Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/4421
DOI URL: http://dx.doi.org/10.21474/IJAR01/4421
ISSN NO. 2320-5407

RESEARCH ARTICLE

ANAEMIA IN TYPE 2 DIABETES PATIENTS WITH AND WITHOUT NEPHROPATHY IN ANAND-GUJARAT –INDIA.

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Manuscript Info

Manuscript History

Received: 09 April 2017
Final Accepted: 11 May 2017
Published: June 2017

Key words:-
Diabetic Nephropathy, Diabetics without Nephropathy, Anaemia, HbA1C, Creatinine and Hemoglobin.

Abstract

Diabetic nephropathy (DN) is the most common cause of chronic kidney disease (CKD). Anaemia is one of the most significant complications of CKD.

A total of 170 participants-enrolled in Shri Krishna Hospital, Karamsad, Anand-Gujarat, were selected. The association between hemoglobin and diabetes was studied with and without nephropathy. Creatinine estimation was done by using (Siemens-Dimension-RXL- and X pand) and Hb by Automatic cell counter. Data were computer-analyzed using (SPSS 20.USA) program. Reference management was done by Endnote X7 program.

Results: In our study of 170 type II diabetes mellitus (DM) participants, irrespective of having nephropathy or not, 66% was anemic and 34% seemed to have normal hemoglobin. In DM patients with nephropathy, 78.1% was anemic and only12.9% had normal hemoglobin. In DM patients without nephropathy, 44.7% was anemic and 53.3% had normal hemoglobin.

The mean level Hb decreased in diabetics with nephropathy (10.4±2.17) compared to those without nephropathy (12.7±1.84). The negative correlation [r]between Hb and creatinine was -0.24 and p-value = 0.002.

Conclusion: Hemoglobin is an independent risk factor for the progression of nephropathy to the End-Stage Renal Disease (ESRD) in type 2 diabetes. This is explained by the effect of Erythropoietin (EPO) production from the kidney.

Introduction:-

Anemia is a more frequent complication of diabetic nephropathy(Thomas, MacIsaac, Tsalamandris, Power, & Jerums, 2003). It has recently been recognized that in diabetics not only anemia is seen in renal failure, but it also occurs in patients with slight impairment of renal function(Ritz & Haxsen, 2005). At any level of glomerular filtration rate (GFR), anemia is more frequent and serious in diabetic compared to non-diabetic patients. The major cause of anaemia is the decreased production of erythropoietin from kidney. Erythropoietin stimulates bone marrow to produce red blood cells (Berns, 2014; Mehdi & Toto, 2009; OsamaEl & EmanEl, 2010; Semeraro et al., 2013).

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The rationale for the use of erythropoietin is based on recent observational data, documenting diabetic patients with chronic kidney diseases but not in those without nephropathy(Makadiya et al., 2013). The presence of anemia amplifies the cardiovascular risk (Vlagopoulos et al., 2005; Weinrauch et al., 2016).When serum creatinine is still normal, the erythropoietin concentration is the indicator of more rapid decreased glomerular function(Drüeke et al., 2006).Recent data clearly indicate that in patients with impaired renal function anaemia is more frequent in patients with diabetes. Moreover, in diabetic patients, anaemia occurs at an earlier stage of chronic kidney disease. The Kidney Early Prevention Program(Bosman, Winkler, Marsden, Macdougall, & Watkins, 2001; El-Achkar et al., 2005), showed that the proportion of patients with anaemia was higher in diabetic compared to nondiabetic patients, even when the estimated GFR was still 60–89 mL min^{-1}. Another study revealed similar data to what was done by Brad C, Astor et al. What was pointed out is that anaemia is consistently more frequent in diabetic patients compared to nondiabetic patients, even when GFR was > 80 mL min−1 1·73 m^{-2}(Astor, Muntner, Levin, Eustace, & Coresh, 2002). In another study conducted by Thomas et al. , it is reported that the proportion of diabetic patients who had anaemia was 10% at a GFR of (60–70 mL min^{-1} 1·73 m^{-2}), 20% at GFR (50–60 mL min^{-1} 1·73 m^{-2}), 30% at GFR (40–50 mL min^{-1} 1·73 m^{-2})and 40% at GFR (30–40 mL min^{-1} 1·73 m^{-2})(Thomas et al., 2003).These findings are remarkable with regard to the past opinion that states anaemia is a major problem only when GFR had decreased to values less than 40 mL min^{-1} 1·73 m^{-2}.

