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ASSOCIATION BETWEEN VITAMIN D STATUS AND INFLAMMATORY BIOMARKERS AMONG DIABETIC DYSLIPIDEMIA PATIENTS: A CROSS-SECTIONAL STUDY

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ABSTRACT

Introduction: Abnormal status of Vitamin D might be the leading cause of the development of various lifestyle disorders including Diabetic Dyslipidemia. Insulin resistance is considered to be one of the main leading factor of diabetic dyslipidemia and adequate glycemic control together with the management of hyperlipidemia shall reduce its future complications. **Aim:** The aim of the study was to demonstrate the association between vitamin D status and inflammatory biomarkers among diabetic dyslipidemic patients in a rural area of Kerala. **Materials & Methods:** A cross-sectional study was demonstrated to correlate the association between vitamin D status and inflammatory biomarkers among diabetic dyslipidemic patients in the study population. The incidence of diabetic dyslipidemia was observed in people of all age groups in adulthood and it was assumed that abnormality in vitamin D status may be one of the contributing factors leading to it. **Result:** The diabetic dyslipidemic patients with normal vitamin D status were found to have better control over glycemic status, also vitamin D deficiency was exhibited negatively associated with the major inflammatory biomarkers such as LpPLA2, AAT, and hs-CRP. **Conclusion:** It shall be concluded that lifestyle modification and appropriate pharmacotherapy together with vitamin D supplementation may be the ideal strategy to improve health conditions as well as the quality of life in diabetic dyslipidemic patients.

KEYWORDS

Cardiovascular Disease, Diabetes Mellitus, Dyslipidemia, Inflammatory markers, Vitamin D.

INTRODUCTION

Diabetes Mellitus is a condition of abnormal glucose tolerance which is characterized by chronic hyperglycemia with disturbances in fat, carbohydrate, and protein metabolism with multiple etiologies. American Diabetic Association (ADA) guidelines say that, a patient is said to be normoglycemic if a fasting blood sugar level is less than 110 mg/ml, postprandial blood sugar level is less than 140 mg/ml, or glycosylated hemoglobin (HbA1c) level below 5.7%[1]. The incidence of diabetes mellitus, dyslipidemia, obesity, and hypertension together presented in a person lead to a serious condition called Metabolic Syndrome. Diabetes is nowadays commonly recognized as a coronary heart disease risk equivalent, mainly attributed to the high rates of dyslipidemia among diabetic patients which are believed to be one of the major factors leading to a high mortality rate among diabetics due to cardiovascular diseases.

Dyslipidemia is a common metabolic abnormality associated with diabetes, which is characterized by a spectrum of quantitative and qualitative changes in lipids and lipoproteins.[2]Numerous studies have documented the association between higher LDL levels with increased risk of cardiovascular diseases among both diabetic and non-diabetic populations. Dyslipidemia is a disorder of lipoprotein metabolism, which includes a number of abnormalities such as hypercholesterolemia, hypertriglyceridemia, and low levels of high-density lipoprotein cholesterol.[3] As per National Cholesterol Education Programme (NCEP) guidelines, dyslipidemia is defined as a serum Total Cholesterol level greater than 200 mg/ml, serum Triglyceride level greater than 150 mg/ml, Serum HDL cholesterol levels less than 40 mg/ml (for men) and less than 50 mg/ml (for women) and LDL cholesterol levels greater than 130 mg/ml. Dyslipidemia represents the major link between diabetes and the increased cardiovascular risk of diabetic patients.[4] Diabetic dyslipidemia is a common pattern of lipid abnormalities together with Diabetes mellitus, which is characterized by elevated fasting LDL cholesterol and triglycerides with low HDL cholesterol, and the predominance of small dense LDL particles.[5] The underlying pathophysiology of diabetic dyslipidemia is yet to be understood completely. Alterations of insulin-sensitive pathways and increased free fatty acid concentration together with low-grade inflammation are assumed to play a major role in increased intestinal and hepatic biosynthesis of cholesterol and decreased catabolism of triglyceride-rich lipoproteins. Insulin resistance is believed to be the main triggering factor for the development of diabetic dyslipidemia.[6] Vitamin D deficiency may be associated with Diabetic Dyslipidemia which may cause increased morbidity and mortality due to various metabolic diseases. Vitamin D is a hormone synthesized mainly by the kidneys, which is conventionally considered to be involved with bone homeostasis and evidently associated to exert a wide range of effects on cardiovascular and nervous systems.[7]

