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Study of Serum Vitamin D, Calcium and Phosphorus in the Uncomplicated and Complicated cases of Type 2 Diabetes Mellitus

Akanksha Menaria[1*], Dr. A K Verma[2], Shilpa Sharma[3]

[1]Biochemist, Department of Biochemistry, RNT Medical College, Udaipur, Rajasthan, India
[2]Professor & Head, Additional Principal, Department of Biochemistry, RNT Medical College, Udaipur, Rajasthan, India
[3]Senior Demonstrator, Department of Biochemistry, RNT Medical College, Udaipur, Rajasthan, India

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***Corresponding author:** Akanksha Menaria

Abstract **Original Research Article**

Background: Diabetes mellitus also described as "Disease of Civilization" is a metabolic anamoly having gruesome impact on quality of life worldwide. The disease has been labeled since ancient times and was recognized as a serious illness. ***Objectives:*** The objectives of the study are to estimate the level of Serum Vitamin D, Calcium and Phosphorus in the Uncomplicated and complicated cases of Type 2 Diabetes Melitus. ***Materials & Methods:*** The work encloses clinical study on Type 2 Diabetes Mellitus. It is broadly categorized into two parts. The first part comprises of Control group study conducted on 100 healthy control subjects. The second part comprises of Clinical Study, further subdivided into two groups. The first group (Group 1) comprises of 100 newly diagnosed or Uncomplicated Type 2 Diabetic cases and the second group(Group 2) comprises of 100 Complicated (Microvascular or Macrovascular) Type 2 Diabetic cases. Patients and controls were selected from the outdoor and indoor area of Endocrinology and Medicine Department of M.B. Hospital, R.N.T. Medical College, Udaipur.Subjects of both Group I and II along with control group were analyzed for serum Vitamin D, S.Calcium, S.Phosphorus, FBS, PPBS, HbA_1C, Lipid Profile and Liver Function Test. After assessing all the values, Mean and standard deviation of all subjects and parameters are analyzed. Statistical analysis is performed with SPSS software. Comparison of categorical variables (among category comparison) is done using Chi-Square Test. Comparison between cases and control is done by independent student's t test. By using t value P value is calculated. P value less than 0.05 ($P<0.05$) is considered significant. ***Results:*** All the categories of Complicated Type 2 Diabetic cases when compared with Control of the same category gave a similar reflection of significantly low Vitamin D ($P<0.05$)Calcium, Phosphorus ($P<0.05$), HDL-C, Total Protein, Albumin ($P<0.05$) and significantly high glycemic status ($P<0.00$), Total Cholesterol, TG, LDL-C, VLDL-C ($P<0.00$), Urea, Creatinine, Uric acid, SGOT and Alkaline Phosphatase ($P<0.00$). The Smokers displayed significantly lower Vitamin D, and raised FBS, LDL-C, Uric acid ($P<0.05$). The Alcoholics exhibited drop in Vitamin D, HDL-C ($P<0.05$) and significant upsurge of Total Cholesterol, TG, LDL-C ($P<0.05$). ***Conclusion:*** Our study clearly indicates significant decline in Vitamin D, Calcium and Phosphorus levels in Type 2 Diabetes and its associated complications. Faulty lifestyle, altered food habits & diminished physical activity culpable for obesity aggravates the pre-existing insulin resistance state. Therefore timely assessing of Vitamin D and various minerals at the start and even before the onset of diabetes will be certainly supportive in diabetes management. Towering figure of this epidemic demands varied biochemical approach along with conventional glucose monitoring goals. Hence, our study strongly advocates the regular assessment of Vitamin D and Minerals for beforehand diagnosis of the diabetes and its vascular adversities.

Key words: Vitamin D, DM, Minerals, smoking,alocohol.

INTRODUCTION

Type 2 Diabetes also called Adult onset Diabetes or Non-Insulin Dependent Diabetes Mellitus includes a group of different metabolic disorder resulting in an elevated blood glucose level (hyperglycemia) secondary to either insulin deficiency or abnormal insulin action. The symptoms of untreated diabetes include excessive urine production, increased hunger and poor healing [1]. Diabetes is a clinical syndrome characterized by hyperglycemia caused by absolute or relative deficiency of insulin.

Vitamin D has an important role in the regulation of cellular Ca^{+2} signalling which is linked to cellular responses, signaling and secretion. Sustained Ca^{+2} signals triggered by 1, 25-(OH) $_{2D3}$ have been researched for the regulation of apoptosis in diseases as obesity and Type 2 diabetes [2]. Moreover, 1, 25-(OH)

$_{2D3}$ induced Ca^{+2} signals (Ca^{+2} oscillations) can regulate insulin secretion from pancreatic β-cells [3]. The rapid increase in intracellular calcium triggers insulin release. The role of 1,25(OH)2D3 in insulin secretion derives from its effect on Ca+2 influx, mobilization, and buffering in pancreatic β-cells [4].

