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Changeable factors for diabetes mellitus type II patients and compare it with healthy control

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Abstract - A study has been analysed "Changeable factors for diabetes mellitus type II patients and compare it with healthy control." A random sample total 150 well-informed, informed consented subjects of either male and female 35-70 years of age group has been selected from Bihar. 100 patients suffering from diabetes mellitus type II were placed in group 1 (serving as cases) and fifty (50) apparently healthy individuals with matched age were place in group 2 (serving as a healthy control). The number of the disease population is believed to be approx. 70-90 per% of apparently healthy Indians have under vitamin D level across all the regions and age groups. In the present, diabetic cases had 19.09±5.34 ng/ml of serum vitamin D level. Healthy control subjects had 26.55±3.61 ng/ml of serum. The results been showed that 53% male and 47% female participants among cases. 40% male and 60% female participants among healthy control. Diabetes mellitus of patients, 17 cases were under good control, 28 were under fair control and 55 were under poor control. Diabetic cases had 19.09±5.34 ng/ml of serum vitamin D level. Healthy control subjects had 26.55±3.61 ng/ml of serum vitamin D.

Keywords: Diabetes mellitus, Vitamin D, Insulin, C-peptide, Risk factors and Type-II patients

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INTRIDUCTION

Diabetes mellitus also known that "Diabetes" was introduced to the public ever since 1st century 'Before Christ' (BC). The disease nomenclature was as diabaineinin Greek; the word was used for "siphon" by Aretus (the Cappadocian). He explained the word diabaineinfor the clinical condition of individual suffering from 'too much of passing urine.'[1] The literature reveals use of the term diabetes (meaning to pass through) for the first time in the year of 1425 AD while much later in the year of 1675 another term mellitus (Greek word) was added after diabetes for the reason being the diabetic suffers urine having characteristics of sweetness in odour and taste. Now keeping aside, a brief history about the term 'Diabetes mellitus' derivation if we define the disease in short then it is the disorder related to physiology of compromised glucose metabolism or disorder in which cells are unable to utilise glucose for energy. Therefore, the above-mentioned illness can be described as increased level of glucose in the blood (hyperglycemia). The problem lying there in formation of the insulin hormone by the pancreas, the insulin hormone not being able to perform its function for decreasing blood glucose status of the body or, the above mentioned both causes may be there in the body at the same time.[1] The uncontrolled and increased glucose level in blood for a period of time in diabetes mellitus can cause detrimental effects in various organs and systems in our body like dysfunction, damage or failure to organs like eyes, kidneys, nerves, heart and even blood vessels.

Diabetes mellitus:- From the mid of 20th century the study for occurrence of diabetes mellitus began formally in India. So far, 7 studies were published regarding the occurrence of the disease by late 1960. It was ICMR (Indian Council of Medical Research) who, in 1971, started first multicenter research for diabetes mellitus. The result of ICMR study showed prevalence of the disease to be 2.1 percent in major cities and 1.5 percent in rural regions near those cities. It was reported to be 9.3 percent in Mumbai and as high as 16.6 percent of diabetic patients in Hyderabad when National Urban Diabetes Study carried research in 6 major metro cities in India.[2] The study was carried after more than 20 years of ICMR study. Various studies were also carried in small towns and rural areas around the same time and diabetes occurrence was reported to be 5.9 percent in small towns and 2.7 percent in rural areas. From 2011, IDF (International Diabetes Federation) estimated the number of diabetic cases in India and the number was summarized from the conclusions from many smaller studies. But these numbers were not thought to be actual representation of number of cases in India. ICMR-India Diabetes (ICMRINDIAB) study is considered to counter this knowledge gap and the estimates the number of cases of diabetes mellitus in India using uniform sampling techniques in rural and urban areas of all states of India. million people suffering from diabetes mellitus and 77 million people having prediabetes were estimated in 2011. Then the IDF re-stated increase in number of diabetic cases from 50 million in 2009 to 61.9 million

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in 2011. These numbers are increasing day by day which is shown by the IDF study in 2015 and this time the number of cases reached to 69.2 million in India.[3]

Diabetic classification earlier was made from clinical observations such as: age- juvenile or adult onset of diabetes ii. model of curing diabetes- insulin dependent vs non-insulin dependent diabetes mellitus It was not until 1979, when NDDG (National Diabetes Data Group) along with WHO decided to postulate new and combined criteria which will help in classification and identifying and recognising cases of diabetes. Therefore, these bodies revised previously available idea of classification and published.[4] This classification was on the basis of the knowledge of clinical sign and symptoms of diabetes, physiological and biochemical explanation of the disease and of course the way of managing and curing the disease.

