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This is great pleasure and honor for us that Life Science Journal of Pakistan (LSJP) is publishing its first issue at PCSIR labs complex. LSJP covers different areas of life science encompassing, biotechnology, cell biology, medical and allied sciences, plant sciences, microbiology, molecular biology, biochemistry, and animal sciences. In Pakistan we have a very limited number of recognized journals, due to which publication time is very long and one has to wait for a very long period of time to get the work published. LSJP was started to share the burden of pre-existing journals and publish the research articles in minimum possible time using the same standard procedure followed by most of the popular journals. There has been a great effort by our team, particularly Journal manager, all section editors, chief editor, and chief executive. Team LSJP made every effort to make the process of manuscript submission, review and publication very user-friendly and convenient, for that we used a similar model as that of journals of repute.

In the first issue, six manuscripts will be published. In medical and allied fields, we accepted a manuscript related to biomedical profile changes of CKD patients during and after hemodialysis. We feel it will be beneficial for clinicians to understand profile changes to maintain the health of patients undergoing dialysis. Another manuscript related to the nutritional profile of school children will be published for the understanding of our policymakers to give serious concerns to child nutrition at school age. In the field of biotechnology, a manuscript related to dextran production was accepted. Dextran is a very important polymer that has multifarious applications, therefore this work could be beneficial for understating the method of dextran production. Two reviews, one related to Rabies virus and the other related to the nutritional importance of dry fruits has also been published in this issue. We will continue addressing scientific trends of life science related all disciplines mentioned in the scope of LSJP, for that we hope more and active participation of scholars from academia and R&D institutes.

In the end, I congratulate LSJP team and I am thankful to all especially Journal Manager for his hard work and sincere efforts to covert our dream of establishing a new journal into a reality. I am also thankful to Chief Executive, Chief Editor, Section Editors and reviewers for their help and support for bringing LSJP at this level of starting its journey by taking the first step towards excellence in life sciences.

At this occasion of joy and fulfillment we are hereby missing one of our founder editors, Prof Dr. Shahjahan Baig (1951-2018) whose sudden and sad demise last year left us in tatters and caused a serious loss to LSJP. We pray to Almighty Allah for forgiving him and granting him a higher place in heaven. He was a nobleman dedicating to his cause of promoting science and technology.

Dr. Yasar Saleem,
Managing Editor,
Life Science Journal of Pakistan

List of Contents – Issue 1, Volume 1. 2019

ORIGINAL RESEARCH

Biochemical profile of patients with chronic kidney disease (CKD) undergoing regular hemodialysis

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Abstract

Kidneys play a vital role in the removal of metabolic waste products in the form of urine. Chronic kidney disease (CKD) is the decline in kidney function for more than 3 months. This study was designed to investigate comorbidities associated with CKD and to evaluate biochemical parameters of patients undergoing regular hemodialysis from different areas of Punjab. This is a population-based study, conducted at Tahir Heart Institute, Chenab-Nagar, Pakistan. In which 26 (15 males and 11 female) patients (mean age 56 ± 6 years) diagnosed with CKD stage-v and initiated dialysis in a regular manner. Their renal function tests, liver function tests, electrolytes, albumin, and vitamin-D were performed, and appropriate statistical analysis was done. Results showed that CKD affects more males than females. Hypertension is leading comorbidity with CKD around 46%, hypertension, and diabetes both 26.9%, diabetes 15.4%, cardiovascular diseases 7.69% and other 3.8%. Level of urea, creatinine, potassium, and phosphorus was higher in a patient with no significant gender difference. Iron and vitamin–D were deficient in all these patients and this deficiency was higher in female patients. Our findings provide comorbidities that leads towards kidney failure and an overview of routine biochemical parameters of CKD stage-v patients with gender differences.

Introduction

Chronic kidney disease is an emerging health issue worldwide. CKD stage v or end-stage renal disease is the decrease in GFR below 15mL/min per1.73m^2 for more than 3 months estimated based on creatinine-based GFR. CKD is also a prognostic disease for many premature cardiovascular diseases (CVDs) and congestive heart failure (CHF) that are a stimulator of each other (1).

Hypertension is leading comorbidity associated with death due to CKD (2, 3). Mortality rate due to hypertension is about 40% worldwide (4). It is commonly noticed with CKD and is poorly controlled in patients undertook hemodialysis (5). Hyperactivity of the renin-angiotensin system (RAS) is associated with the progression of kidneys damage. Kidneys release renin in case of sudden change in blood pressure that converts angiotensin to active form angiotensin I in kidneys that elevate blood pressure, it is converted into angiotensin II by angiotensin-converting enzyme (ACE) in lungs. Angiotensin II acts directly on blood vessels and cause vasoconstriction and elevate blood pressure (6, 7). CKD affects more than 23% of patients who have diabetes mellites and cause numerous complications including cardiovascular diseases (8). *The death rate increase in patients having diabetes than those without diabetes (9).* CKD increases with age as it affects more to older individuals (10, 11).

There are different biochemical parameters that indicate reduced kidney function. Electrolytes perform many important functions in the body within normal limits. Kidneys remove excess of these electrolytes and other uremic toxins. In kidney failure or reduced kidneys function several complications produced due to low fluid excretory capacity. Control of potassium is too much important in cardiac patients (12). Hyperkalemia and hyperphosphatemia are also associated with an increased mortality rate (13).

Measurement of blood urea nitrogen or serum creatinine has been used to assess renal function. Urea comes from the protein in the diet or body's metabolism system. Creatinine comes from muscular activity. Serum creatinine concentrations are affected by several diagnostic interferences; it depends on muscle mass critically. Additionally, creatinine concentration is affected by age, gender, culture, body habitus and nutrition (14). Another study reported that the delay in serum separation from venous blood may alter some creatinine measurements, that cause misclassification of CKD (15). Hemodialysis led to a reduced level of these uremic toxins in these patients and decrease the burden on the kidneys (16).

Patients with CKD usually suffer from the mineral-bone disorder. It is due to modifications in vitamin-D metabolism, one of the key features of mineral bone disorder that has foremost medical and research consequences. CKD patients have a vitamin-D deficiency. So, they are usually prescribed vitamin-D supplements. Deficiency may be due to reduced sun exposure (17), reduced synthesis of vitamin-D due to kidney disease (18) and nutritional restrictions contribute to the high occurrence of vitamin-D deficiency and severe loss of vitamin-D binding protein leads to increased kidney loss of vitamin-D metabolites (19). Iron deficiency is also most common among CKD patients, and this leads toward anemia. It is associated with high hospitalization and high mortality rate (20). This study was designed to investigate about biochemical parameters of CKD patients undergoing regular hemodialysis and find out comorbidities associated with CKD among both males and females.

Methodology

This is a population-based study; conducted at Tahir Heart Institute, Chenab Nagar, Pakistan. A total of twenty-six (15 male; 11 female) patients were selected for the study with age limit 40–60 years. Patients had been informed by written consent following WHO ethics rules 2007. All patients were diagnosed with CKD stage-v and initiated regular hemodialysis from Sharif Dialysis Centre, Tahir Heart Institute before January 2016. Patients excluded from the study if they had passed through a recent surgical procedure or those with reactive hepatitis-B or hepatitis-C. Patients who were recommended any kind of special treatment by a physician also excluded.

Demographic (age, height, weight, blood pressure and body temperature), physical and medical or medication data were collected by questionnaires. Patients were considered hypertensive if they had blood pressure higher than 140/90 mmHg or were using antihypertensive drugs. All patients were advised dialysis 3 times a week by physician and followed-up for a comprehensive period of 10 months. Pre-dialysis 10ml blood sample (5 ml in EDTA vacutainer

and 5 ml in serum separating vacutainer) collected after every 12 sessions of dialysis. The analysis was performed at Masroor Diagnostic Centre, Tahir Heart Institute. For hematological examination EDTA vacutainer was kept on a homogenizer for 10-20 minutes and analysis done at Sysmex XN-1000 analyzer.

Renal function tests (urea, creatinine) and electrolytes (sodium, potassium, magnesium, phosphorus, and calcium) performed by serum separating vacutainer using Elecsys 2010, cobas c311 automated analyzer, Roche diagnostic, Pakistan. Vitamin–D and hepatitis viral load evaluation were performed during follow-up period using electrochemiluminescence immunoassay ECLIA Elecsys 2010, cobas e411 automated analyzer, Roche diagnostic, Pakistan. Patients were advised neither to take cold drinks nor any type of fast food or special treatment. They were provided standard food during each dialysis session. Column statistics was applied using Microsoft Excel 2016.

Demographic data

Total 26 patients studied (males 15; females 11). Mean ± S.D age of these CKD patients was 55.8 ± 5.7 years. For male patients, these values were 55.3 ± 7.5 years, for female patients 56 ± 4.4 years. Average body weight was 64 ± 7.6 kg and 55.7 ± 14.6 kg for male and female patients respectively. Their height was 170.9 ± 9.1 cm and 158.3 ± 10.3 cm respectively. Their body temperature has not shown much deviation. All patients had a normal body temperature. These values were 98 ± 0.6 ºF and 97.7 ± 0.6 ºF for male and female patients. Blood pressure showed much deviation because most of the patients were hypertensive. Average systolic blood pressure measured was 157 ± 26.8 mmHg and 160 ± 20.5 mmHg, and diastolic blood pressure 89 ± 17.3 mmHg and 83 ± 7.1 mmHg for male and female patients respectively. Represented in Table 1.

Table No. 1: Demographic data of male and female CKD patients

Demographic Parameters		Male (n=7)			Female (n=3)		
		Mean ± S. D	Min.	Max.	Mean ± S. D	Min.	Max.
Age (Years)		55.3 ± 7.5	50	65	56 ± 4.4	52	61
Weight (kg)		64 ± 7.6	58	78	55.7 ± 14.6	40	69
Height (cm)		170.9 ± 9.1	154.9	182.9	158.3 ± 10.3	152.4	170.2
Body Temp. (ºF)		98 ± 0.6	97	99	97.7 ± 0.6	97	98
Blood Pressure (mmHg)	Systolic	157 ± 26.8	131	187	160 ± 20.5	137	175
	Diastolic	89 ± 17.3	73	120	83 ± 7.1	76	90

Min: Minimum, **Max:** Maximum, **S.D:** Standard Deviation, **temp:** temperature

Table No. 2: Hematological Examination of CKD patients

Parameters	Units	Male (n=7)			Female (n=3)		
		Mean ± S. D	Min.	Max.	Mean ± S. D	Min.	Max.
RBC's	$(10^6/\mu L)$	4 ± 0.4	3.25	4.44	3.8 ± 0.4	3.47	4.21
WBC's	$(10^3/\mu L)$	6.4 ± 1.3	4.76	8.53	6 ± 2.7	3.66	9.01
Platelets	$(10^3/\mu L)$	185.5 ± 54.2	138	291	163.6 ± 58.3	101.4	217
HGB	(g/dL)	11.3 ± 1.5	9.02	13.68	11.3 ± 1.2	9.95	12.31

Min: Minimum, **Max**: Maximum, **S.D:** Standard Deviation

Results

Hypertension was the major comorbidity associated with CKD 46% (n; 12 = 7M, 5F), Followed by hypertension and diabetes combined 26.9% (n; 7 = 4M, 3F), diabetic nephropathy 15.4% (n; 4 = 2M, 2F), cardiovascular diseases 7.69% (n; 2 = 2M) and other 3.8% (n; 1 = 1F). Present in Figure 1.

Hematological parameters are most important in studying CKD. Red blood cells, white blood cells, platelets and hemoglobin were 4 ± 0.4 $(10^6/\mu L)$, 6.4 ± 1.3 $(10^3/\mu L)$, 185.5 ± 54.2 $(10^3/\mu L)$, 11.3 ± 1.5 (g/dL) for male CKD patients and 3.8 ± 0.4 $(10^6/\mu L)$, 6 ± 2.7 $(10^3/\mu L)$, 163.6 ± 58.3 $(10^3/\mu L)$, 11.3 ± 1.2 (g/dL) for female CKD patients respectively. Represented in Table 2. Urea and creatinine (mg/dL) were 124 ± 23, 8.6 ± 1 and 127 ± 29, 7.3 ± 0.9 among male and female patients respectively. Represented in Table 3, Figure 2(a & b). Electrolytes were normal for both genders except potassium and phosphorus, that was noticed high among patients. Phosphorus was (mmol/L) 6 ± 1.5 and 6 ± 1.3 for male and female patients respectively. Sodium, chloride, potassium, calcium and magnesium were (mmol/L) 134 ± 3, 94 ± 3, 5 ± 0.6, 2 ± 0.2, 1 ± 0.1 and 137 ± 1.6, 96 ± 2, 5 ± 1, 3 ± 0.8, 1 ± 0.2 in male and females respectively. Represented in Table 3, Figure 3(a & b). Liver function tests; alanine transferase, aspartate transferase, alkaline phosphate, albumin and bilirubin were 17 ± 4 (U/L), 26.4 ± 7.9 (U/L), 75 ± 3.3 (U/L), 4 ± 0.5 (g/dL), 0.29 ± 0.08 (mg/dL) in male and 15 ± 6 (U/L), 21 ± 5.9 (U/L), 91 ± 8 (U/L), 3.2 ± 0.6 (g/dL), 0.29 ± 0.1 (mg/dL) in female patients respectively. Represented in Table 3.

Iron and Vitamin-D ware seemed severely deficient in these patients. Their mean ± S.D values were 55 ± 8 (g/dL), 15.8 ± 1.8 (pg/mL) for male and 40 ± 8.9 (g/dL), 13.4 ± 1.3 (pg/mL) for females respectively. Represented in Table 3, Figure 4 (a & b).

Table No. 3: Biochemical examinations of male and female CKD patients.