Calcium is essential for insulin mediated extracellular processes in insulin responsive tissues such as skeletal muscle and adipose tissue with a very narrow range of Ca+2 needed for optimal insulin mediated function. Changes in calcium in primary insulin target tissue may contribute to peripheral insulin resistance (21). Low calcium causes impairment of insulin receptor phosphorylation, a calcium-dependent process, leading to impaired insulin signal transduction and decreased glucose transporter-4 activity [5, 6]. Moreover changes in calcium modulate adipocyte metabolism, which may promote triglyceride accumulation via increased de novo lipogenesis and inability to suppress insulin mediated lipolysis leading to fat accumulation [7, 8]. Changes in serum Ca+2 may also lead to cykotine induced apoptosis [9].

MATERIAL AND METHODOLOGY

The work encloses clinical study on Type 2 Diabetes Mellitus. It is broadly categorized into two parts. The first part comprises of Control group study conducted on 100 healthy control subjects. The second part comprises of Clinical Study, further subdivided into two groups. The first group (Group 1) comprises of 100 newly diagnosed or Uncomplicated Type 2 Diabetic cases and the second group (Group 2) comprises of 100 Complicated (Microvascular or Macrovascular) Type 2 Diabetic cases. Patients and controls were selected from the outdoor and indoor area of Endocrinology and Medicine Department of M.B. Hospital, R.N.T. Medical College, Udaipur.

Subjects of both Group I and II along with control group were analyzed for serum Vitamin D, S.Calcium, S.Phosphorus, FBS, PPBS, HbA_1C, Lipid Profile and Liver Function Test.

Exclusion criteria

Cancer, Renal Osteodystrophy patients **,**Patients having anemia of any cause, serious infections, chronic liver disease or on corticosteroid therapy.Patients receiving medications that affect vitamin D metabolism/ absorption (phenytoin, rifampin, isoniazid, ketocanazole).Patients receiving vitamin D and Calcium supplementation

10 ml of blood from the Control group and Clinical group was drawn from a antecubital vein and collected in plain vial. Serum was separated by centrifugation of blood sample and following parameters were estimated in both Control and Clinical study group.

1) Vitamin D - ECLIA method on Cobas e411 analyzer

2) Calcium - o-crespothalin complexone (OCPC) method on Siemens Dimension R_xL Clinical Chemistry System

3) Phosphorus - phosphomolybdate method on Siemens Dimension R_xL Clinical Chemistry System

4) Blood sugar - hexokinase-glucose-6 phosphate dehydrogenase method on Siemens Dimension R_xL Clinical Chemistry System

5) HbA1C: HbA_1C values are taken from the patient medical record.

6) Lipid profile

- S. cholesterol: enzymatic method on Siemens Dimension R_xL Clinical Chemistry System
- S. Triglyceride: enzymatic method on Siemens Dimension R_xL Clinical Chemistry System
- S.HDL: enzymatic method on Siemens Dimension R_xL Clinical Chemistry System
- S.LDL: enzymatic method on Siemens Dimension R_xL Clinical Chemistry System
- S.VLDL: The value of VLDL-cholesterol is calculated by friedwald's formula. **VLDL-cholesterol =**
- **Triglyceride/5**

7) Liver function test

- SGPT (ALT): IFCC method on Siemens Dimension R_xL Clinical Chemistry System
- SGOT (AST): IFCC method on Siemens Dimension R_xL Clinical Chemistry System
- ALP: IFCC method on Siemens Dimension R_xL Clinical Chemistry System
- Total protein: modified biuret method on Siemens Dimension R_xL Clinical Chemistry System
- Albumin: bromocresol purple (BCP) dye-binding method on Siemens Dimension R_xL Clinical Chemistry System

BMI, Waist circumference (WC), Waist -Hip ratio (W/HR) is measured as per WHO guidelines (WHO, 2014).

Statistical Analysis

After assessing all the values, Mean and standard deviation of all subjects and parameters are analyzed. Statistical analysis is performed with SPSS software. Comparison of categorical variables (among category comparison) is done using Chi-Square Test. Comparison between cases and control is done by independent student's t test. By using t value P value is calculated. P value less than 0.05 ($P<0.05$) is considered significant. Multiple comparisons are done by Post Hoc Analysis of Variance (ANOVA) and Least Significant Difference (LSD) is calculated using Fisher's LSD Method. Using LSD, t and P values are calculated.

RESULTS AND DISCUSSION

Table-1: Age wise distribution of participants

		Controls			**Group**		
						Complicated	
					Uncomplicated Type 2 Diabetes Cases	Type 2 Diabetes Cases	Total
Age (years)	30-45	Count		73	26	5	104
		% within Group		73.0%	26.0%	5.0%	34.7%
	46-60	Count		16	41	40	97
		% within Group		16.0%	41.0%	40.0%	32.3%
	61-80	Count		11	33	55	99
		% within Group		11.0%	33.0%	55.0%	33.0%
Total		Count		100	100	100	300
		% within Group		100.0%	100.0%	100.0%	100.0%
		Value	df	P			
Pearson Chi-Square		1.117	4	0.000			

Table 1 presents the comparison of age group which is divided into three sub group; 30-45 years, 46-60 years and 61-80 years showed valid percentage of 73.0%, 16.0% and 11.0% respectively in Controls, 26.0% ,41.0% and 33.0% respectively in Uncomplicated Type2 Diabetes Cases and 5.0%, 40.0% and 55.0% respectively in Complicated Type2 Diabetes Cases. The value obtained from Pearson Chi-Square test is 1.117 which is statistically significant (P<0.000).

