# Correlation of Serum Erythropoietin Levels with Different Stages of Diabetic Retinopathy

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## ABSTRACT

**Objective:** To determine the correlation of serum erythropoietin concentration with diabetic retinopathy in patients with type 2 diabetes mellitus.

**Study Design:** Cross-sectional study.

**Place and Duration of the Study:** Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from July to December 2021.

**Methodology:** A total of 180 individuals were enrolled in the study and placed in 2 groups as group 1 have 90 cases of type 2 diabetes mellitus and group 2 having 90 age-matched healthy controls. Group 1 was further subclassified into proliferative diabetic retinopathy (PDR) and non-proliferative diabetic retinopathy (NPDR) subgroups by an expert ophthalmologist. Serum erythropoietin, creatinine, blood HbA1c, and haemoglobin were analysed. Correlation between stages of proliferation and serum erythropoietin, creatinine, blood HbA1c, and haemoglobin were analysed. An independent-sample student t-test was applied to compare mean Serum erythropoietin between PDR and NPDR groups. Pearson's correlation was applied among disease severity, and type of retinopathy. A p-value of ≤0.05 was considered significant.

**Results:** The average age of participants in groups 1 and 2 was 45.88±8.6 and 56.6±10.23 years, respectively. More males (n=60, 66.7%) were noted in cases compared to controls (n=42, 46.7%). serum erythropoietin concentration observed in cases (8.4±1.87 IU/L) was higher than controls (6.50±0.9). The mean serum erythropoietin concentration in PDR (9.35±1.74 IU/L) was significantly greater than that in NPDR (7.3±1.38 IU/L, p <0.001). The serum concentration of erythropoietin in group 1 increased linearly with the severity of the disease (r=0.103).

**Conclusion:** Serum erythropoietin concentrations increased in uncontrolled type 2 diabetics more so in proliferative retinopathy cases, and increased with disease severity.

**Key Words:** *Erythropoietin, Diabetic retinopathy, Proliferative diabetic retinopathy.*

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## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterised by chronic hyperglycemia leading to microvascular and macrovascular complications. The International Diabetes Federation (IDF) has estimated the global population with DM to be 463 million in 2019.[1] Diabetes mellitus can lead to some acute (diabetic ketoacidosis and dehydration) and chronic complications (cardiovascular, retinopathy, neuropathy, and nephropathy) as well. Among the chronic complications, diabetic retinopathy (DR) is very common,[2] and is a leading cause of visual disturbance in adults.[3]

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IDF observed 103.12 million cases of DR, with the highest prevalence in Africa (35.90%) and the lowest in South and Central America (13.37%).[1] A prevalence of 6.17% was noted in Asia.[1] In Pakistan, the prevalence of DR is 28.7%.[4]

Ischemia, vascular permeability, and new blood vessel growth are commonly observed in DR.[5] DR may occur as proliferative diabetic retinopathy (PDR) or non-proliferative diabetic retinopathy (NPDR) which are classified into mild, moderate, and severe forms. The degree and duration of hyperglycemia, uncontrolled hypertension, and dyslipidemia are the major risk factors for diabetic retinopathy. High glucose leads to microaneurysms and lack of pericytes in capillaries leading to a degeneration of capillaries in retinal cells resulting in decreased retinal perfusion and contributing to the progression of retinopathy.[6]

From a molecular perspective, various studies have been conducted in the last few decades to understand diabetic retinopathy precisely. Numerous animal models and patients with diabetes have shown biochemical mechanisms involved in

diabetic retinopathy progression. Diabetic patients are thought to have impaired hemostasis in their retina, resulting in neovascularisation.[7] Hypoxia-induced factor -1 causes vascular endothelial growth factor (VEGF) expression during this process of neovascularisation.[8] Hypoxia is primarily responsible for EPO expression which has an erythropoietic effect but also has neuroprotective and angiogenic effects. EPO is expressed in the kidney, liver, uterus, brain, and retina.[9] Other factors in addition to hypoxia-inducible factor-1 leading to diabetic retinopathy are angiogenic factors, chemokines, cell adhesion molecules, and soluble CD 200.[10,11]

Erythropoietin is a systemic angiogenic factor, but its role in ocular angiogenesis and diabetic retinopathy is not yet fully understood.[12] There are few international studies in which Serum erythropoietin level has been correlated with DR,[8,10] but the evidence is lacking in the local population. Therefore, the objective of this study was to determine the correlation of serum EPO levels with diabetic retinopathy in patients of the disease severity group.

