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A cross sectional study on the relationship between serum calcium level and severity of dengue fever

Dr Raveen Titus Thomas
Senior Resident, Department of General Medicine, Government Medical College Kottayam, Kerala

Dr P Sanghamithra
Professor, Department of General Medicine, Government Medical College Kottayam, Kerala
Email: sangamithradr60@gmail.com

***Abstract*---**Background: Dengue is the most rapidly spreading mosquito borne disease in the world. An estimated 50 million dengue infections occur annually. It is caused by a small, single stranded RNA virus belonging to the genus Flavivirus, family Flaviviridae and is divided into four serotypes (DEN-1 to DEN-4). These various serotypes are transmitted to humans through the bites of infected Aedes mosquitoes, primarily Aedes aegypti. Materials & Methods: A cross sectional study was conducted on 72 patients with dengue fever, for a period of 12 months in the medicine wards of Government Medical College, Kottayam. All cases fulfilling the inclusion criteria during the study period were included after SRC and IRB approval. Results: Mean age of patients in the present study was 32.6 in dengue fever and 32.1 in severe dengue. There was no significant correlation between age and serum corrected calcium level (Pearson correlation coefficient – 0.074, p value 0.539). In patients with dengue fever, mean hematocrit was 39.2 %, while in those with severe dengue, it was 40.5 %. There was no significant correlation between hematocrit and serum corrected calcium level (Pearson correlation coefficient – 0.147, p value 0.219). The area under the receiver operating characteristic (ROC) curve was 0.962, indicating a high accuracy for serum corrected calcium level in detecting severe dengue. Conclusion: Serum corrected calcium levels were found to be significantly lower in severe dengue when compared to dengue fever. Serum corrected calcium levels correlate with severity of dengue fever and can be used as a prognostic marker for dengue fever.

***Keywords*---**Serum calcium level, Severity of dengue fever, A cross sectional study.

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Introduction

Dengue is the most rapidly spreading mosquito borne disease in the world. An estimated 50 million dengue infections occur annually. It is caused by a small, single stranded RNA virus belonging to the genus Flavivirus, family Flaviviridae and is divided into four serotypes (DEN-1 to DEN-4).[1] These various serotypes are transmitted to humans through the bites of infected Aedes mosquitoes, primarily Aedes aegypti. As per the Dengue guidelines given by WHO in 2009, Dengue fever has been classified into: Dengue fever with no warning signs, Dengue fever with warning signs and Severe dengue.[2] Although the WHO severity classification of dengue is used here, certain studies show that only WHO criteria of severity may not be sufficient to categorize and treat the patients of dengue, especially those with complications. [3,4]

Calcium ion plays an important role in normal cell signaling and cellular function. Serum calcium level is divided into three fractions:

I. Ionized (free) calcium – 50 % of the total
II. Protein bound (albumin) – 40 % of the total
III. Anion bound (lactate, phosphate) – 10 % of the total

Hypocalcemia is defined as a serum corrected calcium level less than 8.5 mg/dL. Hypocalcemia has been described in dengue fever and is more marked in severe dengue – the precise mechanisms of which are not clearly defined. The probable causes for hypocalcemia that have been proposed include – reduced Na^+-K^+ ATPase activity, acquired parathyroid hormone deficiency, renal one-alpha hydroxylase insufficiency, reduced dietary vitamin D and calcium intake. [5]

Various studies have shown serum calcium to be significantly lower in patients with severe dengue, hence serum corrected calcium level could be used as an important biomarker to assess severity of dengue fever and plan management accordingly. This study aims to correlate the severity of dengue fever with the level of serum corrected calcium.

Objectives

To estimate the relationship between serum, mean corrected calcium level and the severity of dengue fever in patients admitted in medicine wards in Government Medical College, Kottayam.

Materials & Methods

After obtaining due ethical clearance from the Ethical Committee of Government Medical College, Kottayam and Department of General Medicine, a cross sectional study was conducted on 72 patients with dengue fever, for a period of 12 months in the medicine wards of Government Medical College, Kottayam. All cases fulfilling the inclusion criteria during the study period were included after SRC and IRB approval. Informed consent was obtained from the patients.

Sample Size

Using the values of mean and standard deviation from the study done in BIRDEM Hospital, Dhaka by Uddin et al. with 95% confidence interval and 95% power, the minimum sample size calculated was 72 dengue fever patients. [6]
Sample size = 72

Inclusion criteria
All patients admitted in Medicine wards with serologically confirmed Dengue fever (Dengue NS1/ IgM positive) in the age group 18-60 years.

Exclusion Criteria
Patients on calcium supplements or drugs affecting calcium homeostasis.

Study Procedure
A detailed structured proforma was completed and according to the history, examination findings and laboratory investigations, patients were classified into 2 groups: Dengue fever (with / without warning signs) and severe dengue. Serum corrected calcium level was assessed in these patients within 24 hours of admission using electrochemistry (calcium sensing electrode) and then correlated with the group of dengue fever the patient falls into.

Data management and statistical analysis

Data was coded and entered in Microsoft Excel and analyzed using IBM SPSS software Version 22. The significance of the difference between means of the two groups was tested using student's t-test. A 'p' value less than 0.05 was considered significant. Charts and tables were generated with the help of Microsoft Excel.

Results

A total of 72 patients were dengue fever were included in this study. The diagnosis of dengue fever was confirmed with Dengue NS1 antigen or Dengue IgM ELISA.

Table 1 Age & gender distribution of dengue patients

Age group distribution of dengue patients		
Age in years	Frequency	Percentage
≤20	12	16.7
21 – 30	28	38.9
31 – 40	15	20.8
41 – 50	10	13.9
>50	7	9.7
Total	72	100
Gender distribution of dengue patients		
Sex	Frequency	Percentage
Male	51	70.8
Female	21	29.2
Total	72	100

The mean age of the study participants was 30 years. The minimum age was 18 years and maximum age was 60 years. Majority of the study population, 28 (38.9 %) were in the age group of 21-30 years. 51 (70.8 %) of the study population were males.