Table-2: sex wise distribution of participants

Controls					**Group**		**Complicated**	
					Uncomplicated Type 2 Diabetes		**Type 2 Diabetes**	
					Cases		Cases	Total
Sex	Male	Count		64	40	53	157	
		% within Group		64.0%	40.0%	53.0%	52.3%	
	Female	Count		36	60	47	143	
		% within Group		36.0%	60.0%	47.0%	47.7%	
Total		Count		100	100	100	300	
		% within Group		100.0%	100.0%	100.0%	100.0%	
		Value	df	P				
Pearson Chi-Square		11.572	2	0.003				

Table 2 represents the Sex group (Male & Female) comparison of Cases and Controls with valid percentage of 64.0% and 36.0% respectively in Controls, 40.0% and 60.0% respectively in Uncomplicated Type2 Diabetes Cases and 53.0% and 47.0% respectively in Complicated Type2 Diabetes Cases. Chi-Square test yielded a statistically significant value of 11.572 (P<0.003).

Table-3: comparison of BMI between case and control

Controls					**Group**	**Complicated**	
					Uncomplicated Type 2 Diabetes Cases	Type 2 Diabetes	
						Cases	Total
BMI	Normal	Count		67	35	61	163
		% within Group		67.0%	35.0%	61.0%	54.3%
	Over- weight	Count		24	41	33	98
		% within Group		24.0%	41.0%	33.0%	32.7%
	Obese	Count		9	24	6	39
		% within Group		9.0%	24.0%	6.0%	13.0%
Total		Count		100	100	100	300
		% within Group		100.0%	100.0%	100.0%	100.0%
		Value	df	P			
Pearson Chi-Square		29.387	4	0.000			

Table 3 represents comparison of BMI groups categorized into Normal, Overweight and Obese with valid percentage of 67.0%, 24.0% and 9.0% respectively in Controls, 35.0%, 41.0% and 24.0%

respectively in Uncomplicated Type2 Diabetes Cases and 61.0% ,33.0% and 6.0% respectively in Complicated Type2 Diabetes Cases. Chi-Square value obtained is 29.387 with a significant P value (P<0.000).

Table-4: Statistical Evaluation of Vitamin D, Mineral Status, Glycemic Status, Lipid Profile and other Biochemical Parameters among Uncomplicated Type 2 Diabetes cases (Intra group) for the Disease Duration (Years)

Parameters	0-1 Vs >1-5		0-1 Vs >5-10		0-1 Vs >10+		>1-5 Vs >5-10		>1-5 Vs >10+		>5-10 Vs >10+	
	t	signi	t	signi	t	signi	t	signi	t	signi	t	signi
Vitamin D (ng/ml)	2.20	0.05	1.99	0.05	2.33	0.05	5.20	0.05	2.88	0.05	1.89	0.05
Calcium (mg/dl)	2.56	0.05	3.23	0.05	5.12	0.01	2.32	0.05	2.43	0.05	1.11	N.S
Phosphorus (mg/dl)	0.12	N.S	0.18	N.S	0.16	N.S	0.06	N.S	0.04	N.S	0.02	N.S
FBS (mg/dl)	0.81	N.S	5.15	0.01	4.73	0.05	2.33	0.05	1.91	0.05	0.41	N.S
PPBS (mg/dl)	2.20	0.05	6.94	0.01	9.32	0.001	6.74	0.01	8.12	0.005	6.38	0.01
HbA_1C (%)	0.14	N.S	0.60	N.S	1.95	0.001	0.45	N.S	1.89	0.05	1.88	0.05
T.Cholesterol (mg/dl)	0.29	N.S	2.47	0.05	16.75	0.000	1.18	N.S	16.46	0.000	14.28	0.000
Triglycerides (mg/dl)	0.71	N.S	1.33	N.S	2.40	0.05	0.61	N.S	2.33	0.05	2.73	0.05
HDL-C (mg/dl)	0.80	N.S	2.55	0.05	3.40	0.05	0.25	N.S	3.20	0.05	0.95	N.S
LDL-C (mg/dl)	2.83	0.05	3.96	0.05	7.80	0.01	1.79	0.05	6.96	0.01	5.76	0.01
VLDL-C (mg/dl)	0.14	N.S	1.26	N.S	2.68	0.05	1.12	N.S	2.82	N.S	2.94	0.05
SGOT (U/L)	0.57	N.S	0.45	N.S	0.97	N.S	1.02	N.S	1.40	N.S	1.43	N.S
SGPT (U/L)	0.64	N.S	1.38	N.S	1.24	N.S	1.73	N.S	0.88	N.S	0.62	N.S
Alk.Phosphatase (U/L)	0.67	N.S	2.45	0.04	1.51	N.S	1.77	N.S	0.84	N.S	0.21	N.S
Total Protein (g/dl)	1.35	N.S	2.15	0.05	2.47	0.05	3.51	0.05	4.11	0.05	0.62	N.S
Albumin (g/dl)	0.11	N.S	2.07	0.05	2.42	0.05	0.84	N.S	1.88	0.05	0.01	N.S

Table 4 represents the comparison of Duration of Disease divided into 0-1year, >1-5Years, >5-10years and >10+years with valid percentage of 34.0%, 49.0%, 12.0% and 5.0% respectively in Uncomplicated Type2 Diabetes Cases and 13.0%, 35.0%, 26.0% and 26.0% respectively in Complicated Type2 Diabetes Cases. Chi- square value obtained in total 200 subjects is 3.467 and a statistically significant P value (P<0.000).

