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Research Article

Biomolecular Evaluation of Cobalamin (Vitamin B12) in the Diabetic Population of Lahore and Sheikhupura

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Abstract

Aim: Many efforts are being made in Pakistan to cure Diabetes. These efforts are therapeutic cure and therapeutic prevention of Diabetes. One of these efforts is to work on Vitamins. The purpose of our research is the Biomolecular Evaluation of Vitamin B12 in the Diabetic population of Lahore and Sheikhupura.

Methods: The serum samples of diabetics and normal healthy controls were collected. 200 were diabetics from Health Centre, University of Punjab, Lahore and District Head Quarters Hospital Sheikhupura and 100 were healthy controls from both. The biomolecular evaluation of Vitamin B12 was done with SDS PAGE and High Performance Liquid Chromatography.

Results: Vitamin B12 is low in the diabetic population as compared to the controls. Conclusion: The vitamin B12 level of diabetics of Sheikhupura is much low as compared to diabetics of Lahore. This suggests that high vitamin B12 level can be therapeutic prevention of Diabetes.

Keywords: Vitamin B12; Diabetes; Serum sample

Introduction

Diabetes mellitus is a metabolic disorder. In this disease the human being either has elevated blood sugar concentration [1]. Diabetes is the seventh most important reason of death [2]. Type 1 diabetes consider as immune-associated and results in the damage of insulin secreting β cells of pancreas [3]. Type 2 DM begins by resistance of insulin as well as outcome of which is failure in insulin release [4]. The main causes of diabetes are large body mass, autoimmune disorders, hereditary trend of DM and age etc [5]. The symptoms include gain or loss of weight, uncontrolled thirst and hunger, wounds don't heal properly etc. Diabetes can be prevented by changing lifestyle, eating healthy food etc [6]. Number of individuals with diabetes has increased to 422 million [7]. A shocking 7.1 million public have DM and Pakistan is at 7th position for having DM globally [9].

Vitamin is required at little amounts for existence of human being. Vitamin B12 is found within many foodstuffs as well as exists in nutritional medicines as well as recommended direction. It is present with many types [9]. Vitamin B12 acts as a cofactor for methionine synthase and L-methylmalonyl-CoA mutase. Methionine synthase activates the alteration of homocysteine to methionine [10,11]. Methionine is vital for the development of S-adenosylmethionine which is a general methyl donor for roughly 100 different substrates, including DNA, RNA, hormones, proteins, and lipids. L-methylmalonyl-CoA mutase converts L-methylmalonyl-CoA to succinyl-CoA in the destruction of propionate [10-12] which is an important biochemical reaction in fat and protein metabolism. Succinyl-CoA is also necessary for the synthesis of hemoglobin. Nerves contain defensive cover for their protection from pollutants as well as free radicals within blood. Devoid of casing, known as myelin sheaths, bare nerves are injured as well as might expire. Such deceased nerves disturb signals toward as well as away brain as well as might take part in function in nerve associated circumstances. Vitamin B12 assists approach by which the body replenishes this defending casing [13]. The sources of vitamin B12 are beef, fish, chicken, legumes, milk and milk products etc [10,14]. Vitamin B12 with type cyanocobalamin and sometimes hydroxocobalamin could apply parenterally recommended medicine, frequently through intramuscular shot. Parenteral direction can usually cure vitamin B12 [15]. It may accessible like recommendation medicine within gel form which is functional intranasally [16].

The symptoms of vitamin B 12 are Yellowing of the skin, Failure for touch sensitivity, Memory loss, Deficiency of energy, Improper breathing etc [18].

The shortage of vitamin B12 because of pernicious anemia present repeatedly amongst people having type 1 diabetes. People having type 1 diabetes show auto Antibodies to Intrinsic Factor (AIF) type 1 as well as 2 plus Parietal Cell Antibodies (PCA) [18,19]. The PCA hampers release for intrinsic factor leading to pernicious anemia, state that can be 10 times further prevailing amongst people having type 1 DM as well as people don't have DM. Type 1 AIF lead to vitamin B12 deficit inhibiting attachment of vitamin B12 to IF. This inhibits transport toward assimilation spot, terminal ileum. Regardless of better glycemic reducing influence, metformin is revealed for reduction of vitamin B12 status. Metformin induced vitamin B12 shortage amongst people having type 2 diabetes comprise: variations of small bowel motility that induces increased growth of bacteria as well as resulting vitamin B12 deficit, viable reduction and vitamin B12 malabsorption, changes within intrinsic factor status as well as contact to cubulin endocytic receptor [20]. Metformin hamper calcium bound assimilation for vitamin B12-IF complex at the terminal ileum. Such inhibitory consequence can be inverted by calcium medication [21].