A suboptimal level of Vitamin D is thought to be involved in the risk of cardiovascular diseases, by altering the other risk factors such as hypertension, diabetes mellitus and inflammation. Vitamin D deficiency is considered as the leading factor responsible for the development of various complications that may cause insulin resistance, glucose intolerance and endothelial dysfunction leading to diabetic dyslipidemia.[8] Many studies demonstrated in different populations point to the hypothesis that elevated levels of inflammatory mediators including Lipoprotein-associated phospholipase- A2 (LpPLA2), Alpha-1- antitrypsin (AAT), and High sensitive C - reactive protein (hs-CRP), might lead to direct pro-inflammatory effects and contributes to the initiation and progression of diabetic dyslipidemia. The optimum level of Vitamin D is considered to be up-regulated anti-inflammatory markers such as IL-4 and IL-10, and also down-regulate pro-inflammatory biomarkers such as interleukin-1 (IL-1), IL-2, IL-6, IL-23, tumor necrosis factor-α, and interferon-γ.[9] In the Indian Scenario, the prevalence of Vitamin D deficiency is reported to be very high in number and it might lead to the inflammatory process leading to an increase in the risk for cardiovascular disease. The health indicators such as high life expectancy, high literacy rate, low fertility rate, and reasonably better health care system of Kerala are at par with developed countries.[10]

However, the risk factors in CVD such as diabetes mellitus, dyslipidemia, and obesity are filthy high among the Malabar region population of Kerala. The primary management of diabetic dyslipidemia includes following an active lifestyle together with moderate-intensity physical exercise or Medical Nutrition Therapy (MNT), which might reduce the severity of the disease.[11] If the lifestyle modification does not reflect diabetic dyslipidemia, pharmacological interventions with appropriate drug therapy are a must to reduce future risks. Adequate control over glycemic status and lipid profile shall improve the health status and quality of life; and also may cause a decrease in future cardiovascular and cerebrovascular complications. [12]

MATERIALS & METHODS

A cross-sectional study was conducted in a teaching hospital located in a rural area of South India, in order to study the association between Serum Vitamin D status and inflammatory biomarkers level in adult patients with diabetic dyslipidemia. The Institutional Ethics Committee reviewed the research proposal and granted approval (IEC/MES/42/2014) to conduct the study at department of general medicine and department of cardiology of the study center. The study subjects were contacted by telephone and were asked to attend the free medical camp conducted at the study center. Participants of either gender in the age group of 25 to 75 years attending the outpatient department of general medicine and department of Cardiology for cardiac care were considered for the study. Among them, those who meet the diagnostic criteria for both diabetes mellitus and dyslipidemia according to ADA and NCEP-ATP III guidelines respectively enrolled in the study as the test group. Healthy subjects in a match with age and gender having normal Vitamin D status were also enrolled as the control group. Patients who were not on overnight fasting and those with chronic diseases like cancer, infectious diseases, auto-immune disorders and other inflammatory conditions were excluded from the study[13]**.** Also, patients who were not willing to give written informed consent were excluded from the study.

The sample size for the study was calculated with a minimum of 120 subjects in each group, based on the parameters from similar studies. The details of the study were explained well to each participant and written informed consent in vernacular language was obtained from each of them before the enrolment. Demographic details and anthropometric data were collected from each participant by using a predesigned proforma. 10 ml of venous blood was collected aseptically from each participant by venipuncture and serum was separated for estimation of Vitamin D (25(OH)D) levels by Chemiluminescence Immunoassays (CLIA). Based on the Vitamin D status of the test population, they further divided into three sub-groups as Vitamin D deficient group (< 20 ng/ml), Vitamin D insufficient group (20–29 ng/ml) and Vitamin D normal group (≥30 ng/ml)[14].

The quantitative estimation of Fasting Blood Sugar, Total Cholesterol, Low-Density Lipoprotein, High-Density Lipoprotein, Triglycerides, Lipoprotein-associated phospholipase- A2 (LpPLA2), Alpha-1-antitrypsin (AAT) and High sensitive C - Reactive Protein (hs-CRP) were analyzed using suitable kits[15]. The data obtained from the study were spread to an Excel sheet and statistical analysis was performed by using SPSS software (version 21.0). The values were expressed in mean ± standard deviation (SD). The demographic data such as age and gender were analyzed by various descriptive statistics. Statistical analysis was done using One-way ANOVA to find out significance in biochemical parameters between all the groups (Test-1, 2, 3, and the control group). P-value less than 0.05 ($p<0.05$) was considered statistically significant and a p-value less than 0.01 ($p<0.01$) was considered statistically highly significant[16].

