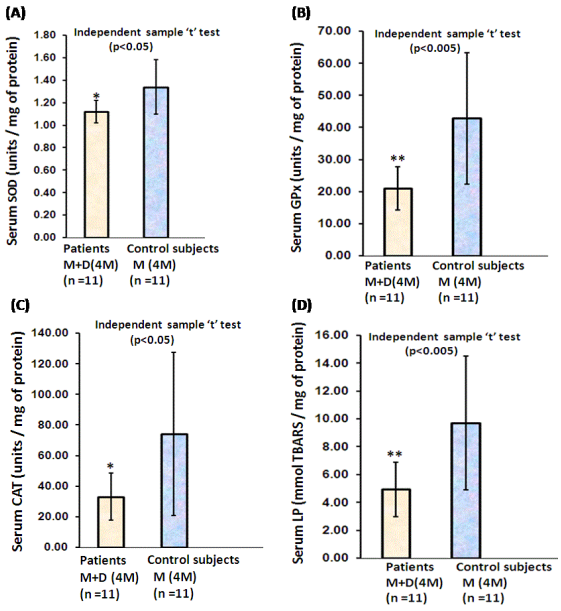

Figure 5: The level of enzyme activity (SOD, GPx & CAT) is significantly reduced (p<0.05 & p<0.005) in serum of patients with muscular dystrophy (n=11) with medication and diet of two months duration as compared to patients with muscular dystrophy (n=11) with medication of two months duration. Level of LP is also significantly reduced (p<0.05 & p<0.005) in serum of patients with muscular dystrophy (n=11) with medication and diet of two months duration as compared to patients with muscular dystrophy (n11) with medication of two months duration (Level of enzyme activity expressed as mean±SD)

Measurement of muscle power

Grading of muscle power on Brooke and Vignos's scales showed the difference for the patients with diet and medicinal treatment vs. only with medicinal treatment after two months duration. But, this difference is not

significant as described in the Figures 6A and 6B. Similarly, the grading of muscle power on Brooke and Vignos's scales showed the difference for the patients with diet and medicinal treatment vs. only with medicinal treatment after four months duration. The observed difference does not reach to the significance shown in Figures 7A and 7B.

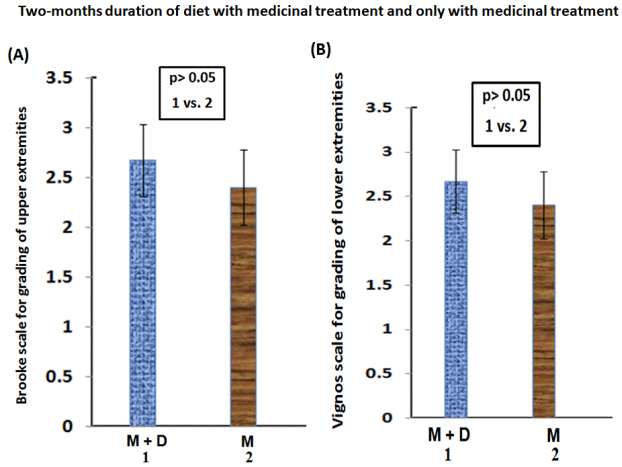

Figure 6: The grading of muscle power on (A) Brooke and (B) Vignos's scales showed the difference for the patients with diet and medicinal treatment vs. only with medicinal treatment after two months duration (Grading of muscle power expressed as mean±SD; p>0.05)

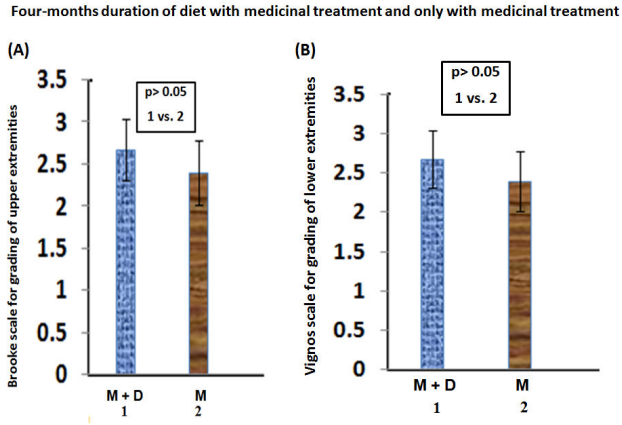

Figure 7: The grading of muscle power on (A) Brooke and (B) Vignos's scales showed the difference for the patients with diet and medicinal treatment vs. only with medicinal treatment after four months duration (Grading of muscle power expressed as mean±SD; p>0.05).

DISCUSSION

Earlier studies prove that oxidative-stress and increased production of reactive oxygen species (ROS) plays a central role in pathogenesis of muscular dystrophies [3-5]. Based on these facts we formulated diet that would quench and nullify the effect of reactive oxygen species. The contribution of the different components of the formulated diet and role in management of muscular dystrophy has been described below. Black gram and mung bean sprouts are highly nutritious. Sprouting also enhances the digestibility and availability of nutrients. Various dormant enzymes are activated during sprouting; proteins are degraded into amino acids, whereas starches are degraded into maltose [32]. Bounded minerals (calcium, zinc and iron) are freely available inside the cells. The amount of vitamins is also increased. Sprouting also increases the essential fatty acid content, thereby enhancing the antioxidant property [32]. Thus sprouted black gram and mung beans provide essential minerals, vitamins and proteins as well as antioxidants to muscular dystrophy patients.