Table 2 showing the age, serum corrected calcium, Hematocrit, platelet count (on admission) & SGPT vs severity of dengue fever

Age vs severity of dengue fever					
		Age (in years)			
Dengue Fever	N	Mean	S.D	t	P
Dengue fever	39	32.6	11		
Severe dengue fever	33	32.1	14	0.167	0.868
Serum corrected calcium vs severity of dengue fever					
Dengue Fever	N	Serum Corrected Calcium (mg/dL)		t	P
		Mean	S.D		
Dengue fever	39	8.9	0.4		
Severe dengue fever	33	8.2	0.2	9.305	<0.001
Hematocrit vs severity of dengue fever					
		Hematocrit			
Dengue Fever	N	Mean	S.D	t	P
Dengue fever	39	39.7	2.7		
Severe dengue fever	33	40.5	5.9	0.839	0.404
Platelet count (on admission) vs severity of dengue fever					
		Platelet count on admission (/mm^3)			
Dengue Fever	N	Mean	S.D	t	P
Dengue fever	39	81923.1	36434.1		
Severe dengue fever	33	39606.1	34734.7	5.016	<0.001
SGPT vs severity of dengue fever					
Dengue Fever	N	SGPT (IU/L)		t	P
		Mean	S.D		
Yes	39	89	59.2	4.894	<0.001
No	33	460.5	470.3		

39 patients of the 72 (54.2 %) belonged to the dengue fever, while 33 (45.8 %) were classified as having severe dengue. Of the severe dengue patients, 4 patients (12 %) developed severe transaminitis (SGPT > 1000 IU/L) and 2 patients (6 %) died during the course of the illness.

Serum corrected calcium level was measured during the first 24 hours of admission. The mean corrected calcium level in severe dengue patients (8.2 +/- 0.4 mg/dL) was significantly lower than that of dengue patients (8.9 +/- 0.2 mg/dL) (p value < 0.001)

There was a statistically significant association between serum corrected calcium level and severity of dengue fever. There was no significant association between age and severity of dengue fever (p velue 0.868) or between hematocrit and severity of dengue fever (p value 0.404). There was a positive correlation (Pearson correlation coefficient + 0.655) between platelet count on admission and serum corrected calcium level (p < 0.001). There was a negative correlation (Pearson correlation coefficient - 0.57) between SGPT and serum corrected calcium level (p < 0.001).

Table 3 Correlation of Serum corrected calcium with individual variables

Correlation of Serum Corrected Calcium with other variables	Pearson Correlation coefficient	P
Age	-0.074	0.539
Hematocrit	-0.147	0.219
Platelet count on admission	0.655**	<0.001
SGPT	-0.570**	<0.001

There was no significant correlation between age and serum corrected calcium level (Pearson correlation coefficient – 0.074, p value 0.539). There was no significant correlation between hematocrit and serum corrected calcium level (Pearson correlation coefficient – 0.147, p value 0.219).

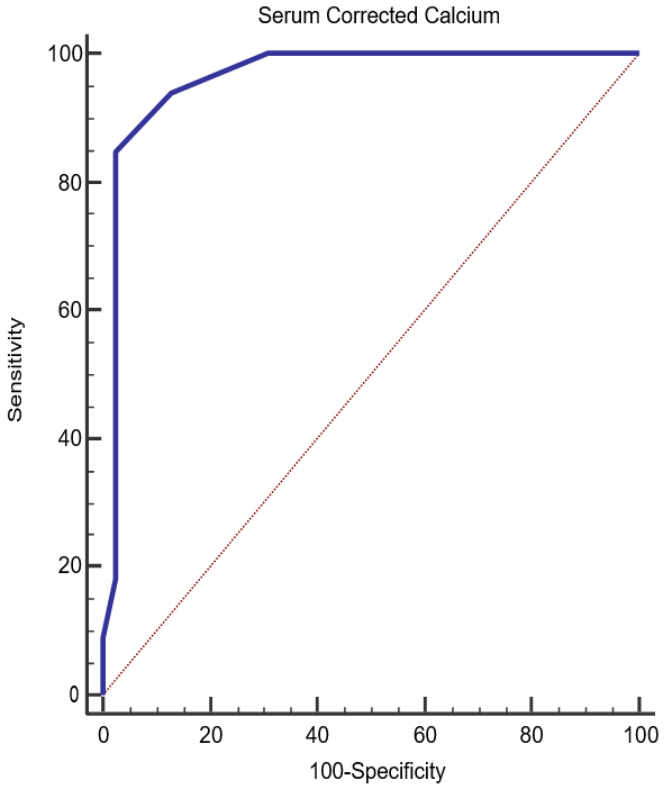

Figure 1 Receiver operating characteristic (ROC) curve of serum corrected calcium

The area under the receiver operating characteristic (ROC) curve was 0.962, indicating a high accuracy for serum corrected calcium level in detecting severe dengue.

Table 4 Analysis of ROC curve of serum corrected calcium

Dengue fever	
Sample size	72
Severe Dengue fever	33
Dengue fever	39
Area under the ROC curve (AUC)	0.962
Standard Error	0.024
95% Confidence interval	0.888 to 0.993
Youden index J	0.8228
Optimum cut off	≤8.4
Sensitivity	84.85
Specificity	97.44

The optimum cut off for serum corrected calcium was calculated as < 8.4 mg/dL with a sensitivity of 84.85, specificity of 97.44, positive likelihood ratio of 33.09,

negative likelihood ratio of 0.16, positive predictive value of 96.6, and negative predictive value of 88.4.