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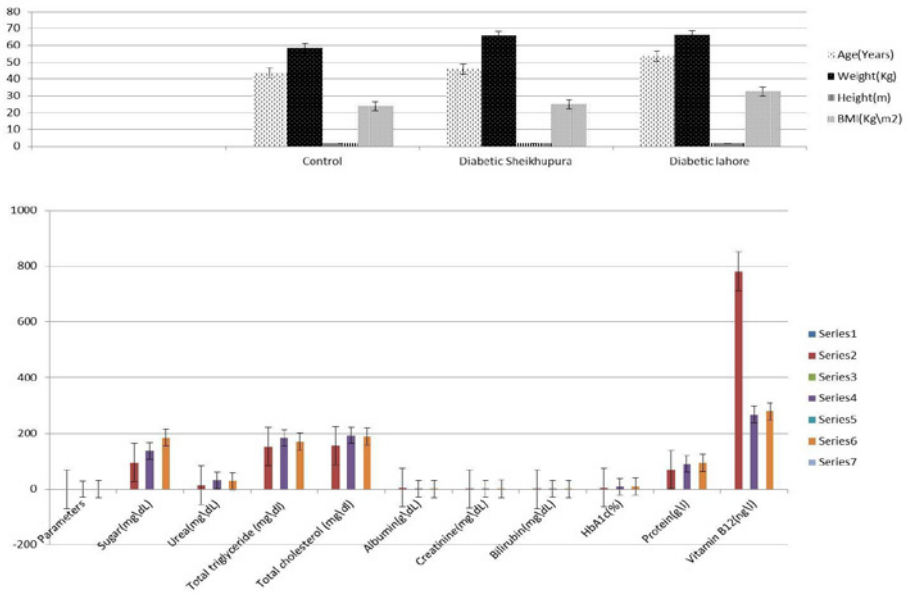

Figure 1: The Comparison of biochemical parameters of control with diabetic patients from Lahore and Shaikupura.

Methodology

Sample collection

Samples were collected from District Head Quarters Hospital Sheikhupura and Health Care Centre, University of Punjab, Lahore. The blood was then centrifuged at 13000 rpm and serum, plasma and blood cells were obtained. Serum was selected for the determination of vitamin B12 level of diabetic patients. All samples were stored at -80°C until further analysis.

Estimation of physical parameters

The physical parameters include age, weight, height and BMI. Age of all the subjects was calculated from the history of the subjects and it is calculated in years. Weight of all the subjects were measured by weighing machine and then recorded in kilograms. BMI was measured by using BMI formula and putting the values of weight and height in it.

Estimation of Biochemical parameters

The biochemical parameters are measured to find out their values in the blood. The glucose level was measured by using glucometre. Triglycerides creatinine, albumin, bilirubin, urea and cholesterol were measured by using kits. The protein was measured by using SDS PAGE and vitamin B12 was measured by using HPLC.

Protein

Protein was measured by using Bradford method. The Dilutions of Standard (BSA) and given protein samples were prepared. The Bradford reagent is added into the dilutions, incubated and observed for the color change. The optical density is measured with spectrophotometer. The graph is plotted between standard given samples and the protein value of the given samples is estimated.

SDS-PAGE

SDS PAGE is used to estimate the molecular mass of protein. The resolving and stacking gel was prepared and loaded in the gel assembly. The samples are prepared by adding the loading dye and loaded in the wells of the gel. The protein marker was also run along with other samples to estimate the mass of the given protein sample. The assembly is connected to the power supply and run. The gel is then stained and destained to observe the bands of the protein samples.