Material and Methods:-

The cross-sectional study included 85 patients having type 2 diabetes with nephropathy and 85 without nephropathy in all stages CKD. All the participants belong to both genders with age > 45yrs.They all were admitted to the Shree Krishna hospital, Karamsad. The period was from 2015 to 2017. All the participants were evaluated for creatinine, HbA1C, and hemoglobin level.

The instruments used were Siemens Dimension Clinical Chemistry Analyzer -RXL-and Xpand.
Estimation of the fasting blood sugar was done by using hexokinase method. Estimation of the creatinine was also done by using Jaffe's kinetic method (Fabiny & Ertingshausen, 1971; Trinder, 1969; J. Wallach, 2007; J. B. Wallach, 2007). Estimation of the Whole Blood Glycated Hb (HbA1c) was done by using Turbidimetric Inhibition Immunoassay(Burtis, Ashwood, & Bruns, 2012; J. Wallach, 2007; J. B. Wallach, 2007). The hemoglobin was estimated using automated hematology analyser Sysmex XN-550(Louderback & Fontana, 1976).

We studied the relationship between the levels of hemoglobin concentration (Hb) in diabetics with and without nephropathy. Anemia was defined using world health organization criteria i.e. < 13g/dl for men and <12 g/dl for women .The classification into four groups according to haemoglobin concentration is as follows: severe anemia < 6g/dl, moderate anemia 6-9 g/dl, mild anemia 9.1-13 g/dl and normal > 13 g/dl.

Glomerular filtration rate (GFR) was calculated according to CKD-EPI creatinine formula(Kojima & Totsuka, 1995; Levey et al., 2009) .
The CKD-EPI creatinine equation is:

$$GFR=141\times\min\left(\frac{Scr}{k},1\right)^{\alpha}\times max\left(\frac{Scr}{k},1\right)^{-1.209}\times 0.993^{Age}\times 1.018\,[if\ female\,]\times 1.159[if\ black\,]$$

K=0.7 if female
K=0.9 if male
α = -0.329 if female
α = -0.411 if male
min = the minimum of Scr/k or 1
max = the maximum of Scr/k or 1
scr =serum creatinine (mg/dL)
The classification stage of renal failure is according to the value of GFR. As per the National Kidney Foundation classification(Levey et al., 2003).

This study was approved by the Institutional Ethics Committee (IEC) in Pramukhswami Medical College (PSMC), Karamsad.

Statistical Analysis:-
Descriptive statistics was computed with percentages and proportion. Group comparisons were done by Chi-square test, Pearson correlation and p value, and the mean plus or minus standard deviation (±SD) by SPSS statistical computerized program.

References management was done by Endnote X7 program.

Aim and objective:-
The aim of the study is to determine the prevalence of anemia in diabetic nephropathy and to evaluate its impact on the progression of renal function.

Result:-

In this study of 170 type Ⅱ diabetes mellitus (DM) participants, irrespective of having nephropathy or not, 66% was anaemic and 34% was having normal hemoglobin. In DM patients with nephropathy, 78.1% was anaemic and only12.9% had normal hemoglobin. In DM patients without nephropathy, 44.7% was anaemic and 53.3% had normal hemoglobin.

The mean level of haemoglobin decreased in diabetics with nephropathy (10.4±2.17) compared to those without nephropathy (12.7±1.84). The negative correlation [r]between hemoglobin and creatinine was -0.24 and p-value = 0.002.

The mean level of serum creatinine concentration increased in diabetics with nephropathy (2.56 mg /dL) compared to diabetics without nephropathy which was (1.03 mg /dL).

Table 1:- Distribution of Diabetics with Nephropathy and Diabetics without Nephropathy according to anemia

			Diabetic type		Total
			diabetics without nephropathy	diabetics nephropathy	
Hemoglobin	**Severe anemia** Hb<**6** g/ dL	**Count & %**	0(0.0%)	1 (1.2%)	1 (0.6%)
	Moderate anemia Hb= **6-9** g/ dL	**Count & %**	2 (2.4%)	20 (23.5%)	22 (12.9%)
	Mild anemia Hb= **9.1-13** g/ dL	**Count & %**	36 42.3%	53 62.4%	89 52.4%
	Normal Hb>**13** g/ dL	**Count & %**	47 55.3%	11 12.9%	58 34.1%
Total		**Count**	85	85	170
		&%	100.0%	100.0%	100.0%

This table shows a comparison between anemia in the diabetic with nephropathy and in diabetics without nephropathy. As the results indicate, anemia in diabetics with nephropathy is more than in diabetic without nephropathy. It showes that (87.1 %) have anemic and (12.9%) are with the normal hemoglobin concentration in diabetics with nephropathy. But in diabetic without nephropathy,it is shown that (44.7%) have anemic and (55.3%) have normal hemoglobin concentration. The severe type of anemia was found only in diabetics with nephropathy.