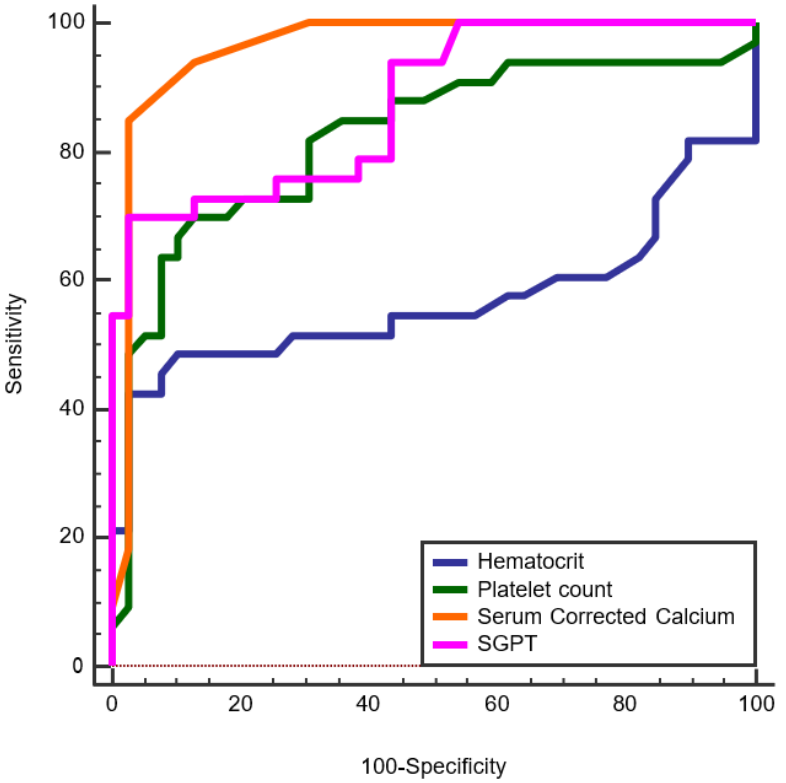

Figure 2 Receiver operating characteristic (ROC) curve of hematocrit, platelet count, serum corrected calcium and SGPT

The ROC curves of Platelet count (Area under the curve – 0.822) and SGPT (Area under the curve – 0.875) also showed high diagnostic accuracies in detecting severe dengue cases.

Discussion

In this study, a total of 72 patients were studied over a period of 12 months, of which 70.8 % were males and majority were in the age group of 21-30 years [38.9 %] and a mean age of 30 years [range 18-60 years]. A similar study was done by Uddin et al in BIRDEM hospital on 84 patients, of which 61.9 % were males, with age ranging between 26 to 63 years. [6] An observational prospective was study done in Amrita Institute of Medical Sciences, Kochi by M.R. Murugan, M. Gopalakrishna Pillai in 2015, on 68 patients, of which 60 % were males, with a mean age of 32 years [range 18 – 50 years]. [7]

Association between serum corrected calcium level and severity of dengue fever

In this study, serum corrected calcium level was found to be significantly lower in severe dengue (8.2 +/- 0.2 mg/dL) as compared to patients with dengue fever (8.9 +/- 0.4 mg/dL), indicating a relationship between serum calcium level and severity of dengue fever. The study conducted by Uddin et al in Ibrahim Medical College and BIRDEM Hospital, Dhaka, Bangladesh in 2002, on 84 dengue patients, showed a mean serum calcium level of 8.69 +/- 0.68 mg/dL in dengue fever, and lower (7.83 +/- 0.66 mg/dL) in dengue hemorrhagic fever– III.[6] The study reported that a significant number of patients with dengue fever had hypocalcemia (some were symptomatic) and concluded that mean serum calcium levels correlated with the severity of dengue fever.

An observational prospective study done in Amrita Institute of Medical Sciences, Kochi by M.R. Murugan, M. Gopalakrishna Pillai in 2015, on 68 patients with dengue fever showed that serum calcium levels were significantly lower in patients with severe dengue (8.48 +/- 0.43 mg/dL) when compared to dengue fever (7.83 +/- 0.42 mg/dL).[7] They concluded that serum calcium can be used as a marker of severity of dengue fever. Platelet counts on admission in this study also showed significantly lower levels in those with severe dengue [39,606] than in those with dengue fever [81,923].

In the study by Uddin et al, lower platelet counts were seen in severe dengue [24,750] (Dengue Hemorrhagic fever Class III as per old WHO classification) than in dengue hemorrhagic fever class I [43,719], suggesting that lower platelet counts were seen in severe dengue [6]. Similarly, in a study done by Aditya Mahajan et al. in A J Institute of Medical Sciences, platelet count in severe dengue [17,791] was significantly lower than that in dengue fever with warning signs [56,050] and in dengue fever without warning signs [1,18,166] [8].

Serum glutamic pyruvic transaminase (SGPT) or Alanine transaminase (ALT) in this study was found to be higher in severe dengue [460.5] when compared to dengue fever [89]. In the study by Uddin et al., SGPT was also found to be higher in severe dengue fever [582] (Dengue hemorrhagic fever class III as per old WHO classification) when compared to dengue fever [140]. [6]

Similarly, the study by Aditya Mahajan et al. also showed higher levels of SGPT in severe dengue [420], when compared with dengue fever with warning signs [129] and dengue fever without warning signs [43], suggesting that higher SGPT values were seen in severe dengue. [8]

Conclusion

Serum corrected calcium levels were found to be significantly lower in severe dengue when compared to dengue fever. Serum corrected calcium levels correlate with severity of dengue fever and can be used as a prognostic marker for dengue fever.

Limitations

The days on which serum calcium assays were done could not be standardized as patients presented on different days after symptom onset.