Parameters	Units	Male (n=7)			Female (n=3)		
		Mean ± S. D	Min.	Max.	Mean ± S. D	Min.	Max.
Urea	mg/dL	124 ± 23	93.6	161.4	127 ± 29	117	158
Creatinine	mg/dL	8.6 ± 1.1	7	9.9	7.3 ± 0.9	6.5	8.9
Sodium	mmol/L	134 ± 3	130	137	137 ± 1.6	136	139
Potassium	mmol/L	5 ± 0.6	4.2	5.9	5 ± 1	3.9	5.4
Chloride	mmol/L	94 ± 3	91	98	96 ± 2	94	98
Calcium	mmol/L	2 ± 0.2	1.6	2.3	3 ± 0.8	2	3.5
Magnesium	mmol/L	1 ± 0.1	0.9	1.2	1 ± 0.2	1.1	1.4
Phosphorus	mmol/L	6 ± 1.5	3.4	8.4	6 ± 1.3	4	7
ALT	U/L	17 ± 4	13.1	24.1	15 ± 6	10	26
AST	U/L	26.4 ± 7.9	14.6	31.7	21 ± 5.9	12.9	25.9
ALP	U/L	75 ± 3.3	70	77.9	91 ± 8	78	99
Albumin	g/dL	4 ± 0.5	3.1	4.4	3.2 ± 0.6	2.7	3.8
Bilirubin	mg/dL	0.29 ± 0.08	0.24	0.45	0.29 ± 0.1	0.21	0.46
Iron	µg/dL	55 ± 8	43.3	64.6	40 ± 8.9	32	53.9
Vit-D	pg/mL	15.8 ± 1.8	13.3	16.6	13.4 ± 1.3	12	14.5

ALT: alanine transferase, **AST**: aspartate transferase, **ALP**: alkaline phosphate
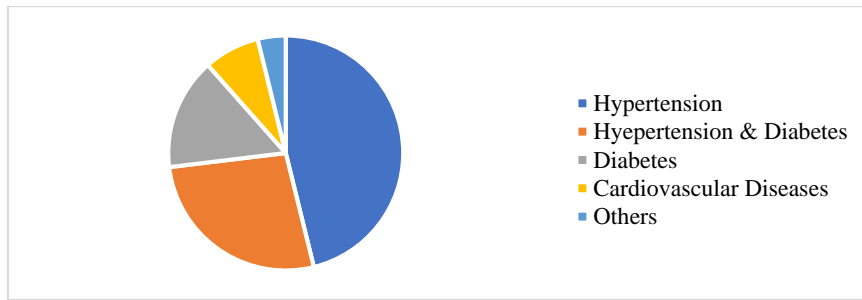
Figure 1 Comorbidities associated with CKD patients.
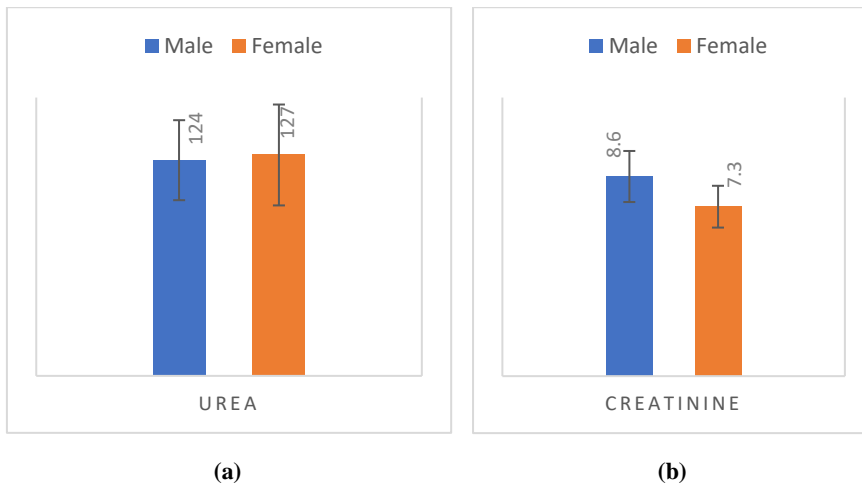

(a)

(b)

Figure: 2 Comparative graph of renal function tests **(a)** urea and **(b)** creatinine, of male and female CKD population.
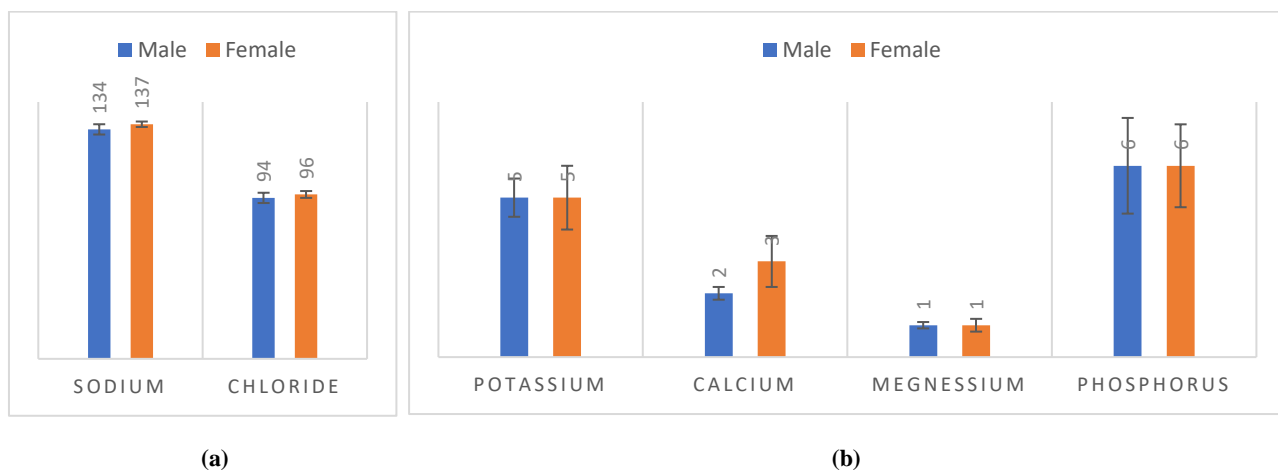

(a)

(b)

Figure: 3 Comparative graphs of electrolytes **(a)** sodium and chloride **(b)** potassium, calcium, magnesium, and phosphorus of male and female CKD patients.
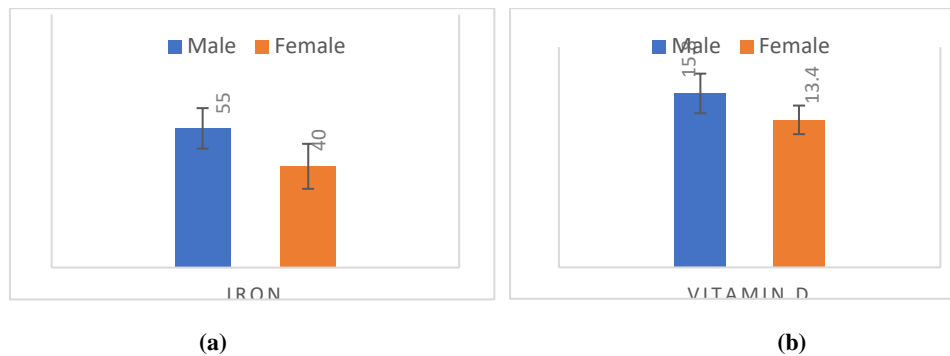
(a)

(b)

Figure: 4 Comparative graphs of **(a)** iron and **(b)** vitamin-D of male and female CKD patients

Discussion

This study found hypertension as leading comorbidity with CKD similar to the findings of Collins et al. (10). Its occurrence persists very high among patients with CKD stage-V. Hypertension noticed among 70% population with CKD. Twelve patients had hypertension (46%) and 7 patients had hypertension along with diabetes mellitus (26.9%) as comorbidities. Management of hypertension is very difficult in hemodialysis patients because much difference exists in pre, inter and post-dialytic period. Systolic blood pressure was prominent in these patients. Males with 157 ± 26.8 mmHg and females with 160 ± 20.5 mmHg which is noticeably very high. One of the possible causes could be the low fluid excretory capacity and volume excess in the body. Hypervolemia causes Hypertension up to 90% in dialysis patients (21). Hemodialytic hypertension is principal cause of cardiovascular mortality. Cardiovascular diseases are a common cause of death in CKD patients (22). During follow-up, one patient died with myocardial infraction having comorbid hypertension.

Diabetes mellitus is the second major cause of CKD. Combined prevalence was 40% in CKD dialysis population. Four patients had diabetes (15.4%) and 3 had diabetes along with hypertension (26.9%) as comorbid disease. Grundy et al. (23) described that diabetic nephropathy affects around 20 – 40 % of people who have diabetes (23).

Kidneys play a vital role in homeostasis by regulating the extracellular fluid composition by plasma filtration constantly in the body, thereby protecting the body's internal environment. Any damage to kidney leads towards the reduced capacity to filter blood that rise the level of metabolic byproducts. Among these, blood urea and serum creatinine concentration are important by-products that indicate kidney function (24). Both were noticed very high among these hemodialysis patients; Male patients showed a high level of serum creatinine 8.6 ± 1.1 (mg/dL) than females (7.3 ± 0.9).

Phosphorus and potassium are also important in CKD. Both were noticed high in the patients due to reduced renal excretory capacity. Potassium mineral controls nerve and muscular function. The heart beats at a normal rhythm because of potassium. Potassium is essential for keeping fluid and electrolyte balance and pH level. To achieve these functions, its level must be kept between 3.5-5.5 mEq/L. Hyperkalemia causes several complications within the body. The kidneys support keeping potassium at a normal level. But due to low excretory capacity, these patients show an increase in electrolytes in the body. Control of hyperkalemia is much important in patients of CVDs (12) so, patients directed to have potassium-free diet. Hyperphosphatemia is related to an increase in CVD risk factor in cardiac patients (25, 26). Patients under investigation showed an increased level of potassium and phosphorus. Sever increase in phosphate level in some patients also reported mineral-bone disorder and very disturbed condition for having joint pain and discomfort.

Modern studies revealed that vitamin-D deficiency is very common in CKD patients' dependent upon dialysis. It can be due to a restricted diet and low exposure to sunlight or metabolic disorders like reduced ability to convert 25-(OH) vit-D into the active form, 1, 25 dihydroxy-vit-D. Vitamin-D deficiency occurs in both male and female patients but the level of vitamin-D significantly lower in female patients (27). These findings are similar with this study as all the patients either male or female, showed vitamin-D deficiency and females were noticed more deficient in vitamin D 13.4 ± 1.3 pg/mL than males 15.8 ± 1.8 pg/mL. Vitamin-D supplements in nutrition should be prescribed to CKD patients on hemodialysis to overcome this deficiency.

Hemoglobin deficiency is also due to deficiency of iron in the blood which has a fundamental role in hemoglobin structure. To overcome this RBCs deficiency in body erythropoietin therapy was given to every patient after each dialysis session. This study found comorbidities with biochemical parameters urea, creatinine and electrolytes (potassium, phosphorus) were higher in CKD patients underwent hemodialysis. Vitamin D and iron were deficient, and this deficiency was higher in female gender than males.

Conclusion

Hypertension was the major comorbid disease in CKD population, following by diabetes and hypertension combined. Biochemical analysis revealed the increased concentration of urea, creatinine, and electrolytes (potassium and phosphorus) and severe deficiency of vitamin D and iron among all patients. Females were

7

noticed more deficient than male CKD population. They found to have a very poor quality of life due to a disturbance in these parameters and for being machine dependent. This study can help healthcare experts to treat CKD population effectively by understanding the complications associated with these subjects.

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ORIGINAL RESEARCH

Life Science Journal of Pakistan
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Impact of school-based nutrition education intervention on consumption of fruits and vegetables among middle school children (age7-10 years)

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Abstract

To evaluate the impact of school-based nutrition education intervention on the consumption of fruits and vegetables (FV) among middle school children. It was a quantitative study. One-eighty students were selected via purposive sampling and divided into two groups, control (G1= 90) and intervention group (G2=90). The intervention consisted of nutrition education lectures related to the importance, benefits, and consumption of fruits and vegetables. The data collection instruments included knowledge, attitude, and practices (KAP) questionnaire and food frequency questionnaire (FFQ). Subjects were measured at baseline and at 2 months post-treatment. Before the intervention, no significant difference was observed between the intervention and control group regarding knowledge, attitude and practices related to fruits and vegetable consumption (P>0.05). However, after the educational intervention, the mean score of knowledge (14.20±2.07), attitude (5.38±0.99) and practices (17.6±2.48) related to fruits and vegetables consumption were significantly higher in intervention group when compared to the control group (P>0.05). Consumption of fewer fruits and vegetables included: banana (87.8%), apple (82.2%), mango (77.8%), cucumber (63.3%), vegetable salad (55.6%) and potato (86.7%) were increased in the intervention group after nutrition education intervention. School-based nutrition education intervention on consumption of fruits and vegetables is most likely to be effective to increase nutrition knowledge and modify attitude and practices of children related to fruits and vegetable consumption. Future researches should be conducted on school-based interventions with longer intervention periods and higher sustainability.

Introduction

Nutrition education is an essential component of nutrition intervention to improve dietary habits, food preferences and to prevent nutritional deficiencies (1). Nutrition education programs are developing for the improvement of nutrition knowledge about sound dietary intake within a community or a particular target population (1). Nutrition plays an important role in physical growth as well as mental development in early life (2).

Nutrition education is very important for the development of lifelong healthy eating behavior (3). Healthy eating behavior may develop during early childhood which will affect child growth and development throughout life. Healthy eating during early childhood may protect against the initiation of diet-related chronic diseases such as overweight and obesity in later life (4). Healthy eating patterns are important to establish during early childhood because they may continue to adulthood (5).

Schools are the best area for promoting healthy eating pattern among young children and adolescents because they may target a large population in an economically efficient way (6). School-based nutrition education intervention might be improving the nutrition and health of school children, their educational performance and learning abilities (7). In the UK, children spend most of their time in school, therefore,

10

schools are a powerful zone for promoting good healthy behavior among children (8). Moreover, children's health status and their educational performance are interlinked with each other. Furthermore, the development of a positive and healthy school environment is very important for the health and wellbeing of children (9).

Food habits are very complicated in nature and various factors are involved in their development. Factors that influence food choices include taste, appetite, cost, income, availability, access, education, time, culture, family, peer, meal patterns, mood, stress, attitude, belief and knowledge related to food. Lack of physical activity and inadequate diet both may be the main causes of poor nutritional status in school going children. Health behavior in school going children (HBSC) survey reported that dietary habits of 90% of European children were very poor (10).

Gao *et al*. (2014) observed that children are likely to eat more snacks, soft drinks, and junk food because they spend most of their time away from home and family (11). Mostly, children replace breakfast and lunch with a snack. Children who eat more snacks and eat less amount of good quality food their chances of becoming overweight and obese are may be very high. Dietary habits of Pakistani school going children are very poor. According to one finding, 8% of children skipped breakfast, 43% were eating snacks and 49% were overweight and obese in Karachi primary school (12). In Pakistan, underweight and overweight co-exist in school children. Warraich et al. (2009) reported that the prevalence of overweight was 70% and underweight was 63% in Karachi school going children (age 9-12 years) (13).

Fruits and vegetables are very useful for human nutrition and health. They are good sources of vitamins and minerals especially, niacin, folic acid, vitamin c, minerals and dietary fiber (14). Ozaki Y and Christy AA (2006) revealed that intake of carotenoids rich fruits and vegetables are linked with decrease severity of cataract (15). Moreover, fruits and vegetables supply the essential vitamins and minerals that could boost the immune system (15).

Aranceta (2004) reported that excessive intake of fruits and vegetables can be associated with lower risk of diseases such as hypertension, diabetes and cardiovascular diseases (CVD) (16). Furthermore, it can prevent most types of cancers. Fruits and vegetable intake in Pakistani school going children is very low. A study conducted by Hakeem et al. (2002) on 8-12 years old rural and urban school going children of Pakistan, finding indicated that consumption of micro-nutrients such as zinc, calcium, folate, thiamine, niacin, and pyridoxine was very low among rural (41%) and urban (43%) children in Sindh Pakistan (17).