Table-5: Statistical Evaluation of Vitamin D, Mineral Status, Glycemic Status, Lipid Profile and other Biochemical Parameters among Complicated Type 2 Diabetes cases (Intra group) for the Disease Duration (Years)

Parameters	0-1 Vs >1-5		0-1 Vs >5-10		0-1 Vs >10+		>1-5 Vs >5-10		>1-5 Vs >10+		>5-10 Vs >10+	
	t	signi	t	signi	t	signi	t	signi	t	signi	t	signi
Vitamin D (ng/ml)	1.91	0.05	0.21	N.S	4.74	0.05	0.39	N.S	3.11	0.05	3.84	0.05
Calcium (mg/dl)	0.71	N.S	2.77	0.05	3.01	0.05	0.69	N.S	0.58	N.S	0.08	N.S
Phosphorus (mg/dl)	1.60	N.S	1.91	0.05	3.28	0.05	0.84	N.S	2.11	0.05	1.97	0.05
FBS (mg/dl)	0.29	N.S	1.00	N.S	3.21	0.05	0.37	N.S	2.89	0.05	1.12	N.S
PPBS (mg/dl)	0.56	N.S	1.21	N.S	2.78	0.05	0.39	N.S	2.52	0.05	2.11	0.05
HbA_1C (%)	0.43	N.S	0.84	N.S	2.43	0.05	0.61	N.S	1.99	0.05	0.32	N.S
T.Cholesterol (mg/dl)	0.11	N.S	0.62	N.S	0.58	N.S	0.33	N.S	0.21	N.S	0.14	N.S
Triglycerides (mg/dl)	0.96	N.S	0.84	N.S	1.80	N.S	0.21	N.S	0.32	N.S	0.27	N.S
HDL-C (mg/dl)	0.19	N.S	0.20	N.S	0.22	N.S	0.10	N.S	0.21	N.S	0.14	N.S
LDL-C (mg/dl)	0.06	N.S	0.37	N.S	0.04	N.S	0.01	N.S	0.29	N.S	0.31	N.S
VLDL-C (mg/dl)	0.96	N.S	0.89	N.S	1.62	N.S	0.12	N.S	0.10	N.S	0.84	N.S
SGOT (U/L)	0.72	N.S	0.84	N.S	0.62	N.S	0.51	N.S	0.18	N.S	0.39	N.S
SGPT (U/L)	0.45	N.S	2.45	0.05	0.12	N.S	0.46	N.S	0.78	N.S	1.89	0.05
Alk.Phosphatase (U/L)	0.47	N.S	0.04	N.S	0.44	N.S	0.46	N.S	0.05	N.S	0.39	N.S
Total Protein (g/dl)	0.86	N.S	2.11	0.05	2.57	0.05	0.99	N.S	2.19	0.05	0.81	N.S
Albumin (g/dl)	1.68	N.S	1.17	N.S	1.10	N.S	0.21	N.S	0.07	N.S	0.01	N.S

Table-6: Macrovascular Complications * Group Cases and Controls (CVD: cardiovascular disease, HT: Hypertension)

Controls					Group	Complicated	Total
					Uncomplicated Type 2 Diabetes Cases	Type 2 Diabetes Cases	
Macro-vascular Complications	CVD+ HT	Count		0	0	19	19
		% within Group		0.0%	0.0%	19.0%	6.33%
HT		Count		0	0	39	39
		% within Group		0.0%	0.0%	39.0%	13.0%
NIL		Count		100	100	42	242
		% within Group		100.0%	100.0%	42.0%	80.66%
Total		Count		100	100	100	300
		% within Group		100.0%	100.0%	100.0%	100.0%
		Value	df	P			
Pearson Chi-Square		2.219	4	0.000			

Table-7: Microvascular Complications * Group Cases and Controls (NRP:neuropathy. RP:Retinopathy ,NHP:Nephropathy)

Controls				Group	Complicated	
				Uncomplicated Type 2 Diabetes Cases	Type 2 Diabetes Cases	Total
Micro-vascular Complications	NIL	Count	100	100	0	200
		% within Group	100.0%	100.0%	.0%	66.7%
RP		Count	0	0	10	10
		% within Group	.0%	.0%	10.0%	3.3%
NRP		Count	0	0	51	51
		% within Group	.0%	.0%	51.0%	17.0%
NRP+RP		Count	0	0	30	30
		% within Group	.0%	.0%	30.0%	10.0%
NHP+RP+NRP		Count	0	0	9	9
		% within Group	.0%	.0%	9.0%	3.0%
Total		Count	100	100	100	300
		% within Group	100.0%	100.0%	100.0%	100.0%
		Value	df	P		
Pearson Chi-Square		3.000	8	0.000		