References

1. Chambers TJ, Hahn CS, Galler R, Rice CM (1990) Flavivirus genome organization, expression, and replication. Annu Rev Microbiol 44: 649-688.
2. World Health Organization. Dengue guidelines for diagnosis, prevention, treatment and control, New edition. Geneva: WHO; 2009.
3. Balmaseda A et al. Assessment of the World Health Organization scheme for classification of dengue severity in Nicaragua. American Journal of Tropical Medicine and Hygiene, 2005, 73:1059–1062.
4. Phuong CX, Nhan NT, Kneen R, et al. Clinical diagnosis and assessment of severity of Confirmed dengue infections in Vietnamese children: is the World Health Organization classification system helpful? Am J Trop Med Hyg 2004; 70(2): 172-9.
5. Zaloga GP, Chernow B. The multifactorial basis for hypocalcemia during sepsis. Studies of the parathyroid hormone vitamin D axis. Ann Intern Med 1987;107:36 41.
6. Uddin KN, Musa AKM, Haque Wasim Md, Sarker RSC, Ahmed AKMS. A follow up on biochemical parameters in dengue patients attending BIRDEM hospital. Ibrahim Med Coll. J. 2008; 2(1): 25–7.
7. Murugan M.R., M. Gopalakrishna Pillai. Hypocalcemia: A Marker of Severe Dengue. Amrita Journal of Medicine. 2016; 12(1):25-29.
8. Mahajan A, Shetty IN. A Study on Correlation between Serum Ionized Calcium and Severity of Dengue Infection. IOSR J Dent Med Sci. 2019 Mar;18(3):44–52.

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Anemia in patients with type 2 diabetes mellitus and its association with the microvascular complications

Dr. Anjaly. V.P
Senior Resident, Department of General Medicine, Government Medical College Kottayam, Kerala

Dr. Sanghamithra. P
Professor, Department of General Medicine, Government Medical College Kottayam, Kerala

Dr. Lijo. K. Mathew
Associate Professor, Department of Genereal Medicine, Government Medical College Kottayam, Kerala
*Corresponding author

***Abstract*---**Introduction: Anemia is more commonly seen in persons with diabetes than in persons without diabetes. There is high risk of microvascular and macrovascular complications in diabetes. Anemia is strongly associated with an increased risk of diabetic complications including nephropathy, retinopathy, neuropathy and heart failure. Aim of the study: To assess the correlation between anemia and the microvascular complications of diabetes mellitus. Materials and Methods: This was a prospective, cross sectional, hospital based study conducted in department of General Medicine at Government Medical College, Kottayam, over a period of one year. Two hundred consecutive cases of diabetes mellitus with anemia were studied for microvascular complications like diabetic neuropathy, nephropathy, retinopathy. Results: Total 200 patients were enrolled with 116 (58%) male and 84 (42%) female patients and male to female ratio was 1.3:1. The patient age ranged from 41 to 86 years and mean age was 60.78±9.38 years. Diabetic neuropathy was absent in 38% and present in 62%. Proliferative and nonproliferative diabetic retinopathy was seen in 18% and 53% cases respectively. Conclusion: Anemia has correlation with the microvascular complications of diabetes. With increasing duration of diabetes mellitus the hemoglobin levels fall progressively. Nonproliferative diabetic retinopathy is more commonly seen in patients with normocytic normochromic anemia. Neuropathy in diabetes is more commonly seen in patients with macrocytic anemia

and obesity is a risk factor for the development of neuropathy. Diabetic nephropathy is more commonly seen in patients with macrocytic anemia than other types of anemia.

***Keywords*---**Diabetes mellitus, Anemia, Microvascular complications, Diabetic retinopathy, Diabetic neuropathy, Diabetic nephropathy.

Introduction

Anemia is more commonly seen in persons with diabetes than in persons without diabetes. The characteristic features of type 2 Diabetes mellitus are hyperglycemia, insulin resistance and relative reduction in insulin secretion. The pathogenesis of diabetes mellitus is multifactorial and heterogeneous and has both genetic and environmental elements. In recent times the prevalence is seeing upward trend attributable to obesity and sedentary lifestyle and other various factors. [1] Insulin resistance and relative insulin deficiency, or a combination of both leads to type 2 diabetes [2] Diabetes is a global disease and India is no exception.

Anemia is strongly associated with an increased risk of diabetic complications including nephropathy, retinopathy, neuropathy and heart failure. It is well known that diabetes has a higher risk of microvascular and macrovascular complications. Anemia is commonly seen in patients with diabetes. The development and progression of microvascular and macrovascular complications in diabetes is associated with anemia. Anemia can falsely lead to low HbA1c levels, which in turn leads to under treatment of hyperglycemia, that further enables the progression of both microvascular and macrovascular diabetic complications. [3] Anemia can be considered as a marker of kidney damage, and anemia in diabetics has increased risk of mortality. To improve performance and quality of life of diabetics, the anemia has to be treated.

The relevance of the study is that anemia and diabetes are both common in general population and microvascular complications are associated with anemia. So early identification and correction of anemia in diabetes can delay the development of microvascular complications. In the present study, we tried to determine the correlation between anemia and the microvascular complications in diabetic patients, attending General Medicine department of our institute.

Aim of the study

To assess the correlation between anemia and the microvascular complications of diabetes mellitus

Materials and Methods

This was a prospective, cross sectional, hospital based study conducted in department of General Medicine at Government Medical College, Kottayam, over a period of one year from January 2019 to December2019. Two hundred

consecutive cases eligible for the study ie patients with diabetes mellitus and anemia attending the General Medicine department formed the study population.

Inclusion criteria:
Adult patients with diabetes mellitus and anemia based on WHO criteria

Exclusion criteria:
- Patients with chronic liver disease, chronic kidney disease stage 3,4,5 due to non diabetic causes, pregnant women, previous history of anemia, patients on treatment for anemia, patients with hematological malignancies were excluded.
- Other factors causing transient albuminuria like urinary tract infection and other acute infections were excluded.

Sample size
Required was of 164 cases based on existing prevalence and in the present study 200 cases were included.

Methodology

After getting Institutional Review Board clearance, 200 patients with Diabetes mellitus and anemia and who have consented to participate in the study were included. A study proforma was prepared and detailed history regarding symptoms, probable cause, examination and investigation findings were noted.