Fruits and vegetable consumption are influenced by age, gender, socio-economic status and family origin (18). Wardle et al. (2000) revealed that fruits and vegetables preferences were different among males 3.26 (FVP 2.6) and females 3.26(FVP 3.0) due to male had poorer nutrition knowledge (19).

Different food items like milk, pulses, grains, butter, and green leafy vegetables may be not liked by most of the children (20). Excessive intake of energy drinks, fewer intakes of fruits and vegetables, high consumption of fats enhance the risk of becoming overweight and obese. Watching television has been affiliated with decreased physical activity, overweight and unhealthy dietary behavior among children (age 10-12 years) (21).

Fruits and vegetable intake during childhood may be protective against cancer and childhood illness (22). In a study which was conducted about nutrition and respiratory health in six Central and Eastern European countries over 20,000 children aged 7-11, finding indicated that fruits and vegetable consumption showed strong association with respiratory symptoms such as cough (OR=1.18) and wheeze (OR=1.14) (23). Currie et al. (2004) revealed that children in Asia are not meeting the minimum suggested intake goals of fruits and vegetables (24). Moreover, children and adolescent have chances of many diet-related diseases like diabetes due to their unhealthy eating pattern (25). Therefore, it is important to develop and enforce effective programs and policies which will elevate the intake of fruits and vegetables in children.

In Pakistan, school going children have no informative knowledge related to fruits and vegetable intake in their daily diet. Furthermore, children who belong to the lower socio-economic status have lower-level nutritional knowledge instead of those who belong to higher socio-economic status. According to the national nutritional survey of Pakistan 2012-2013, 50% of children are malnourished and 49% are anemic due to poor dietary intake (26). Stunting was commonly found among 10-12 years old children of Pakistan. School-based nutrition education programs based on healthy eating in children are very important because they provide an opportunity to combine nutrition education with changes in school environments and due to school-based program parents may also become involved. It is very important to give nutrition education repeatedly in classrooms with the aim to eat a healthy diet (27).

School-based nutrition education interventions on healthy eating have shown some improvement in developing appropriate knowledge, attitude and dietary behavior in children. The present study aims to analyze the effect of school-based nutrition education intervention towards fruits and vegetable consumption in school going children.

11

vegetables, attitude towards fruits and vegetables, practice regarding intake of fruits and vegetables.

Methodology

Study Design

This was a quasi-experimental study, pretest-posttest design. The study was carried out on one hundred and eighty (180) children (Boys=101, Girls=79).

Selection of participants for study

Three primary schools in Lahore, Pakistan were selected on their willingness to participate in the study. These 3 schools were co-educational, shared similar demographic characteristics for gender, number of students, family socioeconomic status, parental support, and school environment. Overall, 180 students were selected through purposive sampling.

Sample size

A sample of 180 participants was calculated by the formula given below:
The sample size from each school was calculated by using Selvin's formula [$n = N/1+ N_ne^2$] [n = Sample Size, N = Total population in this age group, N_n = Total population in school, e^2 = Margin of error] (28). Fifth-grade and fourth-grade students from each branch of school were selected. Then participants were split into two groups, control group (90) and intervention group (90)

Tools for Data Collection

The data was collected in a questionnaire form consisting of close-ended questions (KAP). Demographic factors (name, age, gender, and socioeconomic status) were also collected. Weight and height of students were computed to calculate Body Mass Index [weight (kg)/height (m^2)) (29). Then the Body Mass Index (BMI) of each student has plotted on BMI for age Center of Disease Control (CDC) growth charts.

The data was collected in a questionnaire form consisting of close-ended questions (KAP). In knowledge, attitude and practice questionnaire (KAP), 19 questions were based on knowledge, 6 questions based on attitude and 11 questions based on practices related to fruits and vegetable consumption. The dietary habits of the student were assessed by the use of a Food Frequency Questionnaire. The knowledge, attitude, and practice (KAP) questionnaire was prepared by reviewing other questionnaire applied in similar studies. It was comprised of the following sections: student's knowledge about the importance of fruits and

Pilot study

The pretesting was done to check the validity of the questionnaire. Random children of age 7-10 years were selected for pretesting. Some questions were omitted after the pretest, because of unclearly in those questions. Those questions were rephrased to make sure the clarity of questions.

Nutrition education lectures

Nutrition education lectures were developed and delivered by audio-visual method and demonstration to the intervention group. Nutrition education lectures were delivered after the consent of school administration.
The nine nutrition education lectures were delivered to the intervention group. They were separated by one week's time span and each lecture lasted 30 minutes. During the first lecture, the general concept of nutrition and the importance of healthy eating was introduced to the students. In the second lecture my plate was shown, the definition of my plate and different food groups such as milk, vegetable, meat, and fruits were explained. In the third lecture importance of fruits and major nutrients found in the fruits were explained to the students. In the fourth nutrition education, lecture benefits of fruits were explained. In the fifth lecture the importance of vegetables and major nutrients found in the vegetables were describe to the students. Then in the six lecture benefits about the intake of vegetables was explained. In the seventh lecture serving size of fruits that are consumed on a daily basis was explained to the students. In the eighth lecture serving size of vegetables that are consumed on a daily basis was explained to the students. In the last nutrition education lecture advantages of fruits and vegetable juices and disadvantages, soft drinks were explained to the students. **Duration of study**

A post-test was conducted for both groups (G1=control, G2=Intervention) after two months of study, to analyze the impact of nutrition education intervention on knowledge, attitude, and practices of students. Comparisons were made between two groups to check the impact of nutrition education.
After two months of study student's height and weight were again computed to calculate Body Mass Index (BMI). The food habits of the students were again assessed by the use of a Food Frequency Questionnaire (FFQ) to analyze any change in nutritional status and consumption of fruits and vegetables after nutrition education.

Data analysis

The comparison was made to analyze the change about knowledge, attitude, and practices related to fruits and vegetable consumption by using a paired t-test. SPSS version 20 was used for data analysis. Data was represented in the tables and graphs form. Statistical significance was determined at the level of P=0.05.

Results

Demographic

The baseline parameters of the study participants were weight, height, and Body Mass Index (BMI). Baseline characteristics of children were shown in Table-1. The mean age of the study participants was 9.00±0.50, weight 29.5±2.8 and height 148.8±2.7. The mean Body Mass Index (BMI) of the study participants was 18.5±22.1 indicating that the majority of study participants had normal weight at the time of the study Table-2 shows that according to percentile and frequency distribution of BMI for age, participants of this study were classified as underweight, normal weight, overweight and obese. Percentile and frequency distribution BMI for age boys (n=110) was revealed that 44(35.6%) boys lay in normal weight (>5th and <85th), 20(30%) overweight (>85th and <95th) and 8(16%) were obese (>95th). Percentile distribution BMI for age girls (n=79) was revealed that 57(64.4%) girls lay in normal weight (>5th and <85th), 18(25%) girls were overweight (>85th and <95th) and 10(13%) girls were obese (>95th).

Anthropometric measurements of study participants

After two months, the post anthropometric measurements of study participants from two groups (control group and intervention group) were indicated that the mean age, height, and BMI was the same when compared with baseline data.

Table-4 demonstrated that no significant changes were observed in both groups (control group and intervention group).

Overall Knowledge, Attitude and Practice (KAP) questionnaire score

Table no.3 Illustrated the overall mean knowledge, attitude and practice (KAP) questionnaire score of two groups (control and intervention group) before and after the intervention. After nutrition education intervention on fruits and vegetables (FV) consumption the mean knowledge, attitude and practice score of intervention group 30.01±6.54 were higher as compared to mean knowledge, attitude and practice score of control group 20.73±5.25. The p-value of the intervention group (p=0.0001***) demonstrated a significant difference in mean score of nutrition knowledge, attitude and practice questions before and after nutrition education. The p-value of control group (0.83) indicated non-significant difference in mean score of nutrition knowledge, attitude and practice questions. The results revealed that school-based nutrition education intervention improved nutrition knowledge related to fruits and vegetables (FV) consumption.

Table No. 1: Baseline characteristics of study participants. (N=180)

VARIABLES	MEAN±S.D	BOYS (n=101)	GIRLS (n=79)
Age (years)	9.00±0.50	9.2±0.59	9.4±0.58
Weight (kg)	29.5±2.8	30±2.9	29±2.5
Height (cm)	148.8±2.7	149±2.8	147.2±1.7
BMI (kg/m²)	18.5±22	19±1.3	18±1.3

Table No. 2: Percentile and frequency distribution of study participants (N=180)

BMI	Boys (n=101) f(%)	Girls (n=79) f(%)
Underweight (<5th Percentile)	-	-
Normal weight (>5th Percentile and <85th Percentile)	44(35.6%)	57(64.4%)
Overweight (>85th Percentile and <95th Percentile)	20(30%)	18(25%)
Obese (>95th Percentile)	8(16%)	10(13%)

Table No. 3: Overall knowledge, attitude and practice (KAP) questionnaire score (N=180)

VARIABLES	Control (n=90)			Intervention (n=90)		
	Pre-test	Post-test	P-value	Pre-test	Post-test	-value
Weight (kg)	29.6±2.6	29.6±2.6	0.27	30.1±2.88	30.1±2.88	0.34
Height (cm)	149.4±2.1	149.4±2.1	0.23	149.8±1.94	149.8±1.94	0.20
BMI (kg/m^2)	20.3±2.3	20.3±2.3	0.13	19.0±2.2	19.0±2.2	0.10

***highly significant**

Table No. 4: Anthropometric measurements of study participants

Dietary Habits of study participants

Analysis of two months of food frequency revealed that there was an increase in the intake of certain fruits and vegetables (FV) after the delivery of nutrition education lectures. The percentage (%) of fruits and vegetables are illustrated in the following tables. Though there was an increase in the consumption of certain fruits and vegetables they were not statistically significant.

Fruits

In group2 (intervention) there was significant change observed in the consumption of fruits like banana (87.8%), apples (82.2%), strawberry (46.7%) and mango (77.8%) after nutrition education intervention.

Vegetables

Overall (KAP) score	Control Group			Intervention Group		
	Pre-test	Post-test	p-value	Pre-test	Post-test	p-value
	20.91±5.26	20.73±5.15	0.83	23.18±5.66	30.01±6.54	0.0001***

In group2 (intervention) there was significant change observed in the consumption of vegetables like vegetable salad (55.6%), potato (86.7%) and cucumber (63.3%) after nutrition education intervention**.**

Discussion

The baseline parameters of this study were age, weight, height and BMI. The results revealed that the mean age of the participants was around 7-10years (9.00±0.50), the weight was 29.5±2.8kg and mean height was 148.8±2.7cm. The body mass index (BMI) revealed that most students had normal weight 18.5±22. The BMI of the study sample was also plotted on BMI for age CDC growth charts. According to percentile and frequency distribution, out of total boys (N=110) 44(35.6%) had normal weight, 20(30%) were overweight and 8(16%) were obese. In girls (N=70) 57(64.4%) had normal weight, 18(25%) were overweight and 10 (13%) were obese. Contradicting to present study, a study was conducted in Pakistan to

measure the anthropometric measurements of children. The findings indicated that 5% children were obese and 14% were overweight (30). The possible reasons for overweight and obese children might be that, less physical activity, unhealthy eating patterns, peer group, media and socio-economic status. After two months of nutrition education intervention, post anthropometric measurements had indicated no change in the height, weight and BMI when compared with pre-test. The reasons may be short nutrition education intervention time that contributes to the no changes in anthropometric measurements observed in the study participants.

Overall school-based nutrition education intervention on fruits and vegetables consumption revealed significant changes on knowledge, attitude and practices in children of intervention group after nutrition education lectures as compared to control group. The mean score of correct answers on knowledge, attitude and practices (KAP) before nutrition education was less in both groups (control group 20.91±5.26, intervention group 23.18±5.66). In addition, the mean score of correct answers on knowledge, attitude and practices after nutrition education was high in intervention group (30.01±6.54). Wardle et al (2000) reported that nutrition knowledge is very important to change the behavior, attitude and practices related to food intake among school going children (31). The possible reasons to change in knowledge of intervention group related to fruits and vegetables consumption might be that they gain nutritional knowledge related to fruits and vegetables consumption from lectures which were delivered to intervention group and demonstration from each lecture. The possible reasons of no change in the nutritional knowledge of control group might be that, they receive no lecture and demonstration related to fruits and vegetables consumption.

Powers et al, (2005) study has demonstrated the similar results that school-based nutrition education intervention improves the nutritional knowledge, attitude and behavior of children towards fruits and vegetables consumption in intervention group (32). Contrary to present study Neumark-Sztainer et al, (2003) indicated that availability of fruits and vegetables at home increase the intake of fruits and vegetables (33). Contrary to present study results of Ramussen et al, (2006) revealed that socio-economic position; preferences, parental intake and home availability all were influenced on fruits and vegetable intake (34). Contrary to present study Brug et al, (2008) revealed that availability and opportunity related factors such as knowledge, self-efficacy, parental influence and accessibility of fruits and vegetables were linked with likelihood of fruits and vegetables in children (35). Dietary habits of study participants were also observed before and after nutrition education intervention via food frequency questionnaire.

Analysis of food frequency questionnaire showed that consumption of few fruits and vegetables like apple (82.2%), banana (87.8%), strawberry (46.7%), mango (77.8%), potato (86.7%), cucumber (63.3%) and vegetable salad (55.6%) increased with the passage of time which was no statistically significant. Results from McAleese and Rankin (2007) had demonstrated that school-based nutrition education intervention brings significant change in fruits (1.9 ± 1.4) and vegetables (2.6 ± 1.7) consumption which was rich in vitamin A, Vitamin C and fiber in intervention group children (36). The possible reasons of less consumption of certain kinds of fruits and vegetables explored after nutrition education lectures might be due to following reasons. Firstly, may be due to less availability of fruits and vegetables (FV) at home due to less parental nutritional knowledge or may be due to they can't afford to buy daily fruits and vegetables (FV). Secondly, may be due to their family eating patterns, food preferences, family rules, and family law and culture preferences. Thirdly, may be due to their parental occupational status. Fourthly, may be due to short time period of nutrition education intervention. There was no change observes in the consumption of fruits and vegetables (FV) in control group. The possible reasons might be that, they received no nutrition education lecture related to importance and benefits of fruits and vegetables consumption, socio-economic status, educational level, poor dietary habits, peer group, family environment, diet related beliefs and attitudes, primary health care practices and availability. The current study demonstrated that school-based nutrition education intervention on fruits and vegetables (FV) consumption improved the knowledge, attitude and practices (KAP) regarding fruits and vegetables consumption in school going children. The limitations of present study are that short nutrition intervention time, parents and teachers were not involved, small sample size and small number of fruits and vegetables measured. For future studies parents must be involved, because home environment brings more change in children knowledge, attitude and behavior related to fruits and vegetables consumption.