High performance liquid chromatography

HPLC is performed to further confirm the results of the given protein samples. The technique of chromatography starts when the solute is injected into the injector at the end of the column. When the analytes and mobile phase are pumped through the column, the separation of components takes place. As the peak on the data display, each component elutes from the column. Eluting component's detection depends upon the detector used. The detector's response to every component is displayed on the computer screen or chart recorder and it is known as a chromatogram.

Statistical analysis

The statistical analysis was done to have the final and confirmed results of research work. The study group size was N = 300. First of all the average of all the values were taken. This average shows the mean of the respective values. Then standard deviation was found out to further analyze the data. The statistical analysis was done with SPSS statistical software package (version 22.0). Significance of difference between mean analysis of control and diabetic groups was determined by using ANOVA. At the end the significance difference between the normal healthy controls and diabetics was found out.

Results

Physical parameters

The physical parameters which we assessed include age, weight, height and BMI. The mean age of the controls was 43.66±2.41 years, that of diabetics from Sheikhupura was45.93±2.98 years and that of diabetics from Lahore was 53.53±3.01 years. The difference in the mean age was statistically not significant (p>0.05) as shown in the

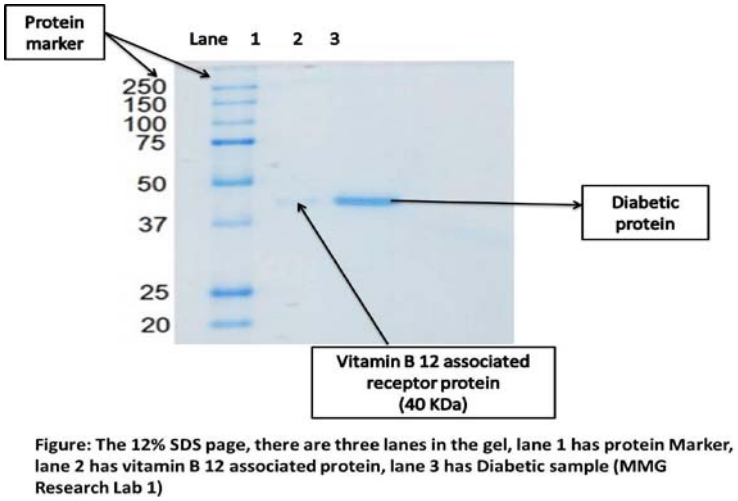

Figure 2: SDS Profile of control with diabetic patients from Lahore and Shaikupura.

table 1.1. The mean weight of the controls was 58.31±3.12 Kg, that of diabetics from Sheikhupura was 68.99±4.55 Kg and that of diabetics from Lahore was 66.06±3.91 Kg. The mean BMI of the controls was 23.91±3.11 Kg\m2, that of diabetics from Sheikhupura was 26.12±3.19 Kg\m2 and that of diabetics from Lahore was 24.26±3.08 Kg\m2. The difference in the mean weight height and BMI was not significant and didn't differ in the comparison between the groups. So it was observed that the three groups were not differing in the mean value of their physical parameters (Table 1).

Table 1.1: Physical parameters of controls and diabetics.

Physical Parameters of Controls as compared to diabetic values				
Sr. No.	Parameters	Controls	Diabetics Sheikhupura	Diabetics Lahore
1	Age(Years)	43.66*±2.41	45.93*±2.98	53.53*±3.01
2	Weight(Kg)	58.31*±3.12	68.99 *±4.55	66.06*±3.91
3	Height(m)	1.60*±0.64	1.62*±0.82	1.65*±0.90
4	BMI	23.91*±3.11	26.12*±3.19	24.26*±3.08