Table-8: Statistical Evaluation of Vitamin D, Glycemic Status, Lipid Profile and other Biochemical Parameters among Control Vs Uncomplicated Type 2 Diabetes cases for Gender and Inhabitance

Parameters	Total			Gender				Inhabitance		
				Male		Female		Urban		Rural
	t	signi	t	signi	t	signi	t	signi	t	signi
Vitamin D (ng/ml)	2.10	0.05	3.14	0.05	2.60	0.05	3.05	0.05	3.20	0.05
Phosphorus (mg/dl)	1.13	N.S	0.904	N.S	1.22	N.S	1.83	N.S	0.72	N.S
FBS (mg/dl)	41.74	0.00	25.24	0.000	16.90	0.000	10.15	0.000	31.15	0.000
PPBS (mg/dl)	70.15	0.00	36.36	0.000	31.71	0.000	14.81	0.000	54.88	0.000
HbA_1C (%)	163.61	0.00	110.67	0.000	53.43	0.000	38.15	0.000	122.43	0.000
T.Cholesterol (mg/dl)	2.59	0.50	0.719	N.S	1.15	N.S	0.30	N.S	5.86	0.005
Triglycerides (mg/dl)	4.31	0.01	0.501	N.S	11.92	0.000	0.89	N.S	3.15	0.05
HDL-C (mg/dl)	2.22	0.50	0.06	N.S	1.65	N.S	1.89	N.S	0.40	N.S
LDL-C (mg/dl)	3.45	0.05	3.35	0.05	7.57	0.001	1.74	N.S	1.88	N.S
VLDL-C (mg/dl)	4.31	0.01	0.50	N.S	11.92	0.000	0.89	N.S	3.15	0.05
SGOT (U/L)	5.64	0.005	1.00	N.S	7.54	0.05	2.97	0.056	2.52	N.S
SGPT (U/L)	0.93	N.S	0.39	N.S	3.72	0.05	1.44	N.S	0.08	N.S
Alk.Phosphatase (U/L)	11.73	0.000	3.95	0.05	9.39	0.000	1.27	N.S	1.30	N.S
Total Protein (g/dl)	0.76	N.S	0.46	N.S	1.50	N.S	0.72	N.S	0.90	N.S
Albumin (g/dl)	1.07	N.S	1.83	N.S	0.361	N.S	1.71	N.S	0.62	N.S

Table-9: Statistical Evaluation of Vitamin D, Mineral Status, Glycemic Status, Lipid Profile and other Biochemical Parameters among Uncomplicated Type 2 Diabetes cases (Intra group) for Educational Status, Family History (FH) and Dietary Habits

Parameters	Illiterate Vs Semi Literate		Illiterate Vs Literate		Semi-Literate Vs Literate		With FH Vs without FH		Vegetarian Vs Non Vegetarian	
	t	signi	t	signi	t	signi	t	signi	t	signi
Vitamin D (ng/ml)	1.53	N.S	0.34	N.S	0.53	N.S	1.10	N.S	0.88	N.S
Zinc (µg/dl)	0.98	N.S	5.85	0.005	3.24	0.005	0.67	N.S	0.57	N.S
Magnesium (mg/dl)	0.81	N.S	1.03	N.S	1.17	N.S	0.87	N.S	0.85	N.S
Calcium (mg/dl)	0.84	N.S	0.54	N.S	1.16	N.S	1.16	N.S	0.89	N.S
Phosphorus (mg/dl)	1.17	N.S	0.22	N.S	0.02	N.S	0.03	N.S	0.33	N.S
FBS (mg/dl)	2.53	0.05	8.56	0.00	1.16	N.S	0.13	N.S	0.31	N.S
PPBS (mg/dl)	1.05	N.S	7.67	0.00	6.37	0.01	0.71	N.S	0.35	N.S
HbA_1C (%)	0.75	N.S	1.37	N.S	0.28	N.S	0.88	N.S	0.11	N.S
T.Cholesterol (mg/dl)	1.93	0.05	2.00	0.05	0.54	N.S	0.46	N.S	1.23	N.S
Triglycerides (mg/dl)	0.83	N.S	1.30	N.S	2.37	0.05	1.49	N.S	1.15	N.S
HDL-C (mg/dl)	0.76	N.S	3.40	0.05	1.53	N.S	0.34	N.S	0.19	N.S
LDL-C (mg/dl)	1.19	N.S	1.44	N.S	1.02	N.S	2.14	0.05	0.80	N.S
VLDL-C (mg/dl)	0.83	N.S	0.26	N.S	2.47	0.05	1.49	N.S	1.15	N.S
Urea (mg/dl)	1.46	N.S	1.86	N.S	1.22	N.S	1.20	N.S	0.03	N.S
Creatinine (mg/dl)	0.82	N.S	0.01	N.S	1.26	N.S	0.87	N.S	1.15	N.S
Uric acid(mg/dl)	2.18	0.05	2.34	0.05	0.29	N.S	1.10	N.S	2.68	0.05
SGOT (U/L)	0.99	N.S	1.48	N.S	1.81	N.S	0.85	N.S	1.50	N.S
SGPT (U/L)	0.15	N.S	1.23	N.S	1.55	N.S	0.35	N.S	1.30	N.S
Alk.Phosphatase (U/L)	0.73	N.S	0.65	N.S	0.94	N.S	1.96	0.05	0.90	N.S
Total Protein (g/dl)	1.02	N.S	0.69	N.S	0.21	N.S	0.86	N.S	1.27	N.S
Albumin (g/dl)	1.68	N.S	0.85	N.S	0.16	N.S	0.99	N.S	1.98	0.05