Conclusion

It was assumed that nutrition education lectures have a positive impact on the knowledge, attitude and practices related to fruits and vegetables consumption in school going children. Regarding that, in this study student's fruits and vegetables (FV) intake was lower than the recommended level, both before and after intervention, so an urgent attention should be given to the quality of students' nutrition. This requires future investment in educating parents and society as well as performing more investigations.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any agency of the Pakistan government. Examples of analysis performed within this article are only examples. Assumptions made within the analysis are not reflective of the position of any Pakistan government entity.

Table No. 5: Analysis of FFQ (fruits) from two groups (control and intervention group)

Fruit Group	Control group								Intervention group							
	Never or <than once/week (%)		2-4 per week (%)		5-6 per week (%)		>Once a day (%)		Never or <than once/week (%)		2-4 per week (%)		5-6 per week (%)		> Once a day (%)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Banana	13.5	14.4	11.2	11.1	1.1	1.1	74.1	73.3	10	6.7	12.2	5.6	0	0	77.8	87.8
Apple	14.4	14.4	7.8	8.9	8.9	8.9	68.9	67.7	16.7	3.3	23.3	6.7	2.2	7.8	57.8	82.2
Peach	27	26.7	18	17.7	21.3	20.6	33.7	34.5	32.2	24.4	16.7	17.8	15.6	27.8	25.5	30
Watermelon	27	24.4	10.1	6.7	17.9	7.8	45	51.1	17.8	12.2	15.5	17.7	23.3	31.1	43.3	38.9
Mango	10.1	12.2	14.6	17.8	15.8	13.4	59.5	56.6	11.1	6.7	14.4	4.4	23.3	11.1	51.1	77.8
Strawberry	22.5	26.7	11.2	13.4	20.2	17.7	46.1	42.2	15.6	12.2	18.9	17.8	36.7	23.3	28.8	46.7
Pineapple	55.1	55.6	11.2	14.4	15.7	11.1	18	18.9	36.7	35.6	11.1	10	26.7	30	25.5	24.4

Table No. 6: Analysis of FFQ (vegetables) from two groups (control and intervention group)

Vegetable Group	Control group								Intervention group							
	Never or than once/week (%)		2-4 per week (%)		5-6 per week (%)		>Once a day (%)		Never or <than once/week (%)		2-4 per week (%)		5-6 per week (%)		>Once a day (%)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Vegetables salad	28.1	30	12.3	12.2	11.3	12.2	12.3	12.2	31.1	**25.6**	14.4	14.5	11.1	4.4	43.3	**55.6**
Spinach *bhujia*	34.8	35.6	17.9	11.1	12.4	12.2	17.9	11.1	36.7	**20**	12.2	**18.9**	10	21.1	41.1	40
Potato	29.2	27.8	13.5	13.4	10.1	10	13.5	13.4	18.9	**4.4**	11.1	3.3	13.3	5.5	56.7	**86.7**
Cucumber	31.5	32.2	13.5	13.3	9	8.8	13.5	13.3	17.8	13.3	8.9	6.7	18.9	16.6	54.5	**63.3**
Ladyfinger *bhujia*	37.1	37.8	11.2	11.2	18	17.7	11.2	11.2	35.6	**16.7**	12.2	23.3	22.2	20	30	40
Loki *bhujia*	60.7	66.7	11.2	10	14.6	11.1	11.2	10	50	**21.1**	12.2	**33.3**	12.2	18.8	25.6	26.7
Brinjal *bhujia*	57.3	16.1	6.7	5.5	18	11.2	6.7	5.5	46.7	35.6	15.6	20	12.2	18.9	25.6	25.6

Conflict of interest

No conflicts of interest.

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ORIGINAL RESEARCH

Production and characterization of dextran from *Leuconostoc mesenteroides* NRRL B-512(f) fermentation

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Abstract

Dextran is a complex, branched glucose polymer carbohydrate with variable glycoside linkages and important with respect to food, clinical and medical point of view. The aim of the current study was to produce dextran from *L. mesenteroides* NRRL B-512(f) fermentation by altering carbon sources in growth media, and comparatively analyze characteristics of resulted dextran samples and its related enzyme i.e. dextransucrase. The desired strain was received from Karachi University, Karachi Pakistan. Dextran samples were fermented by *L. mesenteroides* NRRL B-512(f) using sucrose, dextrose, and fructose as carbon sources in fermentation jars under standard growth conditions. Samples were extracted and purified by treating with chilled ethanol repeatedly and characterized by analyzing physical properties, production yields, gelling properties, melting points, and molecular weights. Molecular weights of extracted dextran samples were determined on FPLC apparatus manually. Dextransucrase (DS) is involved in dextran production in the presence of a rich carbon source. Dextransucrase was extracted and purified by treating supernatants of samples with 25% pre-chilled PEG400 leading to overnight incubation and then centrifugation. The purified protein samples were collected in the form of the palate with gradual monitoring of dextransucrase activity (DSU). Subsequently, its molecular weight was also determined using SDS-PAGE. Dextran can be produced by varying the substrates in fermenting media. But its properties altered by altering the substrate. This alteration may be effective in the context of its applications. As a conclusion to this study, sucrose is the most valuable substrate for fermentation as is gives maximum yield.

Introduction

Dextran is an extracellular bacterial homo-polysaccharide, a complex, branched glucan (glucose polymer) composed of various glucose chains of varying lengths from 3 to 2000 kDa (1). Dextran is uninhibitedly dissolvable in polar solvents like water, methyl sulphoxide, formamide, ethylene glycol, glycerol, and so forth. Some dextran divisions may embrace a specific level of crystallinity and may just be brought into the arrangement by solid warming. Dextran delivered by Leuconostoc *mesenteroides* NRRL B-512(F) comprises of an α-1,6-glycosidic linkage with side chains connected to the 3-positions (α-1,3 linkages) of the backbone in glucose units (2).

Auxiliary and biochemical portrayal of dextran differ by changing its aging conditions like brooding temperature and term, carbon, mineral, and amino corrosive sources. It is delivered at the mechanical dimension by the aging of sucrose-rich media. Raymond and his examination specialists (1993) upgraded the aging conditions for the greatest creation of dextran (3). It has been accounted for before that atomic weight and yield of dextran creation relies upon the procedure factors, for example, temperature, sucrose, and the acceptor focus. It was additionally referenced that medium containing nitrogen source enhanced with various salts expanded its creation (4).

Dextran has heaps of business applications as a medication, an adjuvant, an emulsifier, a transporter, and stabilizer.

Cross-connected dextran is known as Sephadex, which is generally utilized for the detachment and refinement of protein. In sustenance, industry dextran is as of now utilized as jam and dessert thickener. It averts crystallization of sugar, improves dampness maintenance, and keeps up flavor and presence of different sustenance things (5).

Dextransucrase created by the L. *mesenteroides* strains polymerizes the glucosyl part of sucrose into dextran (6). These are extracellular proteins with the double working

19

of beginning polymerization and furthermore, present the numerous sorts of the branch focuses without including anyone catalyst (7). This gathering of glucansucrases is expansive particles of normal mass 160 000 Da (8). Sucrose molecules are hydrolyzed as vitality hotspot for biosynthesis reason. Levans (fructans) are shaped by a comparable procedure in which the fructose buildups are polymerized and the glucose is acclimatized. The protein catalyzing their arrangement is levansucrase. Without sucrose, a few microscopic organisms are fit for the creation of dextran and fructan and different polysaccharides. The significant contrast from the union of bacterial heteropolysaccharides (and different homopolysaccharides) is the absence of inclusion of sugar nucleotides and the extracellular idea of the biosynthetic procedure. (6).

Objective of the study was a comparative characterization of dextran samples by altering fermentation protocols with sucrose, fructose and dextrose (glucose) as carbon sources and then characterize the product dextran by Physical characteristics (color, texture, solubility, etc.), Enzyme activity, Production yield, Gelling property, Melting point and Molecular weight.

Materials & Methods
Strain Revival:
A purified *strain of L. mesenteroides* NRRL B-152(f) was maintained on the slant of on 10% sucrose medium and revive to fresh culture by incubating at 26°C (5).

Confirmation
Confirmation of desired strain was done by selective medium growth (9), biochemical and antibiotic sensitivity tests (10). The 10ml inoculum was prepared with 10% sucrose broth medium and incubated at 30oC for 24 hours then transferred to 90 ml sterile broth and incubates for next 24 hours at 30oC.

Differential fermentation
Differential fermentation of *L. mesenteroides* was carried out by using 10% dextrose, fructose, and sucrose respectively in each 1 litre fermenter as a carbon source with nutrient broth as a medium for dextran production under slandered conditions.

Dextran Extraction & Purification
Each cultured broth was centrifuged at 5,000 rpm for 30 minutes and the supernatant was treated with chilled ethanol in equal volume to precipitate extracellular dextran. For purification, the extracted dextran samples were dissolved in chilled dH$_2$O to make a past then treated with chilled ethanol in equal volume and centrifuged at 5.000 rpm for 10 minutes to collect dextran in pallet form (11). Following method was repeated thrice then resulted purified dextran samples were dried in in dryer oven and used for characterization.

General Characteristics of Dextran
General physiological characters i.e. color, texture, smell, etc., comparative percentage yield by total dry weight (g/L) extracted, relative melting points by melting point apparatus, gelling capacity and molecular weights of dextran samples were comparatively analyzed. Dextran samples were dissolved in dH$_2$O with 0.5, 1, 1.5 and 2 mg/ml concentrations and freeze at 4°C to estimate their increasing thawing time against dH$_2$O.

Molecular weight of dextran
The molecular weight of dextran samples was calculated by FPLC apparatus following the size exclusion chromatography method against the standard blue dextran having molecular weight 200,000 Da with 1ml/min flow rate and 3ml friction size. 5ml column of Sephadex G$_{200}$ was used for this chromatography. Citrate-phosphate buffer with pH 5.0 is used as mobile phase.

Dextransucrase Extraction & Purification
Dextransucrase is an enzyme responsible for the production of dextran was extracted from the supernatant of fermented broth of samples after 5000 rpm centrifugation and treated with pre-chilled 25% PEG-400 respectively followed by 12000rpm centrifugation for 15 minutes and the enzyme was settled down in form of the pallet.

Dextransucrase Activity & Molecular weight
The protocol described by Shukla *et al.* 2010 was followed to measure enzyme activity of dextransucrase regarding each sample in "DSU" unit defined by Miller (13). Incubated at 30°C for 1 hour and ODs were taken at 500nm Slandered SDS-PAGE protocol was performed to determine molecular weights of sample enzymes (14).

Results
Re-cultured growth results of *L. mesenteroides* on sucrose-rich and selective media showed white, shiny, convex colonial growth with lactic acid fermentation and gas production.
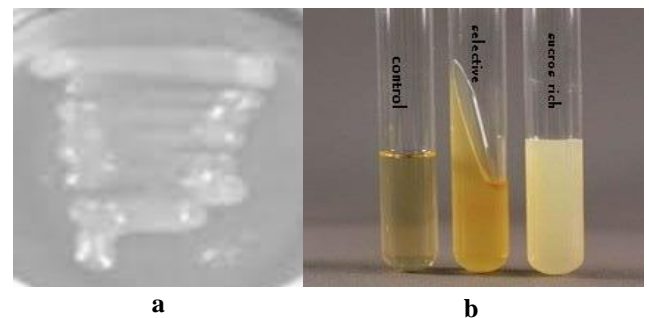

a   **b**

Figure 1: (a) Re-cultured growth of Leuconostoc *mesenteroides* on sucrose rich agar. **(b)** *L .mesenteroides* growth on sucrose rich and special medium along with control broth.

Biochemical tests results include, mannitol +ve and vancomycin sensitivity with 23mm inhibition zone confirmed the presence of *Leuconostoc mesenteroides*.

a   **b**   **c**

Figure 2: (a). In the Vancomycin sensitivity test, *L. mesenteroides* showed 23mm inhibition zone **(b).** *L. mesenteroides* showed the catalase –ve results as no gas formed **(c).** The color change from red to orange showed +ve mannitol results by *L. mesenteroides*

Purified dextran samples were subjected to physical characterization. All dextran samples showed no change in color, smell, texture, and solubility as shown in Table No. 1. While, change in fermentation media affect the percentage yielding, gelling property, melting point and molecular weights of dextran samples. Enzyme activity and molecular weight of dextransucrase extracted and purified from differential fermented broth showed observable variation due to change in carbon source.

Table No. 1: Comparative physical characters of dextran from different substrates

Sr. No.	Characteristics	Ds	Dd	Df
1	Color	White	White	White
2	Smell	No	No	No
3	Texture	Powder	Powder	Powder
4	solubility (H_2O)	5%	5%	2%
5	solubility (ethanol)	insoluble	insoluble	Insoluble
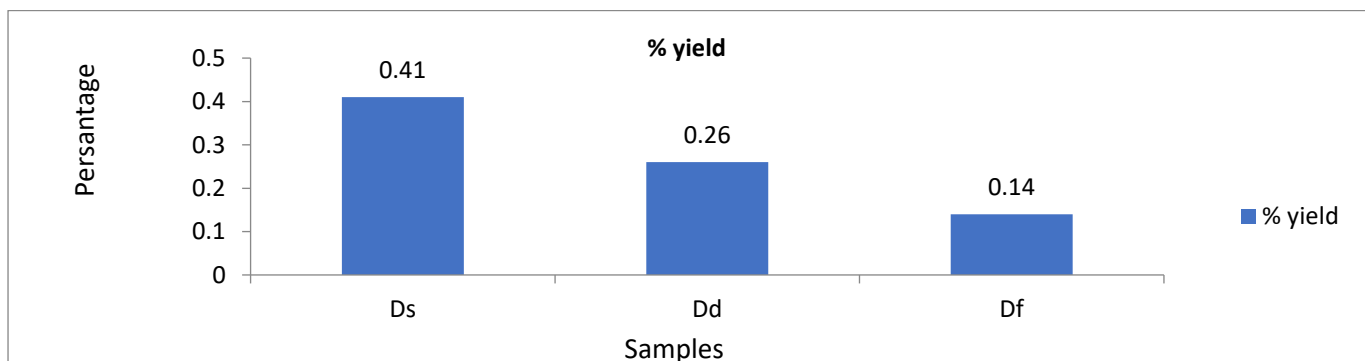
Figure 3: Comparative yield (percentage) of dextran from different substrates