Table 2:- The correlation between Hemoglobin and Creatinine level in Diabetics with and without Nephropathy.

		Creatinine
Hemoglobin	Pearson Correlation	-0.241**
	Sig. (2-tailed)	0.002
	N	170

**. Correlation is significant at the 0.01 level (2-tailed).

This table shows the correlation between hemoglobin and creatinine. The result is negative correlation with Pearson correlation (-0.241) and p- value = (0.002).

Table No 3:- The mean level of creatinine concentration in diabetics with and without nephropathy

Diabetic types	N	**Creatinine** mg/ dL Mean	Std. D	P-Value
Diabetic without nephropathy	85	1.03	0.14	P<0.001
Diabetic with nephropathy	85	2.56	1.34	

Clearly, the mean of serum creatinine concentration increased in diabetics with nephropathy (2.56 mg /dL) compared to diabetics without nephropathy which is marked at (1.03 mg /dL).

Table No 4:- The correlation between Hemoglobin and glomerular filtration rate(GFR) in Diabetics with and without Nephropathy.

		GFR
Hemoglobin	**Pearson Correlation**	0.507**
	Sig. (2-tailed)	0.0001
	N	170

This table shows the correlation between hemoglobin and GFR. The result is positive correlation with Pearson correlation (0.507, and p- value <0.001).

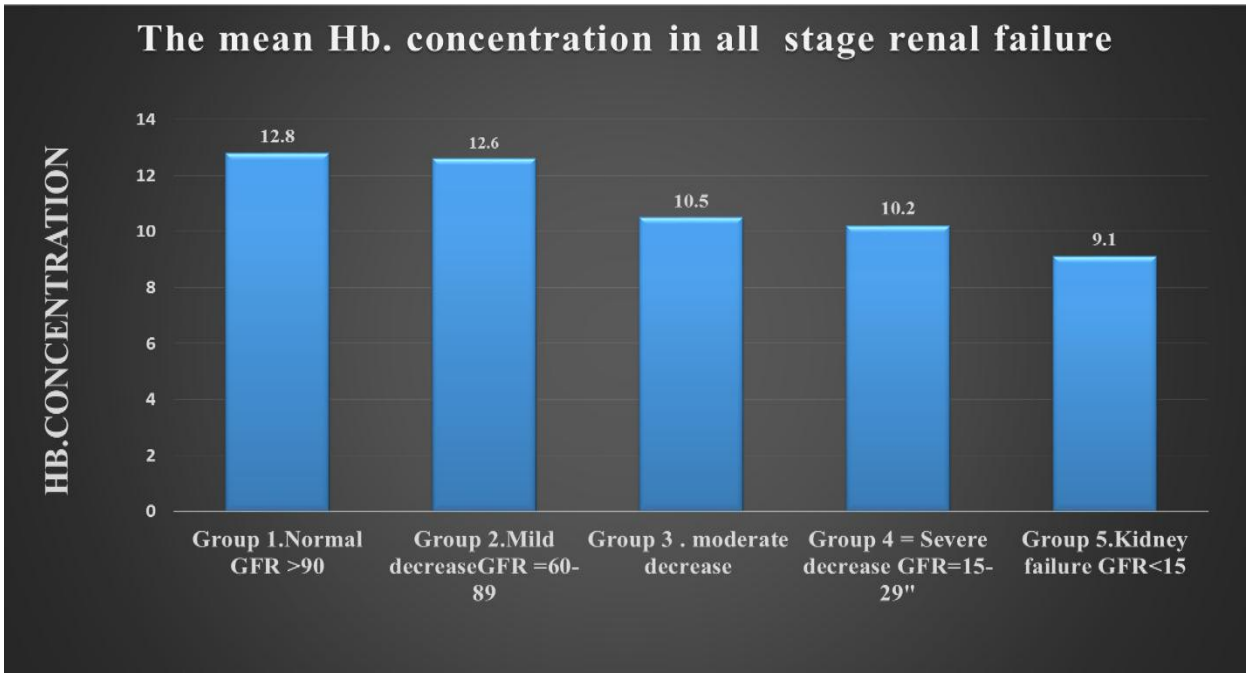

Figure 1:- The mean concentration of hemoglobin level in the different stages of renal failure according to the GFR

In this figure, the results show the mean level of hemoglobin according to the stages of renal failure. From stage 1 to stage 5, we get (12.8 ± 2.06, 12.6 ± 1.70, 10.5 ± 2.33, 10.2 ± 1.84 and 9.1 ± 0.14) respectively. The mean of hemoglobin decreases whenever nephropathy increases to end-stage renal failure. The decreased hemoglobin is synchronous with fall of glomerular filtration rate.