Table-10: Statistical Evaluation of Vitamin D, Mineral Status, Glycemic Status, Lipid Profile and other Biochemical Parameters among Control Vs Uncomplicated Type 2 Diabetes cases for Smoking and Alcohol Habits

Parameters	Smokers				Alcoholic			
	Yes		No		Yes		No	
	t	signi	t	signi	t	signi	t	signi
Vitamin D (ng/ml)	1.38	N.S	1.97	0.05	1.93	0.05	2.07	0.05
Calcium (mg/dl)	2.84	0.05	0.88	N.S	5.37	0.001	0.90	N.S
Phosphorus (mg/dl)	1.09	N.S	0.68	N.S	0.65	N.S	1.08	N.S
FBS (mg/dl)	7.07	0.001	36.32	0.000	21.55	0.000	34.57	0.000
PPBS (mg/dl)	8.03	0.001	63.43	0.000	19.42	0.000	62.57	0.000
HbA_1C (%)	26.42	0.001	137.68	0.000	37.28	0.000	143.02	0.000
T.Cholesterol (mg/dl)	2.15	0.05	1.73	N.S	3.23	0.05	5.45	0.01
Triglycerides (mg/dl)	1.93	0.05	2.87	0.05	3.43	0.05	4.65	0.01
HDL-C (mg/dl)	1.94	0.05	1.07	N.S	2.14	0.05	1.41	N.S
LDL-C (mg/dl)	2.78	0.05	3.74	0.01	1.31	N.S	1.04	N.S
VLDL-C (mg/dl)	1.93	0.05	2.87	0.05	3.43	0.05	4.65	0.01
SGOT (U/L)	1.03	N.S	4.70	0.010	1.50	N.S	1.29	N.S
SGPT (U/L)	0.57	N.S	0.56	N.S	0.11	N.S	0.71	N.S
Alk.Phosphatase (U/L)	3.07	0.05	16.90	0.000	1.93	0.05	2.29	0.000
Total Protein (g/dl)	1.97	0.05	1.14	N.S	1.74	N.S	0.71	N.S
Albumin (g/dl)	1.00	N.S	0.93	N.S	2.40	0.05	0.80	N.S

In Smokers Vs Non Smokers collation, the Smokers showed lower values for Vitamin D (24.20±7.30 Vs 20.96±5.70; P<0.05) and higher significance for TG (119.3±30.3 Vs 103.2±19.3; P<0.01) and VLDL-c (23.86±8.06 Vs 20.6±3.80; P<0.01). No more significant parameter was found in this group. In Alcoholic Vs Non-alcoholic comparison, the latter showed significantly high values for Vitamin D & Zinc (24.58±8.20 Vs 20.34±7.90; P<0.05 and 87.68±9.58 Vs 80.37±8.90; P<0.05 respectively). The Alcoholics showed higher Alkaline Phosphate level (82.73±10.3 Vs 74.63±8.8; P<0.05) when compared to Non-alcoholics. No other significant variable was found in this comparison [Table 10 & 13].

Table-11: Statistical Evaluation of Vitamin D, Mineral Status, Glycemic Status, Lipid Profile and other Biochemical Parameters among Complicated Type 2 Diabetes cases (Intra group) for Gender, Inhabitance and Religion

Parameters	Male Vs Female		Urban Vs Rural		Hindu Vs Muslim		Hindu Vs Christian		Muslim Vs Christian	
	t	signi	t	signi	t	signi	tsigni		tsigni	
Vitamin D (ng/ml)	0.61	N.S	3.03	0.05	3.26	0.05	1.16	N.S	4.84	0.05
Calcium (mg/dl)	2.81	0.05	0.04	N.S	0.23	N.S	1.94	0.05	1.90	0.05
Phosphorus (mg/dl)	0.95	N.S	1.13	N.S	0.20	N.S	1.98	0.05	1.99	0.05
FBS (mg/dl)	0.54	N.S	1.48	N.S	4.78	0.05	0.72	N.S	4.74	0.05
PPBS (mg/dl)	0.35	N.S	1.57	N.S	3.54	0.05	0.61	N.S	1.01	N.S
HbA_1C (%)	0.42	N.S	1.74	N.S	0.31	N.S	0.54	N.S	0.36	N.S
T.Cholesterol (mg/dl)	1.93	0.05	0.45	N.S	1.40	N.S	0.29	N.S	2.10	0.05
Triglycerides (mg/dl)	1.96	0.05	0.18	N.S	0.12	N.S	1.00	N.S	0.94	N.S
HDL-C (mg/dl)	2.10	0.05	2.50	0.010	0.09	N.S	0.74	N.S	0.81	N.S
LDL-C (mg/dl)	3.00	0.05	0.25	N.S	2.29	0.05	1.91	0.05	2.67	0.05
VLDL-C (mg/dl)	1.96	0.05	0.18	N.S	0.12	N.S	0.78	N.S	0.78	N.S
SGOT (U/L)	0.73	N.S	2.04	0.05	0.62	N.S	2.11	0.05	1.01	N.S
SGPT (U/L)	0.35	N.S	1.94	0.05	1.21	N.S	2.78	0.05	1.22	N.S
Alk.Phosphatase (U/L)	0.58	N.S	1.14	N.S	1.36	N.S	9.67	0.01	8.69	0.01
Total Protein (g/dl)	1.06	N.S	1.11	N.S	0.14	N.S	0.74	N.S	0.56	N.S
Albumin (g/dl)	1.43	N.S	0.99	N.S	0.09	N.S	0.31	N.S	0.22	N.S