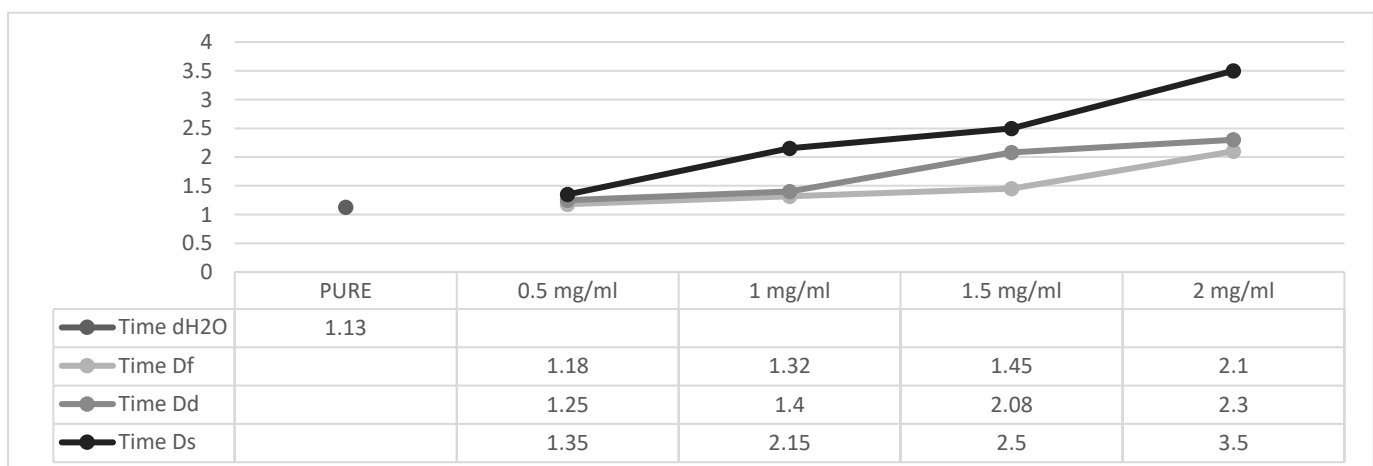|	PURE	0.5 mg/ml	1 mg/ml	1.5 mg/ml	2 mg/ml
Time dH2O	1.13				
Time Df		1.18	1.32	1.45	2.1
Time Dd		1.25	1.4	2.08	2.3
Time Ds		1.35	2.15	2.5	3.5

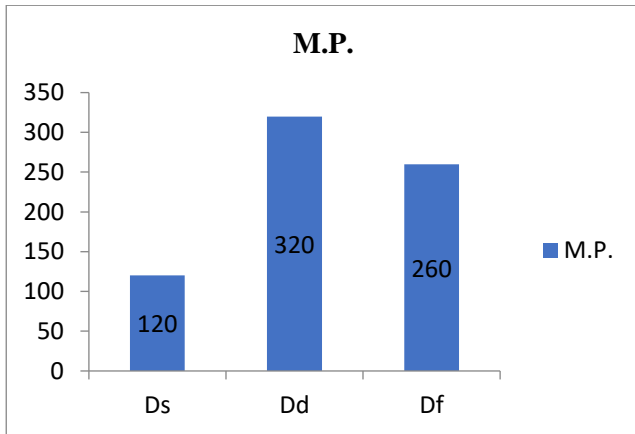**Figure 4:** Gelling property of dextran from variable substrates

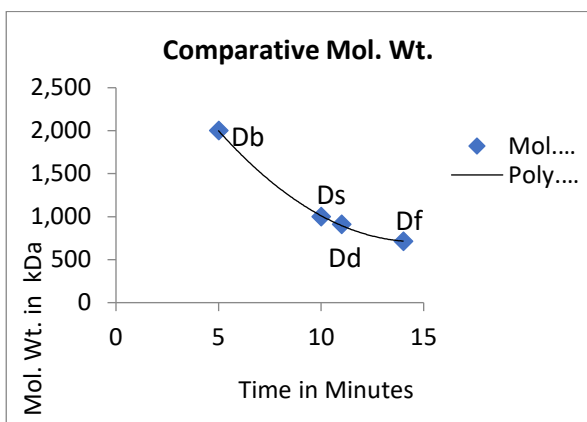**Figure 5:** Comparative melting point of dextran samples

Figure 6: Comparative mol. weight of dextran samples by size exclusion chromatography on FPLC.

Discussion

In 2008 Nasab et al. produced dextran by Leuconostoc mesenteroides NRRL B512 (f) using date extract v/s sucrose as carbon sources. Low molecular weight dextran samples attained by date extract then sucrosesources compared to blue dextran of molecular weight 2,000 kDa (16). While in the present study, sucrose, dextrose, and fructose were used as a carbon source in medium but the same standard, dextran blue was used to analyze molecular weights of samples. Sucrose sample showed the highest molecular weight among all. Dols *et al.* 1997 studied the specific growth rate under aerobic conditions by using glucose and fructose supplements and found that glucose gave a batter yield in anaerobiosis. According to them, both sugars are phosphorylated and catabolized. They also found that in sucrose grown culture, sucrose converted into dextran and fructose and inhibit the growth of *L. mesenteroides* (17). On the current study, dextrose was used instead of glucose against fructose and sucrose for comparative analysis in only aerobic condition and yield of dextrose sample was least as compared to the other two. Maximum yield was attained by the sucrose sample.

Sarwat *et al.* in 2008 analyzed the effect of variable parameters including pH, temperature, %age sucrose content and incubation time and concluded that maximum production was attained with 15% sucrose after 20 hours at 30ºC in 7pH (11). However, in recent work done, the comparison was done between different carbon sources and the best results were received from the sucrose sample.

Dols *et al.* in 1998 produced DS in glucose and fructose instead of sucrose and analyzed by SDS-PAGE. Dextran produced from these enzymes had variable glycosidic linkages (15). Instead of this, in ongoing work done, enzyme activity was analyzed by calculating DSU (dextransucrase unit) in media supernatants by taking ODs before and after incubation.

Purama *et al.* in 2009 studied the dextran produced by Leuconostoc *mesenteroides* NRRL B640 and found that dextran has a novel food gelling and thickening properties (18). It was also observed in the existing study that dextran had some gelling property and increased the thawing time of solutions or medium in which they added.
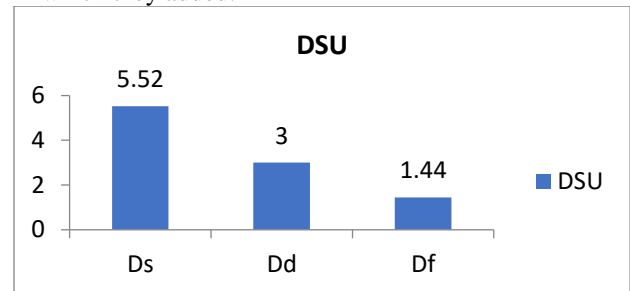
Figure 7: 1 hour incubation at 30ºC, OD at 500nm in 0.1M sodium acetate buffer pH 5.0
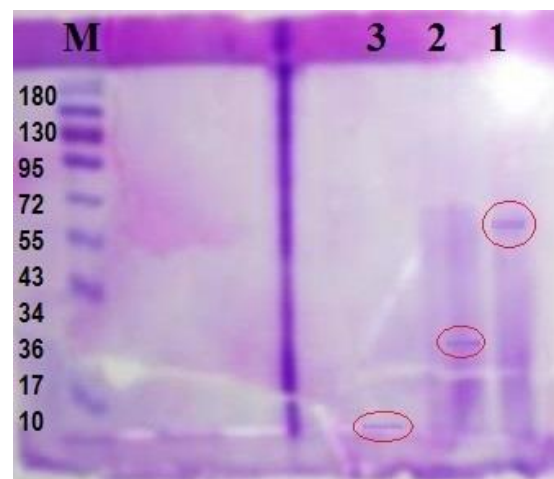

Figure 8: SDS-PAGE analysis, results show that dextransucrase fermented in sucrose medium had higher molecular weight than dextransucrase fermented in dextrose and fructose. M= molecular weight marker, lane 1=DSS (dextransucrase from sucrose fermenter), lane 2= DSF (dextransucrase from fructose fermenter), lane 3 = DSD dextransucrase from dextrose fermen

Conclusion

Findings of this study concluded that dextran, as a biopolymer, its properties can be very by altering the fermenting conditions. Sucrose is used as a basic carbohydrate source in the medium for production. However, it is seen that dextran produced by using substrate other than sucrose but the yield and characteristics vary according to each supplement. Sucrose fermentation gives more effective results as compared to others. It is observed that yield, gelling capacity and molecular weights decreased when fermented with dextrose and fructose instead of sucrose. While melting points were increased. Besides all these, dextran showed the same physical characters include color, texture, and solubility.

Acknowledgment

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REVIEW

Life Science Journal of Pakistan

Comparative assessment of biomedical and nutritional status of dry fruits; A review

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Abstract

Nuts as dry fruits are nutrients rich foods that have high amount of phytochemicals like unsaturated fatty acids, proteins, fibers, carbohydrates, flavonoides, phytosteroides and antioxidants. Other nutritional components that are also present in nuts include vitamins, minerals, and phenolic compounds. Nuts have been proposed as an important component of optimal diets for reducing the risk of chronic heart disease. Anti-inflammatory properties present in nuts, maintain the weight and increase insulin sensitivity. Phytosterols present in nuts have a role in regulating the blood cholesterol level. The essential elements omega 3 and omega 6 fatty acids are also present in walnut and these elements have a more beneficial effect on heart brain and neurotransmitters. Polyunsaturated fatty acids like linolenic acid and monounsaturated fatty acid are also present in nuts which have a potential role in the human body to maintain the low-density lipoproteins and high-density lipoproteins for proper arterial function. Both pine nuts and cashew nuts have antioxidant potential due to the presence of a wide range of phytoconstituents, so this review article has been compiled to summarize the health benefits of dry fruits, phytochemical constituents and their mechanism of actions.

Introduction

Fruits and vegetables are natural elements having antioxidant properties and help to decrease the effect of free radical on the body (1, 2). Free radicals have more harmful effects on the body such as DNA mutation, lipid peroxidation and cross-linking proteins which could be prevented by antioxidation. Nuts as dry fruits are nutrient-rich foods that contain rich amount of unsaturated fatty acid and other compound likes protein, fiber, carbohydrate and antioxidants properties (3). Nutritional compounds that are also present in nuts are vitamins (e.g. tocopherols, niacin, folic acid, and vitamin B6), minerals (e.g., magnesium, potassium, calcium, iron) and phenolic compounds (4). Some bioactive components of nuts i.e. Phytosterols, selenium, folic acid, tocopherols, phenolic compounds, and magnesium, are reported to have antioxidant, anticarcinogenic or anti-inflammatory properties (4). Anti-oxidation properties play an important role to preserve the chronic disease and reduce the risk of cancer. It is described that trend of

obesity is increasing which might be the major cause of cardiovascular diseases and type 2 diabetes mellitus, could be prevented by fruits and vegetables (5).

Phytosterols are present in nuts, fruits and vegetable, and their structure are related to the cholesterol played role in maintaining of blood cholesterol level and it was reported that consumption of 2 g of phytosterols per day reduced LDL cholesterol level by 10%, and are considered to protect cardiovascular complications. Nuts consumption effect on the serum lipid and decreases the low-density lipoprotein and improves the ratio of HDL (6). G-tocopherol and vitamin E are also present in all nuts which also have cardioprotective roles as well as to reduce the risk of heart diseases, hypertension, cancer and Alzheimer's disease (7).

Omega 3 and omega 6 fatty acids are present in walnut and these elements have a more favorable effect on heart, brain, and neurotransmitters. Walnut is the rich source of polyunsaturated fatty acids, basically linolenic acid

and linoleic acid. The particular lipid profile of nuts in general and walnuts, in particular, is likely to be an important contributor to the beneficial health effects of frequent nut consumption. Walnuts improve the blood lipid and potential benefit for oxidative stress and inflammatory marker. Walnuts act as an anti-inflammatory and affect endothelial functioning. Walnuts improve metabolic syndrome and maintain body weight. Nuts are the great source of iron and are mainly determine hemoglobin level in the body. Iron is the most important element for the production of blood. Additionally, iron boosts metabolic processes, essential for growth and development, normal cellular function and also play an important role in the formation of hormones and connective tissues (8).

Pine nuts are most beneficent and protect the body against the diseases of heart, brain, and cancer. Most essential fatty acid like monounsaturated fatty acid and polyunsaturated fatty acid are present both in pine nuts and cashew nuts (7). The body can't synthesize the carotenoids, so carotenoids entered into the body through diet and high amount of lutein and zeaxanthin carotenoids are present in pine nuts which are necessary for healthy eyes and muscles. Cashew nuts also contained antioxidants, anti-inflammatory and cardioprotective substances (9). Comparison of nutritional values per 100 gram of walnuts, pine nuts, and cashew nuts have listed in Table 1

Nutritional status of walnuts

Walnut plays a significant role in medicines which are used to cure heart diseases (10). Walnuts have great antioxidant potential basically due to the presence of phenolic compounds and tocopherols. Omega 3 fatty acids like DHA (docosahexaenoic acid), ALA (alpha linoleic acid), EPA (eicosapentaenoic acid) and omega 6 fatty acids have a beneficial effect on the body (7).

These compounds have a more beneficial effect for heart, brain and blood vessels. Polyunsaturated fatty acids reduce LDL and increase HDL concentration, consequently decreasing the risk for development of heart diseases. Walnuts improve metabolic syndrome and maintain body weight (11).

Health benefits

Antioxidant and anti-inflammatory properties are present in walnut that protect against chronic disease. Walnut consumption has both acute and chronic beneficial effects on vascular integrity (7).

1- Fights depression

Depression remains a serious health problem with significant associated mortality, morbidity, and economic cost. It is stated (12) that omega 3 is present in walnuts and its important function is to protect the brain and help the neurotransmitter functioning. Omega 3 fatty acid is used in healthy diets that reduce the risk of depression. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are polyunsaturated fatty acids. EPA is an omega 3 fatty acid and plays an important role in the treatment of depression. The higher amount of docosahexaenoic acid (DHA) in the membrane and a higher concentration of eicosapentaenoic acid (EPA) in plasma, help to reduce the rate of depression (13).

Mechanism

Many mechanisms have been suggested for the omega 3 fatty acid in antidepressant effect (Fig. 1). Such as: First, the function of omega 3 fatty acid in signal transduction is to regulate the dopaminergic and serotonergic neurotransmitter (14).

Second, the higher level of prostaglandins is related to mood disorder which is caused by inflammation. Prostaglandin E2 and leukotrienes are drives by arachidonic acid, prostaglandin is derived by cyclooxygenase (COX1&COX2) and leukotrienes are drive by lipoxygenase both resulted to produce inflammation and pain. Non-steroid anti-inflammatory drug (NSAIDs) only inhibit prostaglandin E2 but don't inhibit the lipoxygenase pathway. This condition causes several problems such as bleeding, cell damage, and death. It is described that EPA as a reducer of prostaglandins E2 and leukotrienes significantly reduces the pain and inflammation (Figure 1) (15).