RESULTS

Among 1050 subjects who showed the willingness to participate in the study, 376 subjects were diagnosed with Diabetic Dyslipidemia and selected as test population (n=376). The control group was healthy people with normal Vitamin D status (n=120). Vitamin-D status of both control and test group subjects have been statistically analyzed by students t-test and the result is depicted in Table No. 1. The levels of Vitamin D in the control group were significantly higher when compared to the test population. The t-test of the Vitamin D status shows a statistically highly significant difference ($p<0.01$) between the test and control group population. A graphical representation of the vitamin D status of both groups is given in Picture No.1. The control group was healthy people with normal Vitamin D status (n=120). Among 376 diabetic hyperlipidemic participants enrolled in the study were further subcategorized, based on the level of Vitamin D status. The study participant was further grouped into four, one test group and three diabetic dyslipidemic sub-groups with different vitamin D status

· Control = Normal control people. **(n=120)**
· Test-1 = Diabetic Dyslipidemia patients with Vitamin D deficient status (< 20 ng/mL). **(n=129)**
· Test-2 = Diabetic Dyslipidemia patients with Vitamin D insufficient status (20–29 ng/mL). **(n=142)**
· Test-3 = Diabetic Dyslipidemia patients with Vitamin D normal status (≥30 ng/mL). **(n=105)**

The age and gender of the control and test groups were found to be comparable and matched. The distribution of the study groups based on age and gender is given in Table No.2. The mean age of the control group was observed to be 49.6 years, and that of test Test-1, 2, and 3 were 46, 53.5 and 54 years respectively. The control group had a total of 120 subjects with 62 males and 58 females. The Test-1 group had 129 samples with 41 males and 88 females, Test-2 had 142 participants with 81 males, and 61 females, and the Test-3 group had 105 participants with 64 males and 41 females.

The distribution of study groups based on fasting blood sugar level and lipid profile are given in Table No.3. All the parameters had a statistically significant difference between the groups. The significance in biochemical markers of diabetic dyslipidemia such as Fasting blood sugar, Total Cholesterol, Low-Density Lipoprotein, High-Density Lipoprotein, and Triglycerides was statistically analyzed by One Way ANOVA. The mean± SD of Fasting blood sugar level in the control group was 86.25 ± 6.71 and that of test groups, Test-1, 2, and 3 were 146.67 ± 36.53, 131.82 ± 31.85, and 111.22 ± 24.56 respectively. The mean± SD of Total Cholesterol in the control group was 185.29 ± 13.78 and that of test groups, Test-1, 2, and 3 were 263.14 ± 37.2, 243.69 ± 35.88 and 252.38 ± 30.04 respectively. The mean± SD of Low-Density Lipoprotein in the control group was 109.25 ± 13.12 and that of test groups, Test-1, 2, and 3 were 141.94 ± 22.55, 140.15 ± 22.07, and 155.31 ± 22.1 respectively. The mean ± SD of High-Density Lipoprotein of the control group was 48.58 ± 6.13 and that of test Test-1, 2, and 3 were 41.12 ± 4.24, 43.42 ± 5.62 and 44.51 ± 6.03 respectively. The mean± SD of Triglyceride levels in the control group was 103.73 ± 17.18 and that of test Test-1, 2, and 3 were 132.27 ± 34.53, 136.3 ± 40.59, and 168.64 ± 26.64 respectively. The summary of glycemic level and lipid profile of all participants is graphically represented in Figure No.2.

The statistical significance of inflammatory markers such as Lipoprotein-associated phospholipase- A2 (LpPLA2), Alpha-1-antitrypsin (AAT), and High sensitive C - reactive protein (hs-CRP) between different groups was analyzed by One Way ANOVA. The difference between inflammatory biomarkers (Lp-PLA2, AAT, and HsCRP) of different groups was statistically significant ($p<0.05$) between the control and test groups. The mean± SD of Lipoprotein-associated phospholipase- A2 (LpPLA2) levels in the control group were found to be 395.07 ± 69.93 and that of test Test-1, 2 and 3 were 598.76 ± 44.74, 535.14 ± 67.97, and 542.21 ± 77.4 respectively. The mean± SD of Alpha-1- antitrypsin (AAT) levels in of control group was 0.68 ± 0.43 and that of test Test-1, 2 and 3 were 2.02 ± 0.94, 1.54 ± 0.97and 0.95 ± 0.61 respectively. The mean± SD of High sensitive C - reactive protein (hs-CRP) of the control group was 63.85±6.63 and that of test Test-1, 2, and 3 were 72.92±9.49, 72.73±10.28, and 71.24±10.17 respectively. The summary of the above result is graphically given in figure No.3.