Biochemical parameters

The biochemical parameters which we assessed include sugar, protein, urea, creatinine, bilirubin and HbA1c. The mean sugar of the controls was 95.33±9.89 mg\dL, that of diabetics from Sheikhupura was 136.61±10.53 mg\dL and that of diabetics from Lahore was 184.55±14.17 mg\dL. The difference in the mean sugar was statistically significant (p<0.01) in table 1.2.The mean HbA1c of the controls was 5.38±1.27%, that of diabetics from Sheikhupura was 9.26±3.58% and that of diabetics from Lahore was 9.47±3.63%. The difference in the mean was statistically not significant (p>0.05). The mean total triglyceride of the controls was 152.45±12.82mg\dl, that of diabetics from Sheikhupura was 184.16±14.11mg\dl and that of diabetics from Lahore was 170.01±13.10mg\dl. The diabetics show statistically highly significant (p<0.001) difference in triglycerides value than control. The mean albumin of the controls was 5.22±0.90g\dl, that of diabetics from Sheikhupura was 0.19±0.006 g\dl and that of diabetics from Lahore was 0.20±0.007g\dl. The mean Creatinine of the controls was 0.89±0.03mg\l, that of diabetics from Sheikhupura was 0.92±0.04mg\l and that of diabetics from Lahore was 0.91±0.03mg\l. The difference in the mean values is not statistically significant (P>0.05). The difference in the mean values is not significant (p>0.05). The mean bilirubin of the controls was 0.35±0.005 mg\l, that of diabetics from Sheikhupura was 0.71±0.009mg\l and that of diabetics from Lahore was 0.53±0.008mg\l.The difference in the mean values is not statistically significant (P>0.05).The mean cholesterol of the controls was 155.21±10.11mg\dl, that of diabetics from Sheikhupura was 193.12±16.34mg\dl and that of diabetics from Lahore was 189.01±14.70mg\dl. The difference in the mean total cholesterol was statistically highly significant (p<0.001 Table 2).

Vitamin B12

The mean vitamin B12 of the controls was 781.12±25.89ng\l, that of diabetics from Sheikhupura was 267.24±20.61ng\l and that of diabetics from Lahore was 279.52±22.36ng\l. The difference in mean values are statistically highly significant (p<0.001). In normal people the level of vitamin B12 is high but in the diabetics the level is much low as compared to the normal (Figure 1).

SDS-PAGE

The analysis of protein was done on 12% SDS gel. The gel having Vitamin B12 associated receptor protein contains two bands which are much noticeable but the third band is not much clear. These two bands are clear as compared to the other band. The first lane shows the bands of protein marker and the marker is much clear. The second lane has our protein band, the vitamin B12 associated protein band and this band is not clear and visible. The vitamin B12 associated protein has mass of 40 KDa. The second lane contains a band which is very much clear as compared to the other ones. This band is normal protein band (Figure 2).

Discussion

Diabetes mellitus is a multifactorial disease, spanning from genetics to environment and physical factors. In this disease the human being has elevated blood glycemia. This is because insulin making is not adequate, or as the cells of body not act in response to insulin correctly, or both [1]. Diabetes is the seventh most important reason of death. Number of individuals with diabetes has increased

Table 1.2: The Biochemical parameters of Controls and Diabetic.

Biochemical Parameters of Controls as compared to diabetic values				
Sr. No.	**Parameters**	**Control**	**Sheikhupura diabetics**	**Lahore diabetics**
1	Sugar(mg\dL)	95.33**±9.89	136.61**±10.53	184.55***±15.57
2	Urea(mg\dL)	13.51*±4.09	31.86**±6.67	29.25**±5.46
3	Total triglyceride (mg\dl)	152.45***±12.82	184.16***±13.78	170.01***±13.10
4	Total cholesterol (mg\dl)	155.21**±10.11	193.12***±16.34	189.01***±14.70
5	Albumin(g\dL)	5.22*±0.90	0.19*0.006	0.20*±0.007
6	Creatinine(mg\dL)	0.89*±0.03	0.92*±0.04	0.91*±0.03
7	Bilirubin(mg\dL)	0.35*±0.005	0.71*±0.009	0.53*±0.008
8	HbA1c(%)	5.38*±1.27	9.26*±1.58	9.47*±1.63
9	Protein(g\l)	70.13*±3.45	90.53**±5.30	94.64**±7.96
10	Vitamin B12(ng\l)	781.12***±19.89	267.24***±11.61	279.52***±14.36

Data = Mean,± Standard Error, *=P>0.05 (Statistically not significant), **=P<0.01 (Statistically significant), ***=P<0.001 (Statistically highly significant).

to 422 million. The struggle for devising plan to tackle this menace is underway at full throttle [7].

Vitamin is vital for our body. Vitamins are necessary in small quantities. Vitamin B12 is crucial for our body. Its decrease disturbs the regular tasks in the body. Vitamin B12 is decreased in people having DM. This decrease leads to the small number of RBCS resulting in anemia [21]. The small concentration of Vitamin B12 is responsible for nerve destruction which may lead to amputation. Its decrease is also responsible for retinopathy and neuropathy.