## METHODOLOGY

This cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, AFIP, Rawalpindi, from July to December 2021 after getting ethical approval from the Institutional Review Board of AFIP, Rawalpindi (reference number FC-CHP-29/READ-IRB/21/656).

A sample size of 89 was calculated using the WHO calculator, keeping a 5% margin of error, 95% confidence level, and a prevalence of 6.17%.[1] Sampling was done using a nonprobability consecutive sampling technique at the Armed Forces Institute of Ophthalmology (AFIO), Rawalpindi. A maximum number of available participants during the study period were recruited. All participants were between 35 – 80 years. The study population was divided into two groups — the first group comprised 90 cases having type 2 diabetes mellitus for 4 years or more irrespective of gender. Group 2 comprised 90 healthy controls. Exclusion criteria were Individuals with anaemia, dyslipidemia, chronic kidney disease, asymmetrical eye changes, senile cataract, active systemic infection, uncontrolled hypertension, and cases of recombinant erythropoietin therapy. An ophthalmologist (vitreo-retina specialist) subdivided the cases into PDR and NPDR. All patients in PDR and NPDR were examined in detail by an ophthalmologist of AFIO. They were classified according to International Clinical Diabetic Retinopathy Disease Severity Scale (ICDRDSS)[13] as mild NPDR (microaneurysms only), moderate NPDR (microaneurysms, venous beading), and severe NPDR (intraretinal haemorrhages). In the PDR group, three subgroups were mild PDR (neovascularization), moderate PDR (vitreous, pre-retinal haemorrhage), and severe PDR (high-risk cases).

Three ml of venous sample was collected from subjects in both K-EDTA tubes and gel vacutainers. Serum was separated from cells by centrifugation at 3500 RPM for 5 minutes. Separated serum was stored at -20°C for subsequent analysis. Serum EPO level was analysed on random access IMMULITE 2000®, by Siemens Health care Diagnostics USA. Serum creatinine was measured *via* photometric technique on *ADVIA® 1800* Clinical Chemistry System. HbA1c was measured on Sebia Octa using the capillary electrophoresis technique. A complete blood count was done on the haematology analyzer Sysmex XP 100. All parameters were analysed according to the standard operating procedures of AFIP. Data were entered in Microsoft excel and later analysed using Statistical Package for Social Sciences (SPSS) version 23.0. Descriptive statistics were expressed as mean ± standard deviation (SD). An independent-t-sample Student t-test was applied between PDR and NPDR groups, and a p-value ≤0.05 was considered statistically significant. Chi-square was applied between PDR and NPDR and the degree of severity. Pearson correlation was applied among disease severity, type of retinopathy, and serum EPO levels.

## RESULTS

The age range of participants was 35-70 years. The average age of participants in the control group and cases was 45.88±8.6 and 56.6±10.23 years, respectively. The participants were divided into four age groups. The highest frequency of cases was noted in the age group 41-50 years (n= 33, 36.7%), and the least was noted in the age group 30-40 years (n=9, 10%). However, in controls, the group aged between 30-40 years had the highest frequency (n=42, 46.7%).

More males (n=60, 66.7%) were noted in cases as compared to controls (n=42, 46.7%), as shown in Table I along with studied biochemical parameters.

EPO concentration observed in cases (8.41±1.87 IU/L) was higher than controls (6.50±0.91 IU/L). All parameters in PDR and NPDR were subjected to an independent t-test, and a p-value ≤0.05 was considered statistically significant. Table II shows the results of the independent sample t-test.