Clinical

examination including general examination and system examination was done. Fundus examination was done to look for diabetic retinopathy. Laboratory investigations included hemoglobin levels, mean corpuscular volume, RBC indices, platelet count and peripheral smear, renal function tests, blood sugar levels, and urine exam for albuminuria. Vibration sense, ankle jerk and Semmes Weinstein monofilament test was done to look for peripheral neuropathy. Vibration sensation was assessed using 128MHz tuning fork at the base of great toe. 5.07, 10 g Semmes Weinstein monofilament was used to assess the touch sensation. When the filament is placed on the patient's skin (usually the feet) when there is considerable loss of sensation, the patient will not be able to detect the presence of the filament at buckling.

For statistical analysis, the data was entered in Microsoft Excel and analyzed using SPSS software version 18. Quantitative variables were presented as mean and standard deviation. Categorical variables were summarized using frequency and percentage. Statistical testing of association of various factors were done using Pearson Chi square test, independent sample t test and ANOVA. p value less than 0.05 was considered as statistically significant.

Ethical consideration

The above mentioned study was conducted in this institute after obtaining the ethical clearance from the Institutional Review Board of Government Medical College, Kottayam and Department of General Medicine, Government Medical

College, Kottayam. Informed consent was obtained from all subjects who took part in the study. The privacy of the patient and confidentiality of the clinical data was maintained throughout the study. The information collected was used only for the purpose of this study. The study was done at no added cost to the patients, and did not affect the treatment given to them.

Data collection method

Diabetic patients with anemia were identified and the presence of different microvascular complications was studied. The correlation between different types of anemia and microvascular complications was identified.

Observations and Results

A total of 200 patients with diabetes mellitus and anemia were studied to determine if there is any link between anemia and microvascular complications in diabetics.

Demographic characteristics of the study population: Of the 200 patients enrolled in the study, there were 116 (58%) were male and 84 (42%) were female patients and the male to female ratio was 1.3:1. The patient age ranged from 41 to 86 years and the mean age of patients in this study was 60.78±9.38 years. Following is the age distribution: 17% of patients were between 41-50 years, 39.5% patients between 51-60 years, 27% patients between 61-70 years, 15% patients between 71-80 years and 1.5% patients were more than 80 years

BMI of the study population: Among the study population, the BMI of 51.5% patients was between 18.5-22.9, 39.5% of patients between 23-24.9 and 9% of patients more than 25.

Percentage of patients with blurring of vision: Among the 200 patients enrolled in the study, 42% gives history of blurring of vision and 58% doesn't have blurring of vision.

Distribution based on frothing of urine: Among the 200 patients enrolled for the study, 28% had frothing of urine and 72% didn't have frothing of urine.

Distribution based on symptoms of peripheral neuropathy: Among the 200 patients enrolled for the study, 30% had symptoms of peripheral neuropathy and 70% didn't have on symptoms of peripheral neuropathy.

Distribution based on duration of detected diabetes in years: Among the 200 patients enrolled in the study, 127 (63.5%) patients had detected duration of diabetes more than 5 years, (73) 36.5% patients had detected duration less than 5 years.

Distribution based on treatment of diabetes mellitus

Among the 200 patients enrolled for the study, 41.5% were on oral hypoglycemic agents, 10.5% were on insulin and 48% were on both OHA and insulin.

Distribution based on detected duration of diabetes and treatment modality: Among the study population with duration of detected diabetes less than 5 years, 87.5% were on OHA and 12.5% were on insulin containing regimen. Among the patients with duration of detected diabetes more than 5 years 62.5% were on OHA and 37.5% were on insulin containing regimen.

Distribution based on dietary habits of population: Among the 200 patients enrolled for the study 20% was vegetarian and 80% was taking mixed diet.

Distribution of Diabetic retinopathy: Among the 200 patients enrolled in the study, 18% had PDR, 53% had NPDR and the fundus examination of 29% was normal.

Distribution of vibration sensation: Among the 200 patients enrolled in the study, vibration sensation was present in 38% of patients and absent in 62%.

Distribution of ankle jerk: Among the 200 patients enrolled the study, ankle jerk was present in 68% and absent in 32 %.

Distribution of monofilament test: Among the 200 patients enrolled in the study, monofilament test was positive in 49% and negative in 51%.

Distribution of diabetic neuropathy: Among the 200 patients enrolled for the study, diabetic neuropathy was absent in 38% and present in 62%.

Distribution of hemoglobin levels in the population: Among the 200 patients enrolled for the study, the hemoglobin level was more than 11g/dL in 54% of patients, between 9.5g/dL to 10.9g/dL in 29.5%, between 8g/dL to 9.4g/dL in 16% and less than 7.9g/dL in 0.5%.

Distribution of anemia among males: Among the male population, 60.7% had hemoglobin between 13g/dL and 11g/dL.28.2% had hemoglobin between 9.5g/dL and 10.9g/dL.10.3% had hemoglobin between 8-9.4g/dL and 0.9% had hemoglobin less than 7.9g/dL.

Distribution of anemia among females: Among the female population 44.6% had haemoglobin between 12g/dL and 11g/dL.31.3% had haemoglobin between 9.5 and 10.9 g/dL.24.1% had haemoglobin between 8 and 9.4g/dL.