Table-12: Statistical Evaluation of Vitamin D, Mineral Status, Glycemic Status, Lipid Profile and other Biochemical Parameters among Complicated Type 2 Diabetes cases (Intra group) for Educational Status, Family History (FH) and Dietary Habits

Parameters	Illiterate Vs Semi Literate		Illiterate Vs Literate		Semi-Literate Vs Literate		With FH Vs without FH		Vegetarian Vs Non Vegetarian	
	t	signi	t	signi	t	signi	t	signi	t	signi
Vitamin D (ng/ml)	2.81	0.05	2.96	0.05	0.18	N.S	2.15	0.05	1.88	0.05
Calcium (mg/dl)	1.04	N.S	0.86	N.S	0.21	N.S	0.74	N.S	0.70	N.S
Phosphorus (mg/dl)	0.43	N.S	0.88	N.S	0.28	N.S	1.88	0.05	1.29	N.S
FBS (mg/dl)	0.54	N.S	3.11	0.05	0.86	N.S	0.62	N.S	0.18	N.S
PPBS (mg/dl)	0.57	N.S	2.66	0.05	1.24	N.S	0.44	N.S	0.92	N.S
HbA_1C (%)	0.27	N.S	0.31	N.S	0.46	N.S	0.51	N.S	0.57	N.S
T.Cholesterol (mg/dl)	0.12	N.S	0.31	N.S	0.58	N.S	1.02	N.S	1.37	N.S
Triglycerides (mg/dl)	1.24	N.S	1.99	0.05	1.34	N.S	1.00	N.S	1.11	N.S
HDL-C (mg/dl)	0.74	N.S	0.89	N.S	0.78	N.S	1.91	0.05	1.50	N.S
LDL-C (mg/dl)	0.29	N.S	0.36	N.S	0.11	N.S	1.21	N.S	0.73	N.S
VLDL-C (mg/dl)	0.24	N.S	1.89	0.05	1.22	N.S	1.00	N.S	1.11	N.S
SGOT (U/L)	2.56	0.05	0.78	N.S	0.64	N.S	0.17	N.S	0.95	N.S
SGPT (U/L)	1.88	0.05	0.67	N.S	0.50	N.S	0.22	N.S	0.37	N.S
Alk.Phosphatase (U/L)	0.22	N.S	0.31	N.S	0.66	N.S	0.91	N.S	0.47	N.S
Total Protein (g/dl)	0.90	N.S	0.91	N.S	0.31	N.S	1.48	N.S	1.13	N.S
Albumin (g/dl)	1.91	0.05	1.89	0.05	0.51	N.S	0.12	N.S	0.00	N.S

On the basis of Educational Status, the Cases & Controls were categorized into Illiterate, Semi-Literate and Literate group. The inter group comparison of Illiterate group gave out significantly low values of Vitamin D (24.82±7.01 Vs 20.53±4.97; $P<0.05$ significantly high values for FBS, PPBS, HbA_1C ($P<0.000$), Total Cholesterol ($P<0.01$), LDL-C ($P<0.000$)) for the Cases group. For the Semi-Literate group comparison significant low values was recorded for Vitamin D (24.79±10.97 Vs 22.41±6.34; $P<0.05$) and significant high values for FBS, PPBS, HbA_1C ($P<0.000$), Total Cholesterol, TG, LDL-C, VLDL-C ($P<0.05$), Urea ($P<0.000$) and Alkaline Phosphatase ($P<0.05$) by the Cases group. Comparison of Literate Type 2 Cases Vs Literate Controls reflected significant high values for FBS, PPBS, HbA_1C ($P<0.000$), Total Cholesterol, LDL-C ($P<0.01$) and Urea ($P<0.000$) by the Cases group [Table 12].