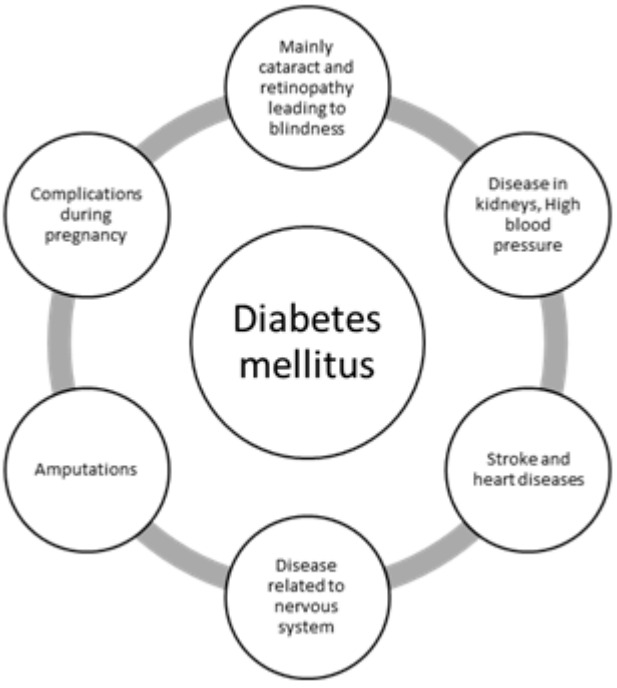
There are two main types of diabetes mellitus- Type 1 diabetes/Juvenile diabetes (insulin dependent): This diabetes is the result of damage to beta cells of pancreas and the damage is caused by autoimmunity in which antibody is produced by own body against beta cells leading to their damage. It is believed to be 10 percent of total diabetes population. And type 2 diabetes (non-insulin dependent): This is another type of diabetes which is common in old age or in obese persons and this is only because in old age insulin n is either not released in required amount from beta cells or unable to perform its function properly resulting in high glucose level in blood. 90 percent of total diabetic population is occupied by this type of diabetes.[5]

Latent autoimmune diabetes of adults (LADA)/ late-onset autoimmune diabetes of adulthood /or aging, slow onset type 1 diabetes /or diabetes type - Nearly fifteen to twenty percent of patients mis-diagnosed with type II diabetes but they actually are suffering from this diabetes. The mis-diagnosis is due to the reason that the occurrence of the disease in older age and patients responds to medicine because of low level of insulin is still being secreted by pancreas. This diabetes occurs among adult population and is slow to show sign and symptoms. Also, gestational diabetes or gestational diabetes mellitus (GDM): Sometimes pregnant lady show increase in blood glucose level during her pregnancy only. High blood sugar level is mostly around 24th week of pregnancy.[6] The high blood sugar is mostly likely to fall under normal level of the delivery of baby.

Manifestations of diabetes mellitus- Diabetes is such a condition in which there might not be any physical indication for much long duration until and unless diagnosis can be made. Most common and noticeable signs and manifestations of diabetes are: In increased frequency of urination- polyuria, patient always feeling thirsty- polydipsia, Polyphagia, weight loss, Weak eye sight and vi. prone to some infections. Diabetes can be threat to life if high blood sugar is not under normal level for long duration and at that time patient shows the complications of diabetes ketoacidosis or even nonketotic hyperosmolar syndrome.