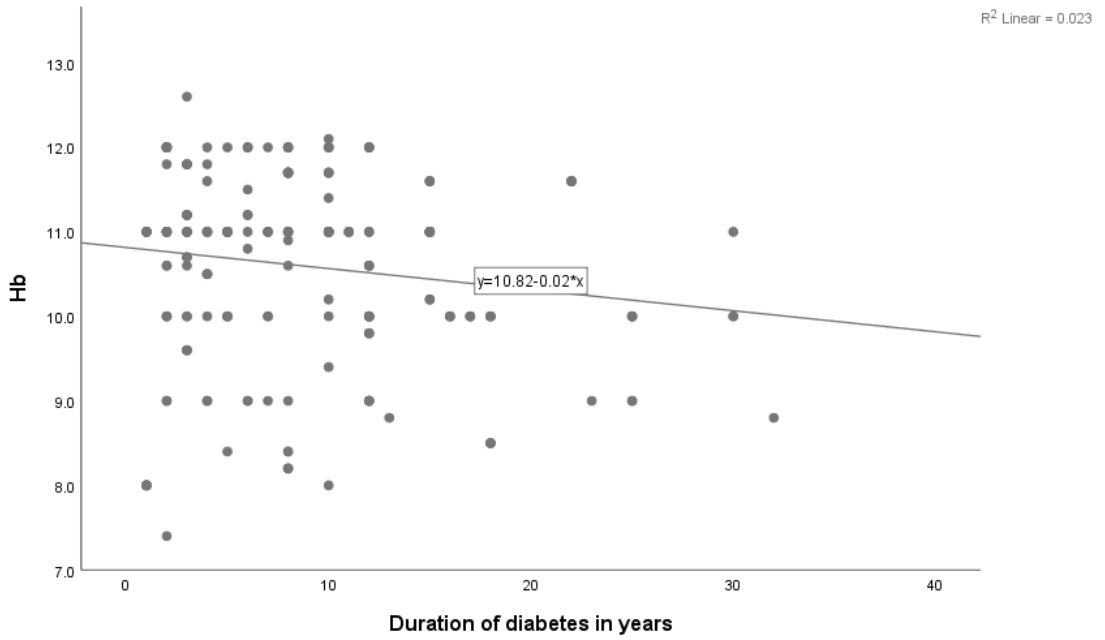

Figure 1 Scatter plot showing correlation between duration of detected diabetes and haemoglobin levels

Among the study population it was found that as the duration of diabetes increases the haemoglobin levels fall.

Distribution of MCV in the study population: Among the 200 patients enrolled, MCV was more than 100 in 15.5%, between 80 to 100 in 51% and less than 80 in 33.5%.

Distribution of peripheral smear results in the study population: Among the 200 patients enrolled for the study, 49% had normocytic normochromic anemia, 30.5% had microcytic hypochromic anemia and 20.5% had macrocytic anemia.

Distribution of serum creatinine levels in the population: Among the 200 patients enrolled for the study, serum creatinine levels was more than 1.2mg/dL in 38% and less than 1.2mg/dL in 62%.

Distribution of significant albuminuria in the study population: Among the 200 patients enrolled for the study, significant albuminuria was present in 78% and absent in 22% cases.

Distribution of Diabetic nephropathy in the study population: Among the 200 patients enrolled for the study**,** diabetic nephropathy was present in 78% and absent in 22%.

Demographic factors associated with Diabetic retinopathy

Table 1 Demographic factors associated with diabetic retinopathy

Variable	Diabetic retinopathy			P value
	PDR	NPDR	Normal	
Age				
Mean(SD)	68.11±10.48	62.01±8.25	53.97±5.49	<0.001*
Sex				
Male	21(17.9)	62(53)	34(29.1)	1.00
Female	15(18.1)	44(53)	24(28.9)	
BMI				
Mean	23.25±1.87	22.73±1.53	23.20±1.40	0.087

PDR, NPDR: Proliferative and Non-proliferative diabetic retinopathy

Among the demographic factors, patients with age group between 47 and 89 years had high risk of PDR, with a p value of 0.001 (statistically significant). Sex and BMI was not important in the development of Diabetic retinopathy

Duration of detected diabetes and relation with retinopathy

Among the 200 patients enrolled in the study, 127 (63.5%) patients had diabetes for more than 5 years, and 73 (36.5%) patients had detected duration of less than 5 years. In the former category, there were zero cases of proliferative diabetic retinopathy (PDR), 27 (37%) cases of nonproliferative diabetic retinopathy (NPDR) and 46 (63%) were normal. In the latter category, there were 36 (28.3%) cases PDR, 79(62.2%) cases of NPDR and only 12 (9.4%) cases were normal. The p value was 0.001 which was significant.

Table 2 Hemogram and relation with retinopathy

Hb	**PDR**	**NPDR**	**NORMAL**	P value
>11	6(5.6)	69(63.9)	33(30.6)	<0.001*
9.5-10.9	20(33.9)	24(40.7)	15(25.4)	
8-9.4	10(31.2)	30(40.6)	9(28.1)	
<7.9	0	0	1(100)	
MCV				
>100	12(38.7)	16(51.6)	3(9.7)	<0.001*
80-100	15(14.7)	62(60.8)	25(24.5)	
<80	9(13.4)	28(41.8)	30(44.8)	

Among the patients with diabetic retinopathy, 63.9% of patients with NPDR had a hemoglobin concentration of more than 11 g/dL. Of the patients with PDR, 33.9% had hemoglobin between 9.5 to 10.9g/dL. Also 60.8% of patients with NPDR had MCV between 80-100.Among the patients with PDR 38.7% had MCV more than 100. So NPDR was more commonly seen in patients with normocytic normochromic anemia than other types of anemia.

Distribution of Diabetic retinopathy in different types of anemia

Among the patients with normocytic normochromic anemia, 10 (10.2%) had PDR and 59 (60.2%) had NPDR. Among the patients with microcytic hypochromic anemia, 8 (13.1%) had PDR and 28 (45.9%) had NPDR. Among the patients with macrocytic anemia, 18 (43.9%) had PDR and 19 (46.3%) had NPDR. The p value was <0.001.

Table 3 Demographic factors and relation with diabetic neuropathy

Variable	Diabetic neuropathy		P value
	Present	Absent	
Age			
Mean(SD)	63.33±10.01	56.61±6.37	<0.001*
Sex			
Male	72(61.5)	45(38.5)	0.991
Female	52(62.7)	31(37.3)	
BMI			
Mean	22.87±1.69	23.11±1.36	0.029*

Based on the age distribution, patients between the age of 43.31 years and 83.35 years had diabetic neuropathy, with a significant p value of less than 0.001. No difference was noted among males and females with diabetic neuropathy (p value-0.991, statistically insignificant). Diabetic neuropathy was significantly associated with body mass index with a p value of 0.029.