Table No. 1: Comparison of nutritional values per 100 gram of walnuts, pine nuts, and cashew nuts

Nutritional value	Walnut	Pine nut	Cashew
Saturated fatty acid	6g	4.9g	8g
Poly-unsaturated fatty acid	47g	34g	8g
Mono-unsaturated fatty acid	9g	19g	24g
Dietary fiber	7g	3.7g	3.3g
Carbohydrate	14g	13g	30g
Protein	15g	14g	18g
Potassium	491g	0.597g	0.66g
Iron	16g	30g	37g
Vitamin B6	25g	5g	20g

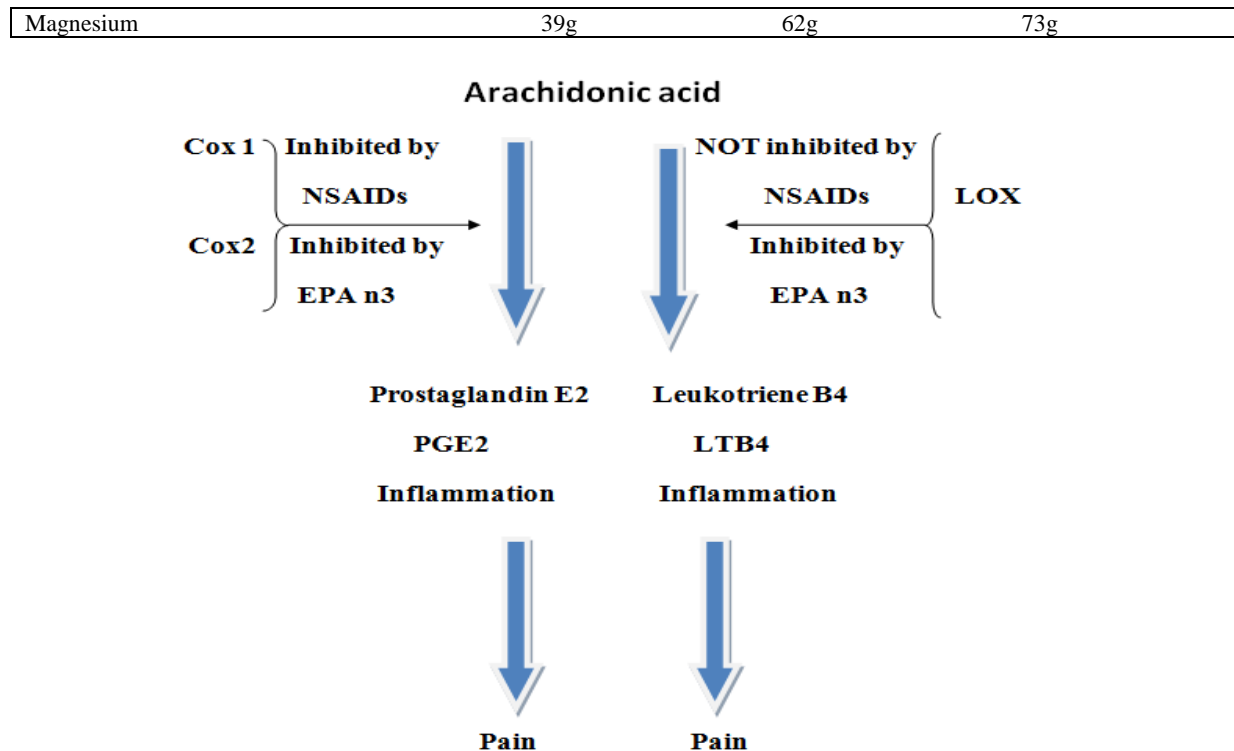| Magnesium	39g	62g	73g

Arachidonic acid

Figure 1: Eicosapentaenoic acid and non-steroid anti-inflammatory drugs are inhibitors of inflammation.

2- Improves brain health and Alzheimer's disease

Alzheimer's disease is a neurodegenerative disease that results in loss of memory. Enzymes act on the APP (amyloid precursor protein) and cut it into fragments (Figure 2) by the β site cleavage enzyme 1 (BACE1), then plaque formation occurs (16). Plaques are found in the brain between the dying cells - from the build-up of a protein called beta-amyloid (the term "amyloid plaques). The overproduction of β amyloid lead due to oxidative stress-free radical produces and cell death. Amyloid-beta fibril process is a multi-step that is produced by oligomerization and aggregation of monomeric then conformational changes occur in alpha-helical and beta-pleated sheet structure (17).

Mechanism

The mechanism of plaque formation causes cell damage and effects on neurotransmitter and brain size is shrinkage in Alzheimer's patients. Antioxidation properties such as gamma tocopherol, melatonin, and phenolic contents are present in walnuts that significantly reduced the risk of brain disease (18). DHA has a more favorable effect on memory, improve brain health and reduce Alzheimer's disease. DHA (docosahexaenoic acid) increases the hippocampus region of the brain. (These regions more adversely effect in Alzheimer's disease) (19). When plaque formation occurs then DHA activates the glial cells, these cells secrete the cytokines and break the peptide bonds of amyloid

plaque. Walnut can inhibit the fibrillization of amyloid-beta synthesis and also solubilize amyloid-beta fibrils. So, omega 3 (DHA) could also have a positive effect on Alzheimer's disease (20).

3- Heart disease

It is stated (21) that omega 3 fatty acids like DHA and EPA reduced the risk of hypertension, high cholesterol, and heart disease. Omega 3 fatty acids decrease the level of triglyceride in the body, consequently, reducing the risk of heart attack and death (22). Low-density lipoproteins are a significant cause of atherosclerosis and cause other disorder such as genetic hyperlipidemias and other familial hypercholesterolemia (FRH) (23). The other several risk factors such as smoking, diabetes, and hypertension are also leading causes of atherosclerosis (24).

Mechanism

Five steps that are involved in this mechanism; (i) chronic endothelia injury, (ii) accumulation of lipoprotein, (iii) monocytes adhesion to the endothelia, (iv) platelet adhesion and (v) lipid accumulation. When a high amount of LDL present on the surface of the endothelial cells it breaks the endothelial barrier and cross the surface where low-density lipoprotein becomes oxidized. When oxidized LDL came to contract with the arterial wall it damage and made deposits on the surface of intima which stimulated the monocytes to the intima. Macrophages in the tissues unable to

26

degrade LDL which further attract the platelets and platelets release the platelet-derived growth factor (PDGF). The growth factors activate the smooth muscle cell. The high amount of lipid contains macrophages that are deposited in the surface of the

intima and then plaque formation occurs (Figure 3). Omega 3 fatty acid can reduce LDL by increasing HDL in return (22).
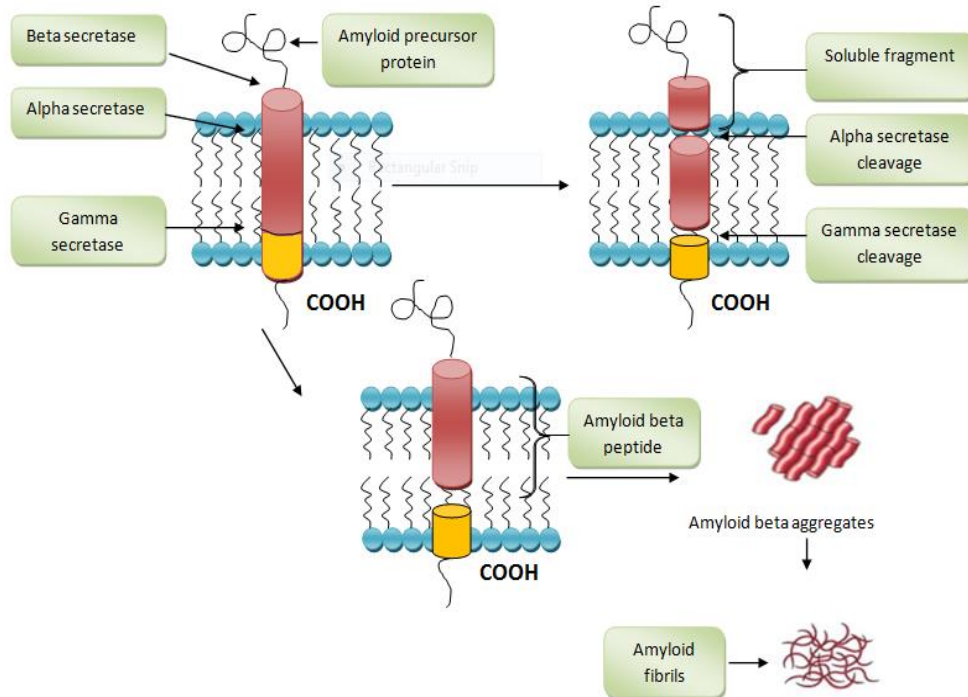

Figure 2: Enzymes act on the APP (amyloid precursor protein) and cut it into fragments. The beta-amyloid fragment is crucial in the formation of senile plaques in AD.
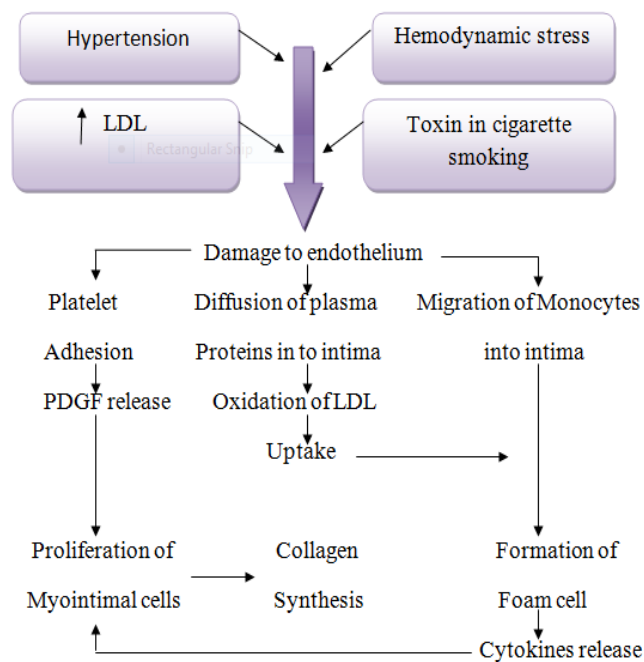

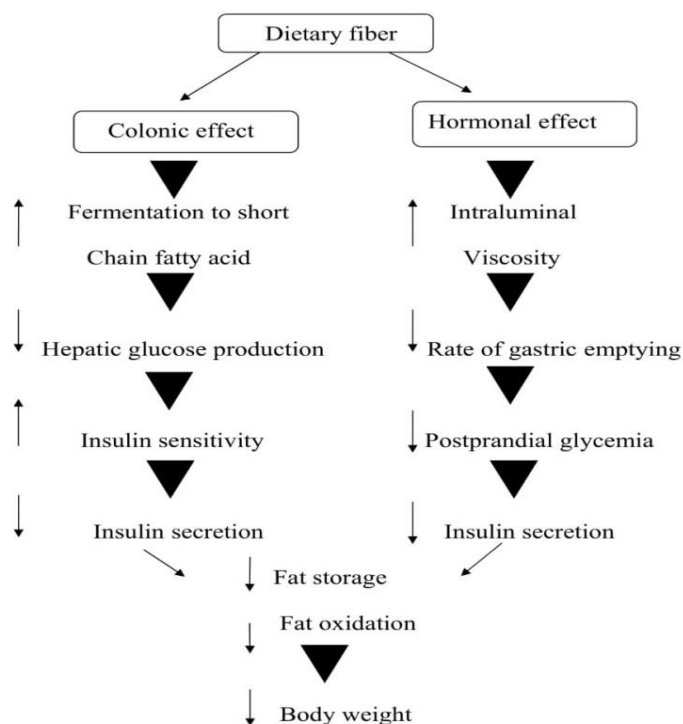**Figure 3:** Mechanism of arterial plaque formation.

Figure 4: Mechanisms through which dietary fiber may aid in body weight regulation.

Weight maintenance

Fibers help to decrease the body weight by three mechanisms such as fiber displaces available calories and nutrients from the diet; fiber increases the chewing process and increase the level of secretion of saliva and gastric juice, and then satiety level increase; and fiber reduces the level of absorption in small intestine as described diagrammatically in figure 4 (25).

Skin protection

Skin is the most important organ in the human body that protects against chemical and mechanical stress, ultraviolet radiation and bacterial infections. The Anti-oxidant properties of Nuts are due to the presence of vitamin C (ascorbic acid), Vitamin E and other phytoconstituents and their role formation and maintenance of skin health by promoting the synthesis of collagen. Oresajo et al, (26) stated the Vitamin C as a cofactor for prolyl 3-hydroxylase 1 (P3H1) and lysyl hydroxylase enzymes catalyzed the cross-linking and stabilized the collagen fibers and collagen fibers ultimately stabilized the structure and function of the skin (27). Vitamin C stabilized the mRNA and increased the gene expression of collagen along with preventing the collagen degradation. Higher intake of Vitamin C decreased the risk of dryness of the skin; skin roughness and ascorbic acid also have an effect on the Trans-epidermal water loss (TEWL) (28). Environmental factors such as pollution and ozone depletion produce free radicals and damage the skin.

Vitamin C provide anti-oxidation and anti-inflammatory properties that reduce the risk of free radical formation (29). Chen et al. (27) stated that oxidative stress occurs when high wavelength UV radiation passes through the skin. UVA (wavelength 320–400 nm) indirectly cause DNA mutation and UVB (280-310nm) are directly damaging DNA by generating reactive oxygen species (ROS) that are developing skin cancer. It is described by Tuong et al, (29) UV irradiation is produced pro-inflammation cytokines growth factor then activates the AP-1 and NF-κB activation as described below. Increase the MMPs expression resulting in degraded collagen and elastin in the skin (30). Increase the productions of ROS effect on the TGF-B growth factor that cause degradation of the collagen synthesis and structural integrity of the skin. Oxidation stress produced aging, wrinkle and pigmentation. Anti-oxidation properties of Vitamin C protect the skin and synthesis of the collagen.

4- Hypertension, cell growth, and others

Potassium helps to regulate the body fluid, sugar level and maintain the blood pressure. Fluctuation, in potassium level, may cause hypertension by influencing the reabsorption capability of kidneys (31). As described by Stolarz-Skrzypek *et al*, (31) that

28

potassium ion present in walnut is the most prevalent cation that has a significant role in normalizing the blood pressure by regulation blood volume.

Folate as coenzymes present in walnut are important for the synthesis of nucleic acid from the precursor of purine and thymidine. Three enzymes are involved in Thymidylate (dTMP) biosynthesis pathway: Dihydrofolate reductase (DHFR), serine hydroxymethyltransferase (SHMT), and Thymidylate synthase (TYMS). Pyridoxal 5 phosphate (PLP), a vitamin B6 coenzyme is required by serine hydroxymethyltransferase (SHMT) which use serine as a one carbon donor for the generation of 5, 10 methylenetetrahydrofolates from THF (32). The latter molecular is one carbon donor for the generation of dTMP from the dump by Thymidylate synthase.

Nutritional Status of Pine nut

Pine nut has a wide range of vitamins such as Vitamin B1, B2, Vitamin E, carotenoids; minerals like phosphorus, magnesium, iron, sulfur, potassium, copper, sodium, zinc, manganese and calcium; and essential macromolecules like carbohydrates, proteins, lipids for maintaining the body's normal health (33). Linoleic acid is more abundant form in pine nuts and is more favorable in maintaining the blood lipid, cholesterol, and blood pressure. It is described by Cabanillas *et al*, (33) that anti-oxidation and anti-inflammation properties are present in pine nuts that protect the brain and heart from many disorders.