Prediabetes- A person is said to be prediabetic if s/he shows impaired fasting glucose level i.e. fasting glucose level more than the normal level but not high enough to declare him or her diabetic. Prediabetics have glycated hemoglobin (HbA1c) level between six percent to 6.4 percent. Prediabetics are not diabetics but they have high risk to become diabetics in future if they do not bring their blood glucose level to normal. Prediabetes and diabetes mellitus type II are most common features and indications of a broader category of disease called metabolic syndrome which is mostly seen among person suffering from dyslipidemia, abdominal obesity, high blood glucose, high blood pressure.[7]

The main health risks and consequences due to diabetes- The quality of life is only better if a person or the family is free of any kind of physical or mental diseases. Disease state brings many risks and problems to the person and even to the family. Diabetes mellitus type II is the disease which has great impact on the standard of life but impact is not in good sense. If the disease is not under control then, after certain duration of time there are many systems of the human body that show malfunctioning and complications start appearing. The complications may be:



Although the above-mentioned consequences of diabetes mellitus are fatal to life and the treatment is costly. But this does not mean the disease is untreatable. [8] If correct diagnosis is done in time and proper medicine is taken with responsibility then diabetes can be defeated. Diabetes can even be prevented if the individual is careful and can spend healthy living. The war with diabetes be won with the

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help of healthy living which includes routine exercises and healthy diet. It is challenging to achieve healthy living in modern life style but not impossible. A healthy diet can be added to a person's kitchen if s/she is cautious about the healthy.[9] One of the most important micronutrients that should be added to one's diet list in order to fight with such disease is vitamin D because vitamin D not only helps in secretion of insulin but also reduces insulin resistance to cells. Vitamin D can also be achieved from sunlight so proper exposure to sunlight is advisable. In this way vitamin D not only can help in prevention of the disease but in prognosis as well. Therefore, it is advisable to maintains optimum vitamin D level.[10] Keeping in mind about such importance of vitamin D we are now going to deal with vitamin D to know about the vitamin such as types, nomenclature, history etc.

Hypothesis: To assess serum vitamin D level in diabetic mellitus type II patients and compare it with healthy control.

METHODOLOGY

A random sample 150 (100 diabetic and 50 healthy control) diabetic mellitus type II patients, in total, from the age group of 35 to 70 years, regardless of their sex, were selected as subjects of the research. These individuals were dividing in 2 groups. Has been selected from state of Bihar. The attendants of patients as well as volunteers act as control of the research, constituted group II. The patients were diagnosed as diabetic by the Department of Medicine on the basis of the following criteria:

Diagnosis measures for diabetes mellitus type II- Patients were diagnosed as diabetic according to the diagnostic criteria for diabetes mellitus issued by American Diabetes Association. The basis of diagnosing diabetes mellitus type II amongst the patients, was taken from the diabetes diagnostic measures published by ADA (American Diabetes Association). The measures are listed below in the table-1 bellow:

Condition	**Fasting glucose (milligrams per deciliter)**	**Post-prandial (2 hours after glucose) (milligrams per deciliter)**	**HbA1c (percent)**
Standard	Less than hundred (100)	Less than one hundred and forty (140)	Less than 5.7
Impaired Fasting Glycemia	100-125	--	5.7-6.4
Impaired Glucose Tolerance	--	140-199	5.7-6.4
Diabetes Mellitus type II	≥126	≥200	≥6.5

Diagnostic measures for diabetes mellitus type II given by ADA. Diagnosis of diabetes mellitus type II can be done by showing any one of the following abnormal biochemical parameters by the individual: Glycated hemoglobin (HbA1c) reading ≥6.5%, Fasting blood glucose reading ≥ 126 milligrams per deciliter, Post-prandial blood glucose which is two hours after a 75gm oral glucose load as in case of oral glucose tolerance test ≥ 200 milligrams per deciliter, And Warnings of increased blood glucose value or hyperglycemic crisis and random blood glucose reading more than 200 milligrams per deciliter.

A detailed history regarding the present or past illness was taken and the general physical examination, local examination and the systemic examination were done as per the proforma attached. Written consent form, both in English as well as vernacular language, was obtained from the participants involved in the research project after informing the participants properly in their understandable language. All, one hundred and fifty (150) subjects, were well-informed about the study. The blood sample was only taken after the patient and healthy subjects' affirmation. No subjects were forced for the participation in the project.