The hypothesis on which this research was based was that diabetes causes the lower level of Vitamin B12 in the body which results in further complications. These complications can be prevented by increasing the level of vitamin B12 and hence this increased level can act as a therapeutic factor to control this disease.

In the given research work, full amount of subjects was 300. 100 were controls, 100 were diabetic from Sheikhupura and 100 were diabetic from Lahore. All these people were male and female both. Therefore three groups were formed. One group is of controls belonging to Lahore and Sheikhupura, second one was of people of Sheikhupura having DM and third one was composed of people of Lahore having DM. The three groups were compared for having vitamin B12 concentration.

The age of diabetics of both cities is increased as compared to controls. This means that the diabetics of Lahore have diabetes in older age because they are somewhat aware of their health issues but the diabetics of Sheikhupura are having diabetes in the younger age as compared to Lahore ones. The weight of the Sheikhupura diabetics is little higher than Lahore diabetics but one thing is noticeable that Lahore diabetics are having low weight at older age as compared to the Sheikhupura ones and diabetics of Sheikhupura have high weight at age younger than Lahore ones. The BMI of Sheikhupura diabetics is higher because their weight as compared to their younger age is higher and the BMI of Lahore is lower because their weight is lower as compare to their older age.

The fasting sugar of diabetics of both cities is increased as compared to controls. But the Lahore diabetics have high sugar as compared to Sheikhupura ones because Lahore ones have high age factor which affect them to have high glucose level. The urea of diabetics of both cities is increased as compared to controls. The urea level is increased in Sheikhupura diabetics as compared to Lahore ones and this increased urea indicates that the diabetics of Sheikhupura have damaged kidney which results in the increased level of urea. The diabetics of Lahore have not kidney much damaged as compared to Sheikhupura so their urea is not much high than Sheikhupura. But the HbA1c of Sheikhupura is somewhat lower as compared to Lahore because their sugar level is also little lower. The triglyceride of Sheikhupura is higher as compared to the Lahore ones and this increased triglyceride is responsible for higher weight and ultimately results in diabetes. But the Lahore ones have lower triglyceride and their weight is also low. The cholesterol of Sheikhupura is higher as compared to the Lahore and this higher cholesterol result in increased weight of Sheikhupura as compared to Lahore which ultimately result in diabetes. The protein concentration of Lahore diabetics is increased as compared to Lahore ones. This is because the sugar level is high among Lahore ones due to which the amount of protein is also increased as compared to Lahore. The vitamin B12 of diabetics of both cities is decreased as compared to controls. The level of vitamin B12 is much lower in Sheikhupura diabetics as compared to Lahore ones so this may lead to the increased risk of neuropathy among people of Sheikhupura as compared to Lahore ones. The lower level of vitamin B12 can be responsible for anemia as it is involved in the decrease of RBCs in blood which results in anemia in diabetics. It can also be responsible for diabetic neuropathy because it is essential for nerves. So its decrease results in the damage of nerves ultimately to diabetic neuropathy. The evaluation of concentration of such physical and biochemical parameters will be useful for not only early diagnosis but also in prediction of diabetes mellitus. So it is essential to evaluate and know these parameters by linking with diabetes.

Conclusion

The conclusion of this research work is that by taking the preventive measures, the diabetes can be controlled. These preventive measures are many which include vitamin B12 level and BMI and Sugar and other parameters. The BMI should be normal in controls but in diabetics it must be controlled to get rid of this dangerous disease. The BMI is mainly responsible for the disruption of the body's normal functions. The sugar level should be controlled in the controls

and diabetics. This is necessary because increased level is responsible for the disturbance of the metabolic system of the body. Vitamin B 12 is the main parameters in our research work so the main focus is on this parameter. The vitamin B12 level should be normal in controls but if someone is diabetic then the level should be maintained to the normal range not toward the borderline. This maintained level of vitamin B12 will control the main complications in diabetics because it can cause many complications like neuropathy, anemia and many more. By taking these preventive measures the diabetes can be controlled and it can be less dangerous.

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