Table 4 Hemogram and relation with neuropathy

Hb	Neuropathy present	Neuropathy absent	p value
>11	53(49.1)	55(50.9)	<0.001*
9.5-10.9	48(81.4)	11(18.6)	
8-9.4	23(71.9)	9(28.1)	
<7.9	0	1(100)	
MCV			
>100	27(87.1)	4(12.9)	<0.001*
80-100	52(51)	50(49)	
<80	45(67.2)	22(32.8)	

Among the patients with diabetic neuropathy, 81.4 % had hemoglobin between 9.5g/dL to 10.9g/dL and 71.9% had hemoglobin between 8g/dL to 9.4g/dL with a p-value of less than 0.001. Also 87.1% of patients with diabetic neuropathy had macrocytic anemia and 67.2% had microcytic anemia. The p value was less than 0.001 which was statistically significant.

Distribution of Diabetic neuropathy in different types of anemia on peripheral smear findings

Among the patients with normocytic normochromic anemia, diabetic neuropathy was present in 46 (46.9%) cases and absent in 52 (53.1%) cases. Among the patients with microcytic hypochromic anemia, neuropathy was present in 44 (72.1%) cases and absent in 17 (27.9%) cases. Among the patients with macrocytic anemia, diabetic neuropathy was present in 34 (82.9% cases and absent in 7 (17.1%) cases. So neuropathy in diabetes was more commonly seen in patients with macrocytic anemia than other types of anemia.

Table 5 Demographic factors and relation with diabetic nephropathy

Variable	Diabetic nephropathy		P value
	Present	Absent	
Age			
Mean(SD)	62.71±9.52	53.93±4.33	<0.001*
Sex			
Male	89(76.1)	28(23.9)	0.542
Female	67(80.7)	16(19.3)	
BMI			
Mean	22.94±1.58	23.02±1.57	0.758

Among those with nephropathy, patients between the age group of 43.67 years to 81.75 years were mostly affected with a p value of less than 0.001(statistically significant). No relation was seen between gender of the patients and BMI to diabetic nephropathy.

Duration of detected diabetes and relation with nephropathy

Among those with diabetic nephropathy, the detected duration of diabetes was more than 5 years in 119 (93.7%) cases and was absent in 8 (6.3%) cases and was statistically significant with a p value of less than 0.001. For those with

duration of less than 5 years of diabetes, nephropathy was present in 37 (50.7%) cases and absent in 36 (49.3%) cases.

Table 6 Hemogram and relation with nephropathy

Hb	Nephropathy present	Nephropathy absent	P value
>11	78(72.2)	30(27.8)	0.023*
9.5-10.9	49(83.1)	10(16.9)	
8-9.4	29(90.6)	3(9.4)	
<7.9	0	1(100)	
MCV			
>100	30(96.8)	1(3.2)	0.002*
80-100	80(78.4)	22(21.6)	
<80	46(68.7)	21(31.3)	

A total of 29 (90.6%) of patients with nephropathy had hemoglobin between 8 to 9.4, and 49 (83.1%) had hemoglobin between 9.5 to 10.9g/dL and 78 (72.2%) had hemoglobin more than 11g/dL which was statistically significant (p value less than 0.001). Also 30 (96.8%) of patients with nephropathy had MCV more than 100, and 80 (78.4%) cases had MCV between 80-100 and 46 (68.7%) had MCV less than 80 with a p value of less than 0.001.

Distribution of diabetic nephropathy in different types of anemia

Among the patients with normocytic normochromic anemia, diabetic nephropathy was present in 72 (73.5%) cases and absent in 26 (26.5%) cases. Among the patients with microcytic hypochromic anemia nephropathy was present in 45 (73.8%) cases and absent in 16 (26.2%) cases. Among the patients with macrocytic anemia, 39 (95.1%) had nephropathy and 2 (4.9%) didn't have nephropathy. P value was 0.012. So, diabetic nephropathy was more commonly seen in patients with macrocytic anemia than other types of anemia.

Discussion

In this cross sectional study, all the 200 patients were subjected to detailed history taking, examination and laboratory investigations. The mean age of patients in this study was 60.78±9.38 years. The maximum number of patients was in the age group of 51-60 years, which is 39.5% of cases and the next highest

number of patients were in the age group of 61-70 years (27%). Males contributed to 58% of the study population. In the study conducted by Rani et al [4] the mean age of the population was 56.32+ 10.02 years and 53.0% were men. In this study, microvascular complications were equally seen in both males and females, which was consistent with the study conducted by Rani et al. [4]In this study it was found that as duration of diabetes increases the hemoglobin levels fall which was consistent with the study conducted by Hosseini MS etal [5]. Normocytic normochromic anemia was the most frequent type of anemia in this study (49%), which was consistent with the study conducted by Hosseini MS et al [5]. In this study, nephropathy was seen in 78%, retinopathy was seen in 71% and neuropathy in 62%. So nephropathy was the most common microvascular complication in this study which was consistent with the study conducted by Hosseini MS et al. [5] When the demographic factors of the study population are compared, patients between the age group of 47 to 89 years had high risk of PDR with a p value of less than 0.001. Among the study population, 71% had diabetic retinopathy with 18% having PDR and 53% having NPDR. Among the 200 patients, 62% with detected duration of diabetes more than 5 years had NPDR. Among the patients with NPDR, 63.9% had hemoglobin less than 13g/dL and more than 11g/dL in males and less than 12g/dL and more than 11g/dL in females. Among patients with PDR, 33.9% had hemoglobin between 9.5 to 10.9 g/dL. NPDR was more commonly seen in patients with normocytic normochromic anemia than other types of anemia. In the study conducted by Hosseini MS et al [5], retinopathy was equally seen in patients with normocytic and microcytic anemia, the difference was observed may be because of the differences in sampling techniques. In the study conducted by Mohan et al [6], diabetic retinopathy is seen in 43% of patients. It was found that with increasing duration of diabetes and fall in haemoglobin levels there is high chance of developing diabetic retinopathy which was consistent with the study conducted by Mohan et al. [6] Among the patients between 25 and 74 years of age, diabetic retinopathy is one of the major cause for the development of impaired vision worldwide. [7] When diabetic neuropathy is taken into consideration, neuropathy was most commonly seen in patients between the age group of 43.31 to 83.33 years which was statistically significant (p value of less than 0.00).81.4% of patients with neuropathy had hemoglobin between 9.5 to 10.9 g/dL and 71.9% had hemoglobin between 8 to 9.4 g/dL (p value less than 0.001).Neuropathy in diabetes was more commonly seen in patients with macrocytic anemia than other types. In the study conducted by Hosseini MS etal [5], neuropathy was more commonly seen in patients with normocytic anemia. In the study conducted by Smith et al [8] patients with diabetic neuropathy had longer duration of diabetes and obesity is a risk factor for the development of diabetic neuropathy. In this study also it was found that high BMI is a risk factor for the development of neuropathy. Presence of significant albuminuria was considered as diabetic nephropathy. Patients with diabetic nephropathy were between the age group of 43.67 to 81.75 years which was statistically significant. The detected duration of diabetes was more than 5 years in 93.7% of patients with diabetic nephropathy (p value less than 0.001).It suggests that as the duration of diabetes increases, the chance of developing nephropathy increases.90.6% of patients with diabetic nephropathy had hemoglobin between 8 to 9.4 g/dL, 83.1% had hemoglobin between 9.5 to 10.9g/dL and 72.25 had hemoglobin more than 11 with a p value of less than 0.001. Diabetic nephropathy was more commonly seen in patients with