Table No 5:- Mean level Hemoglobin in diabetics with and without nephropathy.

Diabetic types	N	Hemoglobin (g/ dL) Mean	Std. D	P-Value
Diabetic without nephropathy	85	12.70	1.84	P<0.001
Diabetic with nephropathy	85	10.40	2.17	

In this table, the results show the mean level of hemoglobin in diabetics without nephropathy compared to diabetics with nephropathy. Hemoglobin concentration is lower in diabetics nephropathy (10.40 ± 2.17) in comparison to diabetics without nephropathy in which hemoglobin concentration is (12.70 ± 1.84) with p-value <0.001.

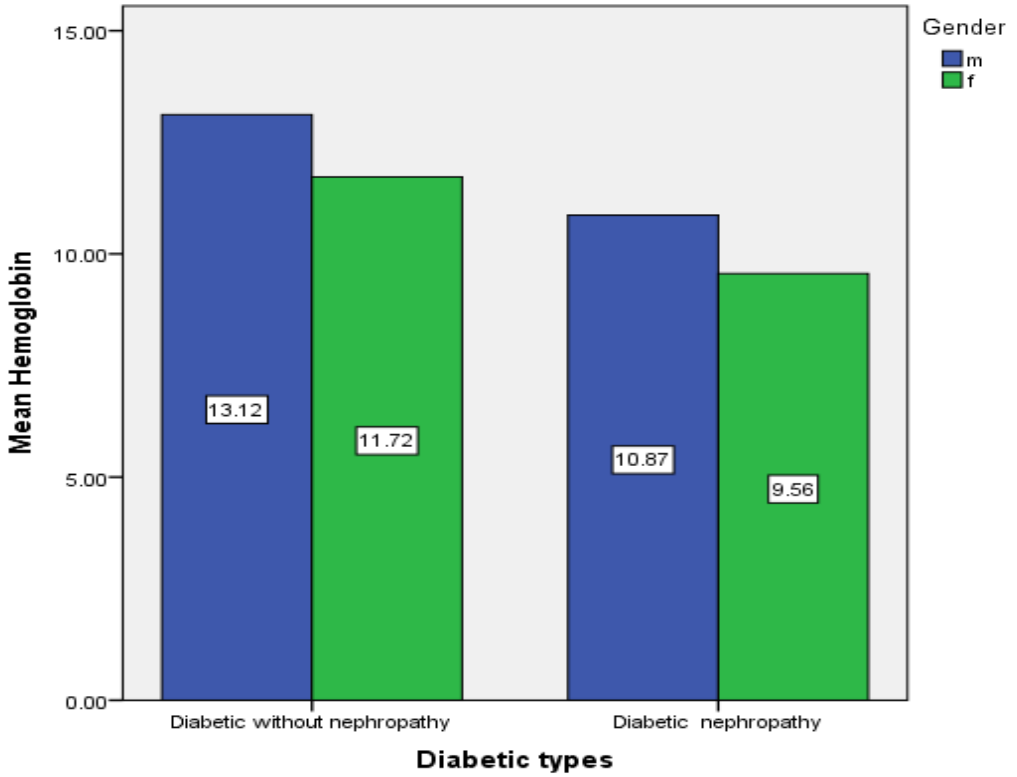

Figure 2:- The mean concentration of hemoglobin in male and female that have diabetes with and without nephropathy.

The hemoglobin concentration decreased in diabetic nephropathy more than in diabetics without nephropathy. The hemoglobin concentration decreased in females more than in males.

Discussion:-

The principal new outcome in this study is that anemia is an independent predictor of progression to End Stage Renal Disease (ESRD) in patients with diabetic nephropathy.

A key question is whether the association of the anemia and subsequent development of ESRD is caused by low hemoglobin with low glomerular filtration rate.

The causes of anemia are multiple, including the relatively benign conditions such as dietary iron deficiency, also serious chronic inflammatory disease or malignancy. The accurate examination is essential to determine the underlying cause accompanied by diabetic microvascular complications (Bach, Schruckmayer, Sam, Kemmler, & Stauder, 2014; Gupta, Copley, Keeney, & Chin-Yee, 2016; Thomas, 2007; Yip & Dallman, 1988). One complication of diabetes is nephropathy, affecting the production of erythropoietin which is responsible for the concentration of hemoglobin (Panjeta et al., 2015).