DISCUSSION

The incidence of Diabetic Dyslipidemia was found similar in all test groups, and slightly more normal vitamin D status. This may be due to the better social and economic status of the population. The levels of Vitamin D in the control group were significantly higher when compared to the test population. The mean age of participants of all groups together was 50 years and the incidence of diabetic dyslipidemia was found slightly higher in males compared with females. Diabetic dyslipidemic patients with normal vitamin D status exhibited less value for Fasting blood sugar (111.22 ± 24.56 mg/dl) when compare with Vitamin D deficient and insufficient groups. That implies that vitamin D has a strong positive association with pancreatic function or insulin sensitivity. Also, the group with normal vitamin d status has a better value for HDL cholesterol (44.51 ± 6.03 mg/dl) when compared with the other 2 test groups, which points to an

assumption that a normal level of vitamin D could have some cardioprotective potential. The Vitamin D deficient group, Test-1, was observed to have a maximum level of inflammatory biomarkers when compared to other test groups as well as the control group. Since the increased level of inflammatory mediators has a negative association with cardiovascular disease, as they make damage to vascular endothelial lining, a normal status of vitamin D might reduce future CVD in diabetic dyslipidemic patients. So clinicians shall prescribe vitamin D supplementation for diabetic dyslipidemic patients in order to maintain cardiovascular health in a better way and thus improve quality of life.

TABLES & FIGURES

Table No. 1: Distribution of test and control groups based on Vitamin-D status

Parameters	Groups		t-test	p-value
	Control (n=120)	Test Group (n=376)		
Vitamin D (ng/ml)	32.18 ± 2.24	23.25 ± 7.69	12.54	<0.01**
Statistical test used: t-test. ** p<0.01 Highly significant, *P<0.05 Significant				

Table No. 2: Age And Gender-wise Distribution Of Different Study Groups Based On Vitamin D Status.

Parameters		Control (n=120)	Test-1 (n=129)	Test -2 (n=142)	Test -3 (n=105)
Mean Age (Years)		49.6	46	53.5	54
Gender	Male	62	41	81	64
	Female	58	88	61	41
Control = Normal control people. Test-1 = Diabetic Dyslipidemia patients with Vitamin D deficiency, Test-2 = Diabetic Dyslipidemia patients with Vitamin D insufficiency & Test-3 = Diabetic Dyslipidemia patients with normal Vitamin D status					

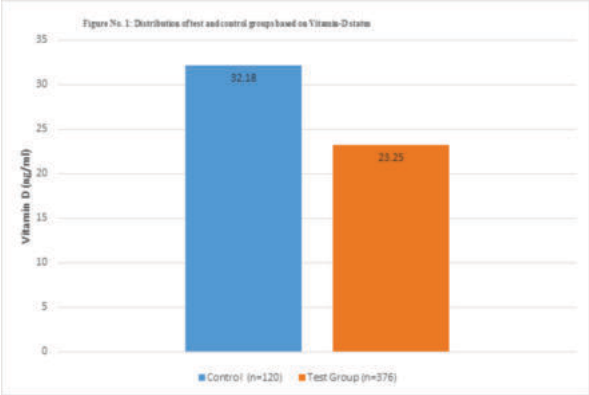
Table No. 3: Distribution Of Study Groups Based On Biochemical Markers Of Diabetic Dyslipidemia