Group 1: Hundred (100) subjects detected with diabetic mellitus type II.

Group 2: Fifty (50) age matched apparently fit and fine, healthy individuals selected to serve as controls.

Ethical Considerations: The study was undertaken subject to approval by Institutional Ethics Committee. Inclusion criteria- Patients suffering from diabetes mellitus type II and patients under treatment of the disease. Age group between 35-70 years of either sex.

Exclusion criteria: Individuals below 35 years, Type 1 diabetes mellitus, and Pregnancy women.

Collection and processing of blood sample- About 5 (five) millilitres of venous blood sample was withdrawn from antecubital vein of the subjects in dry disposable syringe under aseptic conditions. 1 ml of blood was transferred to purple capped (containing anticoagulant) sterile, dry vial for estimation of glycated haemoglobin. Remaining 4 (four) milliliters of the blood sample was dispensed to a dry and sterile red capped vial. The blood was then allowed to stand for half an hour. After the clot formation, the supernatant was centrifuged, and the supernatant fluid was collected for biochemical investigations. Biochemical investigations: Serum vitamin D, Serum

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insulin, Serum C-peptide, Glycated haemoglobin (HbA1c).

Materials required- Micropipettes (10-100 µl and 100-1000 µl). Autoplex ELISA and CLIA Workstation, Lumax CLIA Strip Reader (Luminometer), Vortex mixer, rocking shaker, Timer, Test tubes for mixing of reagents, Eppendorfs for sample preparation, CD Marker, Absorbent paper for blotting CLIA reaction cells, Reaction cell shield during incubation procedure, Glass beaker for wash buffer preparation, Deionized/Distilled H2O, Measuring Cylinder, and 25(OH) Vitamin D CLIA kit etc.

Reagents -

1. 25(OH)D calibrators (A - F): 6 levels of 0.5 ml each Six vials of calibrators (marked as A, B, C, D, E and F). Calibrators A, B, C, D, E and F contain 25(OH)D in a buffer matrix (TBS, Albumin recombinant, NaN3 0.09 percent; yellow in colour) in the concentration of 5, 12.5, 25, 40, 80 and 120 nanograms per milliliter respectively.

2. Controls- 2 levels of 0.35 ml each2 controls, A and B containing 25(OH) vitamin D in a buffer matrix (TBS, Albumin recombinant, 0.09 percent of NaN3), yellow. The concentrations of control A and control B are 8.9 (range <20) ng/ml and 60.64 (range 32-89) ng/ml respectively.

3. Sample diluent- 4 ml Sample diluent, containing 25(OH) vitamin D detector in a buffer matrix (TBS, Albumin recombinant, NaN3 0.09%), green. The chemical is prepared for instant use.

4. Tracer Enzyme conjugate containing streptavidin, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.

5. Releasing reagent- 5 ml Releasing reagent containing iron citrate, sodium toluene sulfonate, sodium citrate, sodium chloride, yellow. Ready to use.

6. Light reaction wells Divisible strips consisting of 8 wells each. Supplied in instant use condition.

7. Wash buffer concentrate- 5 milliliter The supplied wash buffer has chemical composition of Tris, detergent, 0.09 percent of preservative sodium azide. The concentrate should be diluted to 50 times with distilled H2O.

8. CLIA signal A solution- 2 milliliter The solution A is supplied as one vial of luminol (light sensitive) in buffer. The luminol being light-sensitive therefore, supplied in brown bottle. It should be deposited at 2-80 C.

9. CLIA signal B solution- 2milliliter The signal B is supplied as one vial of hydrogen peroxide in buffer in a brown bottle. It is also kept at 2-80 C.

Bringing all chemicals and samples (that were to be used) to 200 to 280 centigrade (room temperature) was 1st step before starting the procedure for estimation.