Previous studies have reported that the incidence of anemia in diabetic patients is higher, occurring at earlier stages of CKD(Loutradis et al., 2016; Palmer, Salanti, Craig, Mavridis, & Strippoli, 2014). Other studies have also compared patients with non-diabetic kidney disease (Babazono et al., 2006; Bosman et al., 2001; Ravanan, Spiro, Mathieson, & Smith, 2007; Shaheen et al., 2011).The main anatomical focus of the lesion of diabetic nephropathy has been the glomerulus. Therefore, the attenuation of renal Erythropoietin responsiveness associated with renal interstitial damage has not been considered as a cause of anemia of diabetic patients prior to progression into advanced diabetic nephropathy (Eschbach, Egrie, Downing, Browne, & Adamson, 1987; Kojima & Totsuka, 1995; Thomas, Cooper, Tsalamandris, MacIsaac, & Jerums, 2005).

However, in this study, the anemic patients with defect in renal function and hemoglobin concentrations were related to the biochemical parameters of renal damage as serum creatinine concentrations. Where the mean Hb concentration was significantly lower in type II diabetic nephropathy patients (10.4 g/ dL) compared to diabetic without nephropathy patients (12.7 g/ dL), the mean serum creatinine concentration was 2.56 mg /dL in diabetic nephropathy compared to diabetics without nephropathy was 1.03 mg /dL.

Babazono et al. observed the low baseline hemoglobin in patients reaching a renal end-stage were 12.0 ± 1.9 g/dL versus 13. 3 ± 1.8 g/dL in diabetics patients with normal proteinuria(Babazono et al., 2006).

However, there are conflicting data regarding their impact on Hb levels in patients with CKD, especially in diabetic nephropathy (Hayashi, Hasegawa, & Kobayashi, 2001; Inoue, Babazono, & Iwamoto, 2008).In the present study we also found that the hemoglobin concentration is lower in diabetics nephropathy (10.40 ± 2.17) in comparison to diabetics without nephropathy in which hemoglobin concentration was (12.70 ± 1.84) with p-value <0.001.This confirms another study in which diabetics with nephropathy were found to be anemia(Mohanram, Zhang, Shahinfar, Lyle, & Toto, 2008).

Another study made a comparison between hemoglobin level in diabetic nephropathy and glomerulonephritis (GN). It revealed that the diabetic nephropathy (DN) patients were anemic (Hb 10.6 ± 0.9 g/dl) in marked contrast to none of the GN patients (Hb 13.7 ± 1.4 g/dl, P, 0.005) (Bosman et al., 2001).

Current national kidney founding guidelines for pre- ESRD participants recommend treatment to goal hemoglobin of 11 to 12 g/dl (Kopple, 2001). Our data suggest that even mild anemia, hemoglobin < 13g/dl, increase the risk for the progression to end stage renal disease (GFR=60-89, Hb=12.6). Previous studies have reported a significant correlation between severity of anemia and various measures of kidney function in the general population that had S. creatinine level above 2.0mg/dl(Hsu, Bates, Kuperman, & Curhan, 2001; Xu et al., 2015).

In summary, this study provides description of the association between kidney function and hemoglobin level across the entire range of kidney function in the Gujarat population. The results demonstrated that participants with estimated GFR below 60 mL/min per 1.73 m2 are much more likely to have anemia in diabetic with nephropathy. The prevalence and severity of anemia increase with declining kidney function. In the moderate kidney dysfunction (GFR= (30 to 59)), anemia increases in diabetic with nephropathy more than without nephropathy. The negative correlation between serum creatinine and hemoglobin in diabetics with and without nephropathy is significant with (r) = -0.24 and p-value = 0.002.The positive correlation between hemoglobin and GFR is also significant with (r) =0.5.7 and p-value <0.001. When GFR decreases also hemoglobin concentration decreases.

Conclusion:-

Our data suggest that there is increased anemia, (Hb <13. g/dL) in diabetic nephropathy more than in diabetics without nephropathy. This is explained by the effect of Erythropoietin (EPO) production from the kidney. Low hemoglobin is synchronous with decrease of glomerular filtration rate in diabetics with and without nephropathy. Management of DM should include mandatory routine haematological tests at follow-up visits enabling aggressive correction of anaemia to prevent other diabetic complications.

Abbreviations:-

CKD Chronic Kidney Disease
ESRD End Stage Renal Disease
DM Diabetes Mellitus
GFR Glomerular Filtration Rate
HbA1C Glycated Hemoglobin

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