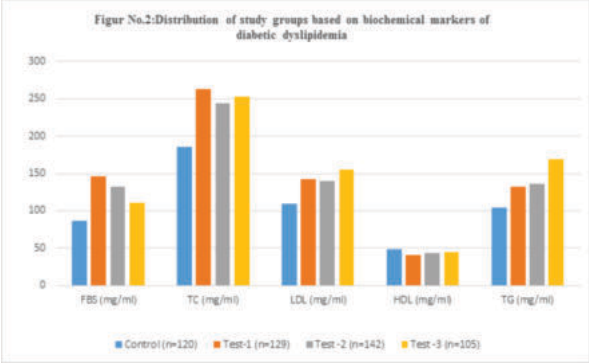
Parameters	Groups				F Value	p-Value
	Control (n=120)	Test-1 (n=129)	Test -2 (n=142)	Test -3 (n=105)		
FBS (mg/ml)	86.25 ± 6.71	146.67 ± 36.53	131.82 ± 31.85	111.22 ± 24.56	110.061	<0.01**
TC (mg/ml)	185.29 ± 13.78	263.14 ± 37.2	243.69 ± 35.88	252.38 ± 30.04	151.918	<0.01**
LDL (mg/ml)	109.25 ± 13.12	141.94 ± 22.55	140.15 ± 22.07	155.31 ± 22.1	105.53	<0.01**
HDL (mg/ml)	48.58 ± 6.13	41.12 ± 4.24	43.42 ± 5.62	44.51 ± 6.03	39.547	<0.01**
TG (mg/ml)	103.73 ± 17.18	132.27 ± 34.53	136.3 ± 40.59	168.64 ± 26.64	78.731	<0.01**
Statistical test used: One way ANOVA. *P<0.05 Significant & ** p<0.01 Highly significant FBS = Fasting blood sugar, TC = Total Cholesterol, LDL = Low Density Lipoprotein, HDL = High Density Lipoprotein & TG = Triglycerides.						
Control = Normal control people. Test-1 = Diabetic Dyslipidemia patients with Vitamin D deficiency, Test-2 = Diabetic Dyslipidemia patients with Vitamin D insufficiency & Test-3 = Diabetic Dyslipidemia patients with normal Vitamin D status						

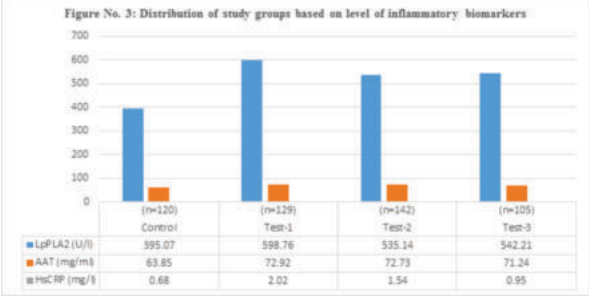
Table No. 4: Distribution of study groups based on level of inflammatory biomarkers

Parameters	Groups				F Value	P Value
	Control (n=120)	Test-1 (n=129)	Test-2 (n=142)	Test-3 (n=105)		
LpPLA2 (U/l)	395.07 ± 69.93	598.76 ± 44.74	535.14 ± 67.97	542.21 ± 77.4	214.033	<0.01**
AAT (mg/ml)	63.85±6.63	72.92±9.49	72.73±10.28	71.24±10.17	39.70	<0.00**
HsCRP (mg/l)	0.68 ± 0.43	2.02 ± 0.94	1.54 ± 0.97	0.95 ± 0.61	71.191	<0.01**
Test used: One way ANOVA *P<0.05 Significant, ** p<0.01 highly significant. HsCRP = High sensitive C-reactive protein. Lp-PLA2 = Lipoprotein associated phospholipase-A2. AAT = Alpha-1- antitrypsin						
Control = Normal control people. Test-1 = Diabetic Dyslipidemia patients with Vitamin D deficiency, Test-2 = Diabetic Dyslipidemia patients with Vitamin D insufficiency & Test-3 = Diabetic Dyslipidemia patients with normal Vitamin D status						

Figures







SUMMARY AND CONCLUSION

Diabetic Dyslipidemia is considered one of the leading causes of developing cardiovascular diseases and which might cause morbidities and mortalities across the world. It has been observed that the incidence of Diabetic Dyslipidemia is kept on increasing high in the Kerala population and Vitamin D deficiency may be associated with an increased risk of CVD including Diabetic Dyslipidemia. The cross-sectional study was carried out to identify if there were any association plays between Vitamin D status and inflammatory biomarkers in the Kerala population with Diabetic Dyslipidemia. The role of specific inflammatory biomarkers for the early prediction of CVD among Diabetic Dyslipidemia patients with different Vitamin-D levels was well studied in a rural population of the Malabar region of Kerala. The result of the study showed that in group Test-1, the participants with Vitamin D deficiency exhibited the highest level of inflammatory biomarkers which points out that Vitamin D status and inflammatory biomarkers are highly associated with the development of Diabetic Dyslipidemia. A randomized controlled trial with Vitamin D supplementation among Diabetic Dyslipidemia patients needs to be conducted in order to establish the role of Vitamin D in cardiovascular diseases. A prospective-follow up study in the future shall be highly recommended to establish the role of Vitamin D in the pathogenesis of CVD. Also, a routine Vitamin D checkup together with routine blood sugar and lipid profile is highly advisable among individuals who are at high risk of cardiovascular diseases. Even so, appropriate drug therapy together with vitamin D supplementation is a must in order to reduce