Health benefits

Essential fatty acids present in pine nut that reduce the risk of heart diseases, pancreatic cancer and maintain the blood pressure and also maintain eye health. As described by Champagne *et al,* (34) high magnesium reduces the risk of high blood pressure, that high blood pressure causes negative effects in heart, kidney and another important organ in the body. Magnesium maintains the blood levels in veins and arteries. High fats deposit in blood vessels and made a plaque, due to plaque formation blood vessels may rupture. Pine nut is reducing the plaque formation that reduces the risk of cardiovascular disorders. Due to calcium arteries and veins muscles contracts normally. Deficiency of calcium cause muscles not to contract that cause high blood pressure and gradually hypertension.

1- Improves eye health

High energy light (blue light) have a negative effect on the retina which causes stress due to the formation of oxidative free radicals. Blue light causes damage to the photoreceptor and muscular degeneration. Two types of Carotenoids like Lutein and zeaxanthin carotenoids, present in pine nuts have anti-oxidant potential which reduces the muscular degeneration. Lutein reduced the inflammation in eyes by scavenging the free radical's body cannot synthesize these nutrients, so the body gets these nutrients through

diet and supplement. Retinal pigment epithelium (RPE) transfers the LUT in the blood of eye by the xanthophyll-binding protein (XBP). And a large amount of lutein present in pine nuts also protects the eyes by absorbing harmful blue light (35).

2- Lowers risk of pancreatic cancer

Magnesium present in pine nuts reduces the proliferation of cancer cells by reducing the acylation of DNA. Uncontrolled expression of Insulin-like growth factor (IGF) which causes pancreatic cancer but magnesium ions from the dietary sources influenced the expression of this growth factor. So, magnesium has a role to reduce the IGF level then cell proliferation is halted (36).

3- Nutritional status of Cashews

Cashews are a basis nutritional elements and most important role in the human body to cure the heart disease. The cashews *(*Anacardium occidentale) are a light color and kidney-shaped nuts. Cashews present in the antioxidant properties and work against the hearth disease and diabetes. The iron and copper are a help to blood cells, fats can inhibit diabetes, anti-oxidation properties can protect against cancer (37). Cashew nuts are made up of monounsaturated fatty acid (oleic acid) and a smaller quantity of polyunsaturated fatty acid (largely present in linoleic acid). Cashew reduces bad cholesterol and increase good cholesterol and help to decrease blood pressure (38). The high consumptions of plant food such as cashews reduce the risk of heart disease, obesity, and diabetes.

4- Bone metabolism

Cashews improve the bone metabolism due to the presence of mineral (potassium, magnesium, and calcium). Cashews are the rich form of vitamin K. Vitamin K a fat-soluble is needed for the post-translation modification of certain protein (39). Vitamin K1 (phylloquinone) is synthesized by plants and used for blood clotting and Vitamin K2 (menaquinone) produced by bacteria in the large intestine and improve bone metabolism. Osteoclasts present in the surface of the bone responsible for the breakdown of bone and Osteoblasts are responsible for the formation of bone. Osteoblasts and osteoclasts are involved in the repairing of bone. Nutritional diet maintains the bone if not intake the nutrition diet then ruptures the bone cell and causes the osteoporosis. Osteoporosis is a public health issue, especially postmenopausal woman. Osteoporosis patients increase the risk of cardiovascular diseases due to vascular calcification (40).

Calcium deposits in arteries and increase the risk of blood plasma, heart attack, aortic stenosis and ischemia of the lower limbs. Vitamin K maintains the bone structure, function and supports the mineralization. Vitamin K is essential for blood coagulation and regulating of bone metabolism (39). Vitamin K2 increases the activity of osteoblasts, modulates the target

29

gene of bone mineralization in osteoblast (41) and induces the osteoclast apoptosis. Vitamin K is needed for the chemical modification of certain protein the process is called carboxylation (42).

5- Lowers the risk of diabetes

Cashews have a great amount of monounsaturated fats and maintain the glucose level in the body. Kim *et al.* (43) stated that monounsaturated fatty acid takes beneficial foe type 2 diabetes mellitus and maintain the glucose level. The saturated fatty acid affects in enzyme activity, gene expression and glucose metabolism (44). The high amount of monounsaturated fatty acids and polyunsaturated fatty acids has an effect on lipogenic gene expression and binding nuclear receptor such as peroxisome proliferator-activated receptor. The free fatty acids played a significant role in glucose homeostatic. GPR40 and GPR120 are particularly effected in insulin secretion, and short chain fatty acid is activating the GPR41 and GPR43 (45). This receptor is a target for diabetes prevention and treatment.

6- Maintains healthy skin

The skin acts as a protective barrier against the environment and plays an impotent role to prevent infection and maintain the body temperature. Cashews acted as a high source of copper, which help to maintain the skin and hair color called melanin (46). Melanins are producing by melanocytes are only present in cuproenzyme called tyrosinase. Intake the copper can help to protect the skin eyes and hair color. Copper synthesize the plasma proteins, collagen, and myelin. Myelin sheath protects the neurons (46).

7- Prevention from cancer

Cashews are a good source of anti-oxidant compounds and prevent the body from free radicals. The free radicals cause oxidation stress and DNA damage so it may cause tumor formation. Cashews nut shell liquid having anacardic acid with anti-cancer and anti-oxidation properties (47). Anacardic acid acts as a mitochondrial decouple of oxidative phosphorylation and inhibition of histone acetyltransferase activity, this is because cancer cells not produce (48).

Conclusion

Dry fruits are natural nutritive sources rich in unsaturated fatty acid and other compound like protein, fiber, carbohydrate, and anti-oxidant compounds. Other nutritional compounds that are also present in nuts such as vitamins (e.g., tocopherols, niacin, folic acid, and vitamin B6), minerals (e.g., magnesium, potassium, calcium) and phenolic compounds. Nuts have been proposed as an important component of optimal diets that reduces the risk of cardiovascular diseases, hypertension, neurodegenerative disorders, bone demineralization, skin disorders, eyes diseases, and

many other health-related issues. Dry fruits are a very important component of the human diet as it contains very high nutritional values as well as biomedical important phytochemical constituents. This review article has described the nutritional and biomedical importance of components of cashew nut, pine nut, and walnuts.

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ORIGINAL ARTICLE

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Impact of nutrition education interventions on milk consumption among students (age 20-22 years)

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Abstract

The purpose of this study is to determine changes in nutrition knowledge, attitude, and practices of 20-22 years students after the nutrition education intervention. It was a pre-post-test study and we selected 153 students from three institutions of Lahore city. Eight nutrition education lectures were delivered to the students and the effectiveness of these lectures was evaluated by pre and post testing of students. The data collection instruments were KAP (knowledge, attitude and practices) questionnaire and FFQ (food frequency questionnaire). First post-testing was done after the nutrition education lectures. Second post-testing was done after two months to evaluate the retention span of nutrition education lectures on student's knowledge, attitude and practices. Data was analyzed by SPSS version 20.0. The mean score of correct answers of KAP (knowledge, attitude and practices) questionnaire was (6.75 ± 2.26) at pre-test and mean score of correct answers of first post testing was (23.81 ± 2.49) and the mean score after second post testing was (23.79 ± 2.58). Results indicated that there was significant increase (P>0.05) in KAP (knowledge, attitude and practices) of students after nutrition intervention. While the results of two post testing showed that there was no significant difference (p=0.732) in overall KAP score of participants. Results of food frequency tables showed that consumption of fresh milk and milkshake was increased after nutrition education intervention. In conclusion, the study showed that nutrition education interventions were effective in improving the KAP of students regarding milk consumption patterns. It is hoped that improvements would be sustained throughout their lives.

Introduction

Nutrition interventions are those activities that are planned and implemented for the purpose of solving the nutrition problems prevailing in a society. They are aimed at improving the nutrition knowledge, food intake and eating habits of a specific population (1). Nutrition intervention involves activities that utilize economic, political and institutional resources of a community for the purpose of changing the food habits of a target population. (2).

In nutrition intervention, basic foods are selected. Milk is a basic food in human diet. Nutritionists consider dairy products as a focus group and milk is called as a 'complete food' that should be a part of balanced diet (3). It has different place among other foods as it is full of high-quality proteins, vitamins and minerals. Milk contains sufficient amount of calcium that is required for building strong bones and teeth. Protein of milk is regarded as quality protein that is required for body growth and body building purposes (3). Phosphorous, zinc and potassium of milk help in regulating fluid

33

balance and keep the skin, bones and hair healthy. Vitamin A of milk is good for vision and vitamin D is required for absorbing calcium. Other B vitamins such as niacin, riboflavin and B12 are also found in milk (4).

Since the body growth is at its peak during adolescent years, so there is a great demand of body building foods and high-quality nutrients. Studies on assessing the eating habits of adolescents suggested that poor nutrition at this time can lead to the delayed sexual maturation and increase the risk of chronic diseases such as cardiovascular disease, osteoporosis and cancer (5).

Marias and Glasauer, 2014 conducted a research study to assess the dietary habits of college and university students and concluded that poor dietary habits and wrong food choices acquired in youth may have lifelong health consequences. Increased availability of carbonated beverages, experiences of life, beliefs, preferences, changes in life style as well as influence of peer group are the factors that lead to the formation of turning point of food habits in college and university students (4).

Holloman *et al*, 2009 conducted a research study to evaluate the effectiveness of nutrition education interventions on changes in soft drink consumption among students. The study used a pre-post test design and concluded that class-based nutrition interventions were effective in decreasing soft drink consumption in students. Total soft drink consumption of students was 8.53 (fl.oz) at pretest which was reduced to 3.62 (fl.oz) at post testing after the nutrition intervention. Whereas calcium intake contributed by milk consumption was 156.75 mg at the pretest and 233.0 mg after the intervention (6).

A survey conducted at Agha Khan University to assess the knowledge and practices of medical and non-medical students of Karachi and the results of the survey concluded that 'lack of time' was the major contributor to the bad eating habits of 49.7% of medical and 58.4% of non-medical students (7).

In Pakistan there is a need to provide nutrition education on milk consumption among college and university students. Focus should be on promoting healthy eating behaviors in college and university students because it is the most crucial period of life. Behaviors and practices acquired at this time may have lifelong implications.

Materials and Methods
Participants for Study

The study was conducted in three different institutions of Lahore city. The sample size calculation (n =153) was included in this study. Participants in the study were selected from institutions and were not older than 20 years, irrespective to their color, class and social status.

Data Collection:

A KAP (knowledge, attitude and practices) questionnaire was designed. The questionnaire consisted of 3 parts
1. **Anthropometrics:**
   Age, weight, height and BMI of the participants were included.
2. **KAP questions:**
   a. Questions about nutrition knowledge
   b. Questions assessing attitude
   c. Questions about dietary practices
3. **Food frequency questionnaire** was also filled by the students to access the daily consumption of milk and milk products.

Study Design

It was a pre-post test study.

Sample size

A sample of 160 participants was calculated by formula given below. The sample size from each institution was calculated by using Solvin's formula [n= N/1+Ne^2] and 7 students were dropped out, so our sample size remained to 153.

Duration of study

The duration of the study was 6 months. Pre testing was done at baseline to investigate the current KAP of students. Post testing was done two times. First post-testing was done after the nutrition education lectures and second post testing was done after the two months using the same questionnaire to evaluate the retention span of nutrition education lectures on student's knowledge, attitude and practices.

Nutrition education lectures

Eight nutrition education lectures were delivered to the students. The duration of each lecture was 30 minutes with the interval of one week.

Statistical analysis

Non-Parametric Wilcoxson Ranked Test was used to compare the mean scores of pre and post testing of knowledge, attitude and practices Percentages of food frequency questionnaires were calculated and comparisons were made between pre and post testing. Statistical significance was

determined at a level of p= 0.05. The statistic software SPSS version 20 was used for data analysis. Data was represented in the form of tables and graphs.

Results
Demographic

153 participants (60 from college A 60 from college B and 40 from university) was included in this study, all females were 20-22 years of age. The baseline parameters of the study participants were weight, height and Body Mass Index (BMI). Baseline characteristics of participants were shown in table no1. The mean age of the study participants was 20.7±0.78, weight 51.0±10.96 and height157.98±5.6. The mean Body Mass Index (BMI) of the study participants was 20.0±4.0 kg/m² indicating that majority of the study participants had normal weight at the time of study and only a slight difference was appeared in height, weight and BMI of the participants during the pre and post testing.

In Table 2, nutritional status of students was evaluated by using BMI (body mass index). The results of the table 4.1 showed that most of the study participants fall in the normal weight category and there is only a slight difference between the BMI of pre-test and post-test.

The table 2 showed that according to the BMI most of the study participants fall in the normal weight category and there was a slight increase in the participants of normal weights between pre and post test. On the other hand, there was also a slight decrease in the participants having over weight and underweight after pre and post testing. While the number of obese although it was very less but remained same.

Overall Knowledge, Attitude and Practice (KAP) questionnaire score

The overall KAP (knowledge, attitude and practices) questionnaire consisted of 30 questions. The mean score of correct answers prior to nutrition education intervention was (6.75± 2.26) and the mean score of correct answers of first post testing was (23.81±2.49) and the mean score of correct answers after the second post testing was (23.79±2.58).

Non parametric Wilcoxon ranked test was applied and the results showed that there was a significant difference (p=0.000) in the mean score of KAP of students after the nutrition education intervention. While the results of the two-post testing showed that there was no significant difference (p=0.732) in the overall KAP score of the participants.

Dietary habits of the study participants

Pre and post analysis of food frequency of study sample regarding the amount of food consumed from each group revealed the following results. Analysis of food frequency revealed that there was an increase in the consumption of certain milk products after the delivery of nutrition education lectures.