Pomegranate juice contains a diverse range of bioactive compounds such as phenolics, flavonoids, proanthocyanidin compounds. These bioactive compounds are active antioxidant system, which scavenger's superoxide radicals [22].

These antioxidants of pomegranate juice help to reduce oxidative-stress induced and prevent oxidative stress induced degeneration of muscle in muscular dystrophy.

The diet recommends mixed flour composed of wheat, maize, barley, gram and soya bean. Wheat, barley, gram is the rich source of carbohydrates and proteins [25,26]. Soya bean is the richest source of protein and phospholipids [27]. High protein diet provides amino acids for muscle regeneration and the phospholipids are required to construct the cell membranes [17,33]. Genistein and isoflavone, also found in soya bean helps inhibit the production of ROS and pro-inflammatory mediators. Effectiveness of genistein in muscular dystrophy is evident from a study where the forelimb strength of mdx mice showed improvement after treatment with genistein [34]. The muscle pathogenesis is decreased with the 20% decline in the level of serum CK in the genistein treated mice [34].

The protein content of cheese and egg provide an additional source of essential amino acids [28,30]. Egg and cheese also contain vitamins and minerals required for, proper and regulated growth and development [28,30]. The egg yolk is rich source of phospholipids and cholesterol required for formation of the muscle membrane [30,33]. Butter prepared from cow milk contains higher dioleo-monounsaturated fatty acid content required for development of strong immune system [35]. Besides Cow milk derived butter has high content of Oleic and linoleic acid, vitamins such as vitamin B complex, vitamin D, vitamin E and vitamin K and minerals [35].

Cow milk is a balanced diet and complete dietary requirement [18,25,26]. These properties make cow's milk essential dietary component for growth and development of muscular dystrophy patients. Turmeric contains Curcumin, a highly potent antioxidant [31]. There is a significant reduction is observed in NF-κB activation by inhibiting the translocation of the p65 subunit of NF-κB to the nucleus through the curcumin. Oxidative-stress is a key contributor to DMD pathogenesis and NF-κB is a pro-inflammatory mediator which is responsible for the production of NO in oxidative stress [34]. Curcumin treatment showed the improved muscle contractile properties compared to controls in mdx mice [34]. In this way, turmeric with milk (a complete and balanced food), i.e., turmeric mixed with milk and produce the colloidal solution [18]. This colloidal form easily absorbed by the human body and helps to produce more effect.

Groundnuts (peanuts) are the richest source of mono-unsaturated fatty acids and this is required for the development of the immune system. This also contains protein and minerals [18,25,26]. In this way, peanut is more important for growth and development. The clinical significance of patients was determined by the level of antioxidant enzymes and LP. A significant increase in the level of antioxidant enzymes and LP in the serum of patients with muscular dystrophy as compared to control subjects were shown the indication of enhancement of oxidative-stress. This is already reported in one of our studies [5]. This also proved that oxidative-stress is one of the major causes for the degeneration of muscle.

Two months medicated (corticosteroid deflazacort medication) patients with muscular dystrophy as compared to patients with muscular dystrophy without medication did not show the significant difference in the level of antioxidant enzymes and LP in serum. But the level of antioxidant enzymes and LP were decreased in the patients with muscular dystrophy with medication. This is due to medication, which helps to reduce the oxidative-stress in a limited extent.

Patients treated with a combination of medications (corticosteroid/Deflazacort) and diet show significant decrease in antioxidant enzyme activity and low serum LP levels as compared to muscular dystrophy patients only on medication after two months of treatment. Similarly, a significant decrease in serum antioxidant enzyme activity and LP in muscular dystrophy patients with medication with prescribed diet as compared to patients only on medication after four months of treatment. This again proved that the diet with medication reduces oxidative-stress in patients with muscular dystrophy as compared to the patients with muscular dystrophy only on medication.

Muscle power measurement confirmed the patients with medication and diet have more preserved muscle as compared to patients with medication because grading of muscle power (without showing significant difference) is slightly better in patients with medication and diet as compared to patients with medication after two and four months duration [35]. So, the proposed diet may be helpful to reduce the muscle degeneration with reduction of oxidative-stress as described above.

The proposed diet provided the antioxidant components to scavenge the oxidative radicals as well as proteins and other requirements for the regeneration process [17,25,26]. This diet also provides the abundant source of phospholipids, which is also helpful for the regeneration of muscle membrane [18,33]. Minerals, vitamins, carbohydrate, proteins and lipids provided by the diet for patients with muscular dystrophy are helpful to reduce the cause of oxidative-stress as well as enhance the regeneration process without creating any side-effects [17,25,26,36].

This formulated diet was designed in such a way that it could be helpful to provide all the nutritional requirements as well as helpful to eliminate oxidative-stress and promote the muscle regeneration process. The proposed diet includes easily available and naturally produced edible components, eliminating any harmful side effects. It is a well-known fact, that genetic diseases are incurable, only temporary medications are available that delay the deterioration in patients with muscular dystrophy [34,37-39]. The proposed diet chart might prove helpful to clinicians for better and effective managements of dystrophy patients.

CONCLUSION

The formulated diet-chart for patients with muscular dystrophy is the outcome of the present study. The experimental results showed the significant reduction of oxidative-stress in patients with muscular dystrophy due to consumption of formulated diet. This is well-known fact that there is no complete cure of the disease and reducing the burden of disease is only one option in the hand of the clinicians. In this regard, this formulated diet may be beneficial for effective and better management of patients with muscular dystrophy.

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