future cardiovascular and cerebrovascular complications in diabetic dyslipidemic patients.

REFERENCES

1. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. The Journal of Clinical Endocrinology & Metabolism. 2004 Jun 1;89 (6):2595-600.
2. Bardini G, Rotella CM, Giannini S. Dyslipidemia and diabetes: reciprocal impact of impaired lipid metabolism and Beta-cell dysfunction on micro-and macrovascular complications. The review of diabetic studies: RDS. 2012;9(2-3):82.
3. Kwiterovich Jr PO. The metabolic pathways of high-density lipoprotein, low-density lipoprotein, and triglycerides: a current review. The American journal of cardiology. 2000 Dec 21;86 (12):5-10.
4. Tóth PP, Potter D, Ming EE. Prevalence of lipid abnormalities in the united states: the national health and nutrition examination survey 2003–2006. Journal of clinical lipidology. 2012 Jul 1;6(4):325-30.
5. Wu L, Parhofer KG. Diabetic dyslipidemia. Metabolism. 2014 Dec 1;63(12):1469-79.
6. Patel P, Abate N. Body fat distribution and insulin resistance. Nutrients. 2013 Jun;5(6):2019-27.
7. Mozos I, Marginean O. Links between vitamin D deficiency and cardiovascular diseases. BioMed research international. 2015 Oct;2015.
8. Kaur R, Kaur M, Singh J. Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: molecular insights and therapeutic strategies. Cardiovascular Diabetology. 2018 Dec;17(1):1-7.
9. Georgiou AC, Crielaard W, Armenis I, de Vries R, van der Waal SV. Apical periodontitis is associated with elevated concentrations of inflammatory mediators in peripheral blood: a systematic review and meta-analysis. Journal of endodontics. 2019 Nov 1;45(11):1279-95.
10. Kontush A, Chapman MJ. High-density lipoproteins: structure, metabolism, function and therapeutics. John Wiley & Sons; 2011 Nov 30.
11. Di Renzo L, Cinelli G, Dri M, Gualtieri P, Attinà A, Leggeri C, Cenname G, Esposito E, Pujia A, Chiricolo G, Salimei C. Mediterranean personalized diet combined with physical activity therapy for the prevention of cardiovascular diseases in Italian women. Nutrients. 2020 Nov;12(11):3456.
12. Herrera MC, Subhan FB, Chan CB. Dietary patterns and cardiovascular disease risk in people with type 2 diabetes. Current obesity reports. 2017 Dec;6(4):405-13.
13. Chehimi M, Eljaafari A. Beneficial effects of fasting on white adipose tissue inflammation and metabolic syndrome in obese subjects: review. Endocrinology & Metabolism International Journal. 2017;4(6).
14. Gotsman I, Shauer A, Zwas DR, Hellman Y, Keren A, Lotan C, Admon D. Vitamin D deficiency is a predictor of reduced survival in patients with heart failure; vitamin D supplementation improves outcome. European journal of heart failure. 2012 Apr;14(4):357-66.
15. Avgeris M, Kokkinopoulou I, Maratou E, Mitrou P, Boutati E, Scorilas A, Fragoulis EG, Christodoulou MI. Blood-based analysis of 84 microRNAs identifies molecules deregulated in individuals with type-2 diabetes, risk factors for the disease or metabolic syndrome. Diabetes research and clinical practice. 2020 Jun 1;164:108187.
16. Bhattacharyya S, Jain N, Verma H, Sharma K. A Cross-sectional Study to Assess Neutrophil Lymphocyte Ratio as a Predictor of Microvascular Complications in Type 2 Diabetes Mellitus Patients. Journal of Clinical & Diagnostic Research. 2021 Aug 1;15(8).