Table-13: Statistical Evaluation of Vitamin D, Mineral Status, Glycemic Status, Lipid Profile and other Biochemical Parameters among Control Vs Complicated Type 2 Diabetes cases for Smoking and Alcohol Habits

Parameters	Smokers					Alcoholic		
	Yes	No		Yes		No		
	t	signi	t	signi	t	signi	t	signi
Vitamin D (ng/ml)	3.38	0.05	3.97	0.05	2.33	0.05	2.07	0.05
Calcium (mg/dl)	8.84	0.001	2.88	0.05	7.37	0.001	1.90	0.05
Phosphorus (mg/dl)	1.09	N.S	0.68	N.S	2.65	0.05	2.08	0.04
FBS (mg/dl)	7.07	0.001	36.32	0.000	21.55	0.000	34.57	0.000
PPBS (mg/dl)	8.03	0.001	63.43	0.000	19.42	0.000	62.57	0.000
HbA_1C (%)	26.42	0.000	137.68	0.000	37.28	0.000	143.02	0.000
T.Cholesterol (mg/dl)	2.15	0.05	2.73	0.05	3.23	0.05	2.45	0.05
Triglycerides (mg/dl)	1.93	0.05	2.87	0.05	2.43	0.05	4.65	0.01
HDL-C (mg/dl)	2.24	0.05	3.07	0.05	3.14	0.05	2.41	0.05
LDL-C (mg/dl)	4.78	0.05	3.74	0.05	1.91	0.05	3.04	0.05
VLDL-C (mg/dl)	1.93	0.05	2.87	0.05	2.43	0.05	4.65	0.010
SGOT (U/L)	0.03	N.S	4.70	0.01	1.50	0.242	4.29	0.01
SGPT (U/L)	0.57	N.S	0.56	N.S	0.11	N.S	0.71	N.S
Alk.Phosphatase (U/L)	0.07	N.S	16.90	0.000	4.06	0.05	12.29	0.000
Total Protein (g/dl)	1.97	0.05	0.14	N.S	1.94	0.05	0.71	N.S
Albumin (g/dl)	1.00	N.S	0.93	N.S	1.40	N.S	0.80	N.S

DISCUSSION

Type 2 DM epidemic has been imputed to urbanization and environmental transition promoting sedentary behaviour and overnutrition. In 2017, global urban diabetic population was 279.2 million and is expected to increase to 628.6 million by 2045 chiefly due to globalisation [10]. In 2019, over 30 million people have been diagnosed with diabetes in India.

Type 2 Diabetes Mellitus is more common in males rather than females while the risk for developing CVD and other complications are higher (25-50%) in women compared to men [6, 30]. As per Government survey of India (2015-2019) prevalence of Type 2 DM in males was 12% and 11.7% in females [11]. In our result we recorded 40.0% males and 60% females with UnComplicated Type 2 Diabetes. Complicated Type 2 diabetes was recorded in 53.0% males and 47.0% females (Table 2).

Duration of Diabetes is the strongest predictor for the developement and progression of vascular complications of the disease. Poor glycemic Control in Indian diabetic population alongwith lack of timely screening predisposes the patients to severe vascular diabetic complications, sometimes even at the time of diagnosis of disease. In our study we observed 34.0% UnComplicated Type 2 Cases and 13.0% Complicated Type 2 Cases in 0-1 years duration of diabetes. 49.0% and 35.0% Cases (both groups respectively) were observed in >1-5 years group, 12.0% & 36.0% Cases (both groups respectively) in >5-10years group and 5.0% and 26.0% Cases in >10+ years group (Table 5).

Smokers Vs Nonsmokers Control subjects reflected significantly low values of Vitamin D ($P<0.05$) and high TG, VLDL-C ($P<0.01$) in the Smokers. Cigarette smoke decreases the production of the active form of Vitamin D and also affects the expression levels of the vitamin in lung epithelial cells. This effect intensifies with higher pack years of smoking and causes chronic inflammation and deterioration of lung functions. Additionally smoking is a well-known risk factor for arteriosclerosis and diabetes mellitus. Many studies have reported high TG, LDL-C, VLDL-C and Low HDL-C concentrations in smokers [12, 13] similar to our study results.

Alcoholic Vs Non-Alcoholic comparison reflected significantly high value of Alkaline Phosphatase ($P<0.05$) by the former group. Exposure to excessive ethanol adversely affects the metabolism of Vitamin D by depleting enzymes involved in converting $25(OH)D_3$ to $1,25(OH)_2D_3$ thus reducing Vitamin D levels as observed in our study [14] A.V Skalny et al., [15] demonstrated that use of alcohol induces modulation of Zinc transporters resulting in decreased Zinc levels in lungs, liver, gut and brain. Zinc deficiency in different organs causes systemic inflammation, endotoxemia, alcoholic liver disease and accumulation of neutroxic metabolites. Alcoholism is usually accompanied by mild increase in liver enzymes usually indicative of Alcoholic Liver Disease. In our study we found slight increase in Alkaline Phosphatase in Alcoholics when compared to Non-Alcoholic Control subjects. No other Parameter was found to be significant in this comparison.

CONCLUSION

Our study clearly indicates significant decline in Vitamin D, Calcium and Phosphorus levels in Type 2 Diabetes and its associated complications. Faulty lifestyle, altered food habits & diminished physical activity culpable for obesity aggravates the pre-existing

insulin resistance state. Therefore timely assessing of Vitamin D and various minerals at the start and even before the onset of diabetes will be certainly supportive in diabetes management. Towering figure of this epidemic demands varied biochemical approach along with conventional glucose monitoring goals. Hence, our study strongly advocates the regular assessment of Vitamin D and Minerals for beforehand diagnosis of the diabetes and its vascular adversities.

AUTHORSHIP

All Authors have done Equal contribution for research work.

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