macrocytic anemia than other types of anemia. In the study conducted by Hosseini MS et al [5], nephropathy was more commonly seen in patients with normocytic anemia.

In the present study, no relation was seen between gender of the patients and BMI to diabetic nephropathy. Modifiable risk factors and ethnic disparity plays role in the development of diabetes mellitus. Data from the prospective Nurses' Health Study (NHS) collected over 20 years, it was found that the risk for developing diabetes in women, corrected for BMI, was increased for Asians, Hispanics, and African Americans (relative risk [RR] 2.26, 1.86, and 1.34, respectively) compared with whites. [9]

Altered glomerular hemodynamics results in activation of the renin-angiotensin aldosterone system (RAAS) and elevated glomerular filtration rate (GFR). [10] There is deposition of advanced glycosylation end products in basement membranes in diabetes due to oxidative stress and inflammation which are intimately related to each other. [11, 12]The findings of our study concur with the literature though there are some limitations in our study as follows:

Limitations of the study

The etiological factors related to anemia were not studied. Being a cross-sectional study, duration of anemia and its long term effects on diabetes mellitus couldn't be differentiated. Also, as this was a hospital based study, the results can't be applied to the general population.

Conclusion

Anemia has correlation with the microvascular complications of diabetes. With increasing duration of diabetes mellitus the hemoglobin levels fall progressively. Nonproliferative diabetic retinopathy is more commonly seen in patients with normocytic normochromic anemia. Neuropathy in diabetes is more commonly seen in patients with macrocytic anemia and obesity is a risk factor for the development of neuropathy. Diabetic nephropathy is more commonly seen in patients with macrocytic anemia than other types of anemia.

References

1. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet Lond Engl. 2005 Apr 9; 365(9467):1333–46.
2. Robertson RP. Antagonist: diabetes and insulin resistance--philosophy, science, and the multiplier hypothesis. J Lab Clin Med. 1995 May; 125(5):560–4; discussion 565.
3. Camargo JL, Gross JL. Conditions associated with very low values of glycohaemoglobin measured by an HPLC method. J Clin Pathol. 2004 Apr 1; 57(4):346–9.
4. Ranil PK, Raman R, Rachepalli SR, Pal SS, Kulothungan V, Lakshmipathy P, Satagopan U, Kumaramanickavel G, Sharma T. Anemia and diabetic

retinopathy in type 2 diabetes mellitus. J Assoc Physicians India. 2010 Feb; 58:91-4.
5. Hosseini MS, Rostami Z, Saadat A, Saadatmand SM, Naeimi E. Anemia and Microvascular Complications in Patients With Type 2 Diabetes Mellitus.
6. Mohan VA, Nithyanandam S, Idiculla J. Microalbuminuria and low hemoglobin as risk factors for the occurrence and increasing severity of diabetic retinopathy. Indian J Ophthalmol. 2011; 59(3):207–10.
7. Willis JR, Doan QV, Gleeson M, Haskova Z, Ramulu P, Morse L, et al. Vision Related Functional Burden of Diabetic Retinopathy Across Severity Levels in the United States. JAMA Ophthalmol. 2017 01; 135(9):926–32.
8. Smith AG, Singleton JR. Obesity and hyperlipidemia are risk factors for early diabetic neuropathy. J Diabetes Complications. 2013 Oct; 27(5):436–42.
9. Shai I, Jiang R, Manson JE, Stampfer MJ, Willett WC, Colditz GA, et al. Ethnicity, obesity, and risk of type 2 diabetes in women: a 20-year follow-up study. Diabetes Care. 2006 Jul; 29(7):1585–90.
10. Hostetter TH. Hyperfiltration and glomerulosclerosis. Semin Nephrol. 2003 Mar; 23(2):194–9.
11. Tonneijck L, Muskiet MHA, Smits MM, van Bommel EJ, Heerspink HJL, van Raalte DH, et al. Glomerular Hyperfiltration in Diabetes: Mechanisms, Clinical Significance, and Treatment. J Am Soc Nephrol JASN. 2017; 28(4):1023-39.
12. Pichler R, Afkarian M, Dieter BP, Tuttle KR. Immunity and inflammation in diabetic kidney disease: translating mechanisms to biomarkers and treatment targets. Am J Physiol Renal Physiol. 2017 01; 312(4):F716–31.
13. Hojs R, Ekart R, Bevc S, Hojs N. Markers of Inflammation and Oxidative Stress in the Development and Progression of Renal Disease in Diabetic Patients. Nephron. 2016; 133(3):159–62.