RESULTS

Blood hemolysis and preparation of hemolysate. Two tubes were marked as control (C) and Test (T) and 0.5 milliliter of lysing chemical was pipetted into both the tubes. 0.1 milliliter of above prepared standard was pipetted into the tube marked as ‘C’ and same volume of well mixed blood was also dispensed into tube marked as ‘T’. Homogeneous solutions in both the tubes were made by proper mixing. This allowed complete and proper hemolysis. These tubes were left stationary for 5 minutes of time.

GHb separation -

1. ‘C’ for standard and ‘T’ for test was marked again in the tubes containing ion-exchange resin after removing the lid of the resin tubes.

2. Hemolysates prepared in above step A were dispensed into the appropriately labelled ion-exchange resin tubes. The volume of dispense was 0.1 milliliter.

3. Resin separator was implanted into each tube. It should be done in such a manner that the rubber sleeve should roughly be one centimeter above the resin suspension level.

4. Vortex mixture was brought into the use for proper and nonstop mixing of the contents in the tube up to five minutes of duration.

5. After vortexing the contents, settling down the resin was allowed. Resin separator was pushed slowly to the extent until firmly packing of resin was observed.

6. The Floating liquid obtained from both the tubes was pipetted into separate cuvettes and their optical density was measured against optical density of distilled H2O.

Total Hemoglobin (THb) fraction- 5.0 milliliters of D/W (distilled H2O) was pipetted into test tubes labelled as ‘C’ for standard and ‘T’ for testing blood sample. Hemolysates prepared above in step A was dispensed into the suitably labelled test tube. The volume dispensed was 200 microliters, and mixed appropriately and uniformly. Optical density of each hemolysate was recorded against the optical density of D/W (distilled H2O).

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Reference Range: [Normal: <5.9%], [Good Control: 5.9 to 6.8 percent], [Fair Control: less than equal to 7.6 percent]. And [Poor Control: more than 7.6 percent].

CONCLUSION

The supplied wash concentrates had to be diluted for working. The final working wash solution had to be thinned 50 times by adding distilled H2O since the original wash supplied, was fifty times concentrated. Therefore, one milliliter of the concentrated wash solution was thinned by adding 29 milliliter of distilled H2O making final volume of 50 milliliter and was kept at room temperature of 200 to 28º Centigrade (room temperature) before its use. As per the requirement of the working signal solution, the preparation was performed accordingly. The final working signal solution was ready by mixing both signal A and signal B in one dry and sterile vessel in 1:1 proportion. 1 milliliter of signal A was mixed with equal volume (1 milliliter) of signal B. The unused portion was discarded, else it was stored at 2-8º C for 36 hours for using next time. It would be useless after the mentioned duration. Direct sunlight exposure was avoided. Also the samples were stockpiled, and pooled serums were deepfreezed at minus 20º centigrade of temperature. Thus deep-freezed serums were thawed and mixed thoroughly just prior to assay. Required number of eppendorfs were taken and marked; 6 eppendorfs were marked as A, B, C, D, E and F for 6 calibrators, 2 eppendorfs were marked as CA and CB for Control A and Control B and other eppendorfs were marked as 1, 2, 3, ... for samples, as per number of samples being assayed. 40 µl of calibrators, controls and samples were pipetted into the respective marked eppendorfs and similarly, 40 µl of sample diluent was pipetted into the each eppendorfs. The contents were mixed well in a vortex mixture. 80 µl of releasing reagent was pipetted into each of the eppendorfs and the contents were mixed well in vortex mixture again. Patients under poor glycemic control had lower vitamin D level and they had higher serum insulin and C-peptide concentration. Patients under good control had highest vitamin D level and lowest serum insulin and C-peptide concentration.

The present case-control research will help the clinicians for effective treatment of diabetes mellitus type II. This will also help in fast recovery from the disease due to the knowledge that supplementing this vitamin helps to improve insulin secretion and function. Therefore, it is suggested that serum vitamin D should be under optimal level for prevention of the disease and for better prognosis of the disease. The data from the study might be helpful for the government authorities for implementing vitamin D fortification program. It is suggested to optimize one's vitamin D level to stay healthy so that individual could help in the betterment of the society and the country.

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