Table No.1: Baseline characteristics of study participants. (N=153)

Parameter	MEAN±S.D		
	Pre-test	**Post-test**	**Post-test1**
Age (years)	20.7±0.78	20.7±0.78	20.7±0.78
Weight (kg)	51.0±10.96	51.1±10.91	51.22±10.82
Height (cm)	157.98±5.6	158.10±5.46	158.10±5.46
BMI (kg/m²)	20.0±4.0	20.43±4.09	20.45±4.06

Table No.2: Nutritional status of sample according to BMI (N=153)

Status	Pre-test	Post-test	Post test 1
Underweight (<18.5)	34.6%	34.6%	34.6%
Normal weight (18.5-24.9)	52.3%	53.3%	53.3%
Overweight (25.9-30.9)	11.1%	10.1%	10.1%
Obese (>31)	2%	2%	2%

Table No.3: Overall knowledge, attitude and practices (KAP) questionnaire score

Parameter	Pre-test-Post-test 1	Pre-test Post-test 1	Post-test 1-Post-test 2
KAP	6.75±2.26	23.81±2.49	23.79±2.58

*Significant, ***highly significant, ns not significant

Table No.4: p-value comparison of KAP score

KAP	Pre-test-Post-test1	Pre-test post-test 2	Post-test1 post-test 2
p-value	0.000***	0.000***	0.732

*Significant,***highly significant, ns not significant

Table No.5: Frequency distribution of milk consumption (pre-post comparison)

Milk products	Daily			2-3 per week			2-3 per month			Never		
	Pre	Post 1	Post 2	Pre	Post 1	Post 2	Pre	Post 1	Post 2	Pre	Post 1	Post 2
Fresh milk	7.2	79.7	63.4	32.6	17	32.7	32.7	3.3	3.9	27.4	0	0
Low fat milk	4.6	21.5	20.9	21.6	34.6	34	31.4	14.3	13.7	42.5	29.4	31.4
Butter milk	7.9	35..3	24.2	28.7	28.8	37.9	31.4	18.3	19.6	32	17.7	18.3
Yogurt	12.5	7.3	54.9	28.7	28.1	33.4	32.1	2.6	9.8	26.8	2	2
Cheese	11.8	16.4	9.2	33.3	51.6	51	22.3	20.9	24.2	32.6	11.1	15.7
Milkshakes	22.8	78.5	71.9	27.4	20.9	24.2	22.9	7	2.6	26.8	0	1.3

Discussion:

To compare the effectiveness of nutrition education intervention pre and post tests were designed. Initially a pre testing was done to access the milk consumption patterns in young adults and then changes in mean scores were analyzed and compared with post test. The changes in the knowledge, attitude and practices regarding milk consumption patterns were shown in table 3. The results of the P value were p<0.05, showing the statistically significant results.

According to the present study most of the students did not consume milk. The results of our study were consistent with Jafari *et al*, 2014 who concluded that 78.1% of the participants did not consume enough dairy products (8). Tarakc *et al*. also reported that the average milk consumption of students was only 100 g, which was only one third of their recommended daily amount. According to Rizzoli, 2014 2-3 servings of dairy were required to meet the recommended calcium intake for bone health and fitness (9). A study was conducted at Kafkas University to access the milk consumption patterns of students and the results concluded that only 33% of them consume milk while 67% of them did not consume milk throughout the study (10) Similar findings have been done by the reports of National Health and Nutrition of America that 39% of men and 43% of women did not consume even a single serving of milk and dairy products on the daily basis (10).

College and university life were said to be the time during which the eating habits often change. Lack of knowledge about healthy diet, personal likes and dislikes, lack of time, increased availability of carbonated beverages, as well as the study burden were said to be the factors that contribute to the wrong food choices towards milk in college and university students.

Milk is regarded as a 'complete food' and the results of the present study showed that most of our young adolescents did not know about the importance of milk for our health. Hence the intake was poor or limited. So, the 'lack of knowledge' was said to be the most powerful factor that effects the consumption of milk.

The results of our study suggested that personal likes and dislikes were the behavioral factors towards the lower consumption of milk. (11) also suggested that the consumption of low-fat milk products in adolescents was directly linked with behavioral intention. Several other studies also reported the significant relationship between behavioral intention and the consumption of fruits, vegetables and dairy products.

The unpleasant taste of milk was another factor effecting milk consumption. According to our study bone health was the positive belief and unpleasant taste of milk is the negative belief affecting consumption of

milk. (12) had also reported that most of the people did not consume milk due to its taste.

According to Forshee *et al*, 2003 decreased milk consumption was associated with obesity and it was indirectly linked to wrong food choices and negative dietary patterns (13). A second positive finding of this study was that, participants mostly choose fat free milk choices. But the total milk consumption was even less than the recommended levels. Students often perceive that milk was fattening and they avoid milk and milk related products instead of choosing low fat or fat free milk.

College and university life have been regarded as the 'busiest' due to the study burden and tough schedule. (14) reported that many factors such as food preferences, expectations as well as changes in lifestyles and increased availability of fast foods were responsible towards the selection of foods in young adolescents. College life is a period where students gain independence from their parents and this period is said to be the turning point of food habits that have their implications through the adulthood. Sakamaki *et al*, 2005 worked on university students in China and reported that only 7% of the university students follow the concept of healthy eating while selecting food (15).

Nutrition education for Pakistani students is essential to change their dietary behavior because only few of them know about the healthy eating. Findings of our study suggested that nutrition education interventions were effective tool to change the nutrition knowledge, attitude and practices among students. Similar findings had been done by Power *et al*, 2005 that through proper nutrition education food preferences and eating habits of the students could be significantly changed (16).

This study suggested that nutrition interventions were important in improving the diet quality by changing the nutrition knowledge as well as food habits of the target population. Class based nutrition interventions help students to improve their nutrition knowledge and dietary practices.

According to Hoelscher a sufficient gap between the pre and post testing is required to bring changes in nutrition knowledge, attitude and practices of the participants (17). Contento also suggested that adequate implementation period is required to produce the large effect (18). Blom-hoffman recommended that 5-13 weeks was sufficient time to improve nutrition knowledge of the participants (19). However, another study has consistently reported that a longer implementation period is required to achieve better behavioral outcomes. In our study gap of two months between pre and post testing produced significant difference in the KAP. (20)

Food frequency questionnaire was filled by the students to evaluate their dietary practices. It was

consisted of list of milk and milk products to indicate usual frequency of consumption over the time period. Results of our study showed that there was a significant increase in consumption of fresh milk and milk products especially in milkshakes after the nutrition education intervention. A survey was conducted among school pupils in Belgium to check the frequency of limited number of food items. Results showed that frequency of intake of milk and milk products were higher. They also reported that food frequency questionnaire was an effective tool that can be used to check the frequency of food items over a certain time period. The possible reason for increase in the consumption of milk and milk products was might be that the nutrition education lectures improved nutrition knowledge of the participants which ultimately changed their attitude and practices towards healthy eating. (21)

Effective teaching strategies are important for successful implementation of nutrition interventions. We used traditional lecture method, video presentations and demonstrations to enhance students' learning.

So, the present study concluded that well planned nutrition education program is an excellent medium to enhance students' knowledge and help them to translate into healthy food choices. There are some limitations of the study. This study was conducted on selected colleges and only female students were included and the duration of study was only 4 months.

Conclusion:

The present study concluded that nutrition education interventions have significant impact on improving nutrition knowledge, attitude and practices of the students. Nutrition knowledge and skills are important in establishing healthy eating behaviors that extend throughout the adulthood. Nutrition education is an effective method to promote dietary changes. Family, school, colleges, media, food industry and govt should work together to incorporate healthy eating behaviors into their lifestyles.

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Disclaimer

The interpretations and views stated in this article are those of the authors and do not inevitably imitate the official policy or position of any agency of the Pakistan government. Analysis done within this article are only examples. Assumptions made within the study are not reflective of the position of any Pakistan government entity.

Conflict of interest

No conflicts of interest.

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MINI REVIEW

Rabies virus: A mini review

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Abstract

Deadly rabies virus belongs to the Rhabdoviridae family of viruses. Rabies is a bullet-shaped enveloped negative-sense RNA genome virus. It causes diseases mostly in animal and humans. It is transmitted to human by bites of dogs and bats mostly and causes hydrophobia and eventual death. The virus uses nerve cells for their multiplication and produces mutations in the nervous system. Each year 59 thousand people die due to the rabies virus, the ratio in China is much more. There is no treatment for rabies. Although vaccination for dogs is available. We can't curve this but we can avoid this by taking preventive measures. The purpose of this mini-review is to summarize the rabies virus and its medical importance.

Introduction

Rabies is the dreadful disease which causes infection both in human and animals. The causative agent is rabies or lyssavirus which is usually found in the saliva of infected animals and is the most lethal of all the infectious diseases. There is no cure for rabies however it can be prevented by vaccination of affected person (1). The word rabies comes from the 'Sanskrit Rajas' that means 'to do violence'. It is said that rabies is derived from the word 'Rabha' which means violence. Rabies virus is transmitted by bites of sharks, bats, and raccoons but most common is the bite of a dog. (2) The disease shows symptoms in 3 to 5 days and common symptoms are fever, malaise, agitation, irritability, irregular breathing and consequently death (3). Patient exhibits hydrophobia due to inability to swallow which causes paralysis of the throat and facial muscles. Rabies virus belongs to the Rhabdoviridae family which is an enveloped RNA virus having bullet shaped coiled capsid and inside it, a negative sense single-stranded linear genome resides. The virus binds to acetylcholine receptor cells where it replicates itself and moves along the peripheral nervous system then moves toward central nervous system from where it spreads in other organs of the body and also shows its presence in the salivary glands. It affects CNS and particularly causes inflammation of the brain (4).

History

Rabies disease was first recognized in Egypt in the fourth century by Aristotle who declared that there was something dangerous in a dog bite. Later on, it was discovered by the physician Girolamo Fracastoro in the 16th century and he called it the incurable wound. Eventually, in 1912, it was confirmed in Kenya (5) and in these days the veterinary cases were reported more than human cases. Many emerging microbial threats of rabies disease were seen in Kenya in the year 1996 (6). However, it also posed a major public health issue in 2009 in the United States and Canada. Bat-related rabies cases have also been seen in persons without being contaminated or bite by a bat (7). A recent statistic from China shows that about 108,412 persons have died due to this disease from 1950 to 2004 (8). Other than dog and bat, fox related cases of rabies virus have also been reported in Asia and Europe. The vaccination against this virus was first prepared by Louis Pasteur in 1885 (9).

Prevalence in the world

According to the World Health Organization (WHO), in the year 2016, 59 thousand people died due to rabies in which 95% of cases were from Africa and Asia. Among these 99% cases were dog-mediated rabies. Every year approximately 30,000 to 70,000 people die from this disease predominating the number of children as compared to adults (10).

Virus structure and features

Rabies is a bullet-shaped virus with 180nm length. It consists of five proteins, an envelope, and a negative sense RNA genome inside the capsid. The five proteins are N nucleoprotein, M matrix protein, P phosphorous protein, G glycoprotein, and L polymerase protein. The G protein is present in the shape of spikes on the outer surface of the envelope, inside this M protein make inner membrane in which N, P, L protein and RNA in inner linked in the chain structure (11). The virus belongs to Rhabdoviridae family and its genus is lyssavirus and it consists of six other viruses which cause diseases in different species like foxes, raccoons, bats, and dogs. Rabies is the enzootic disease which also affects the humans (1). There are two types of rabies which are urban and sylvatic. It is an encephalitis virus and causes acute highly contagious fatal disease. It has the ability to affect peripheral nerves and transfer to CNS and consequently cause inflammation of the brain and responsible for the neurological disease (4, 12). In response to invasion by the virus, the host initially recognizes RNA viruses by cellular sensors and recognition receptors that send an impulse to produce type I IFN (alpha and beta IFN family). Interferon is the signaling protein that is made or released when the body realizes the presence of a foreign pathogen (13). Although the virus is destroyed by simple environmental factors, including temperature, UV light, sunlight, 70% alcohol, ether, deoxycholate, and trypsin. Rabies is transmitted by the bite of an animal without bite there are the very rare cases (14).

Genome structure

Rabies virus is the negative sense RNA virus with 12000 nucleotides that code for the five proteins named nucleoproteins, matrix protein, phosphorous protein, glycoprotein, and polymerase protein. These all protein have a direct and indirect role in pathogenicity. Lyssavirus genus has 6 different rabies-related viruses. Virus RABV is a member of genotype 1, these five genes code for five protein (7). Gene N (nt 59 to 1483), gene P (nt 1486 to 2475), gene M (nt 2481 to 3283), gene G (nt 3289 to 5355) and gene L (nt 5380 to 11853) (15).

Nucleotide protein is directly bound to viral genomic RNA. N protein is responsible for the encapsulation of anti-genomic and genomic RNA. N protein is bind strongly to leader RNA (16).

Phosphorous protein is multifunctional essential for viral replication, in RNA synthesis act as a cofactor for L protein. P protein antagonizes the type 1 interferon IFN mediated antiviral responses by inhibiting signaling pathway for IFN induction and response suppresses activation of interferon regulatory factor 3. It inhibits the nuclear translocation and DNA binding with IFN and inhibits IRF 3 activation (17).

Glycoprotein has a predominant role in pathogenicity. It is organized as trimmer and is a sole protein exposed on the surface of the virion. G protein promotes viral entry from the peripheral to the central nervous system it spreads trans synaptic trigger apoptosis which cause premature state of the host cell (18).

Matrix protein has a clear function in the pathogenicity but has a regulatory role in viral transcription and replication. It lacks pathogenesis however helps in virus budding (19).

Polymerase protein has a cooperative role with the P protein it works as an RNA polymerase in the infected cell (20).

Life cycle

During virus assembly ribonucleotide protein RNP is enclosed in an envelope that contains an inner layer of M protein and G protein. Rabies virus transfer to the host from the host usually through the bite of infected animals or by the saliva. It uses network of nerves cells in peripheral neurons which linked various body parts to the central nervous system. The virus needs to bind to P75 and TR receptor present on the tips of peripheral neurons, in order to move quickly (21). After the entry it attacks on junction of the muscles from where they go to the neural pathway and enter into the cytoplasm of a nerve cell by retrograde and axoplasmic transport. P protein of virus bind to dynein protein which travels in and outside in cytoplasm of nerve cells by retrograde axonal transport. The virus hijacks transport system of neurons and exploits axonal transport machinery. At this place it starts transcribing itself, transcription is in the form of polymerase shuttering that is the process of transcribing nucleotides several times by polymerase enzyme that shuttles on mRNA (22).

Hydrophobia

Actual it is not the fear of water except infected person has a fear of swallowing any liquid that is called dysphagia. Rabies affects the neurons of respiration and swallowing, generally, it effects neural network and CPG's that have the function of swallowing, mastication, and respiration. It has an effect on respiration and swallowing by the brain stem respiratory neurons and DMRC dorsal medullary reticular column. The virus uses our nervous system to avoid water because in case of water intake virus is diluted and it has the chance of diminished transmission and its life cycle stops which is an evolutionary mechanism (23).

41

Vaccination of dogs

Risk of rabies is more in the unvaccinated dogs, those roaming outside on the roads. There is not any treatment method for this disease after the onset of symptoms. However, the vaccination method before the symptoms appear can save an infected person. The disease can be prevented via the administration of the vaccine to the population at risk. DNA vaccination is injected via intradermal in ear pinnae that protects dogs for one year (24). Recombinant rabies-canine adenovirus type 2 vaccine in the bait is used orally in dogs that give long-term immunity to dogs (25).

Preventive measure

Rabies can be prevented on national level. To prevent and eliminate the brunt of rabies WHO suggest that 70% of the dog in the population should be immunized (26). Human rabies can be prevented by the use of post-exposure prophylaxis (PEP) in an emergency situation to victims of rabid animal bites (27). To avoid rabies firstly vaccinate the pets (dogs and cats) according to the law of your country (28). Stay away from stray dogs they have more risk than vaccinated pets. Don't keep wild animals and avoid their contact with your pet (29).

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