The mean serum EPO concentration in PDR (9.35±1.74) was greater than that in NPDR (7.33±1.38) and was statistically significant (p-value <0.001). Among clinical stages, cases with severe disease showed the highest concentration of serum EPO as compared to mild and moderate cases. Mean serum EPO was 6.136 ±0.247 in the mild group (n=12,13.33%), 8.05±0.185 in moderate (n=32, 35.55%), and 9.80 ±0.207 in severe cases (n=46,51.11%) with p-value <0.001. Out of a total of 51 patients with PDR, 32 had severe diabetic retinopathy and 15 had moderate diabetic retinopathy. Whereas a total of 32 patients had NPDR out of which only 14 had severe diabetic retinopathy and 17 had moderate diabetic retinopathy. Pearson's chi-square had a value of 7.027 with a p-value <0.001 which was significant. A significant correlation was found between erythropoietin levels and the severity and type of diabetic retinopathy (Table III).

**Table I: Characteristics of patients in the control group and case groups with DM with analysed parameters.**

| S.No. | Study Parameters | Case Group 1 (n=90) | Control Group 2 (n=90) |
|---|---|---|---|
| 1 | % | 90(100%) | 90 (100%) |
| 2 | Age (mean ± SD) in years | 56.6±10.23 | 45.88±8.65 |
| 3 | Male | 60(66.7%) | 42(46.7%) |
| 4 | Female | 30(33.3%) | 48 (53.3%) |
| 5 | Erythropoietin (IU/L) | 8.41±1.87 | 6.50 ±0.910 |
| 6 | Serum Creatinine (µmol/l) | 119.97±18.7 | 91.92±13.50 |
| 7 | HbA1c (%) | 8.93±1.6 | 5.22± 0.21 |
| 8 | Hb (g/l) | 130.44 ±1.6 | 130.33± 1.61 |

**Table II: Independent Sample t-test between subgroups PDR and NPDR.**

| S.No | Study Parameters | PDR | NPDR | T | 95% Confidence Interval | | p-value |
|---|---|---|---|---|---|---|---|
| | | | | | Lower | Upper | |
| | N | 51 (56.67%) | 39(43.33%) | | | | |
| 1 | Age | 50.18±9.520 | 53.8518±9.637 | -2.07 | -0.78 | -.016 | 0.041 |
| 2 | Duration of disease (in years) | 2.29±0.67 | 2.15±0.779 | 0.915 | 0.164 | 0.44 | 0.362 |
| 3 | Hb (g/l) | 130.81±1.12 | 120.94±2.00 | 2.61 | 0.20 | 1.53 | 0.010 |
| 4 | HbA1c (%) | 8.70± 1.31 | 9.23±1.96 | 1.51 | 1.213 | -0.16 | 0.133 |
| 5 | Creatinine (µmol/l) | 117.94±20.26 | 122±16.48 | | -12.59 | 3.24 | 0.244 |
| 6 | EPO (IU/L) | 9.35±1.74 | 7.33±1.384 | 5.42 | 1.274 | 2.75 | <0.001 |

**Table III: Pearson correlation among disease severity, EPO, and type of retinopathy.**

| | Severity | EPO | Type of Retinopathy |
|---|---|---|---|
| Severity | r 1 | r 0.103 | r -0.276 |
| | | *p* 0.336 | *p* 0.008 |
| EPO | r 0.103 | r1 | r -0.56 |
| | *p* <0.001 | | *p* <0.001 |
| Type of retinopathy | r -0.276 | r -0.56 | r 1 |
| | *p* 0.008 | *p* <0.001 | |

## DISCUSSION

Diabetic retinopathy, a common complication of Diabetes mellitus, is one of the leading causes of visual impairment and blindness across the globe.[14] The pathophysiology of the development of diabetic retinopathy has not been completely clarified despite thorough investigations. Initially, it was associated with local ischemia, but with time, systemic vaso-proliferative angiogenic factors were noted in diabetic patients. These systemic proliferative factors promote and increase diabetic vascular changes and are responsible for retina neovascularisation in diabetic patients.[15]

Results of the present study (n=180) have shown that serum Epo in group 1 was significantly increased which comprised of type 2 diabetics mean ± SD 56.6±10.23.

In this study, the average age of cases was 56.6±10.23 years. Jahangir *et al.*[16] in a local study done in Pakistan, observed mean age of 52.93 years in diabetic patients with retinopathy, which is close to that observed in this study. Shan *et al.*[17] in a cross-sectional study done in the year 2021 in China, found a mean age of 63.89 ±10.48 years, which is slightly greater than that noted in the present study. Out of 395 Diabetic cases with retinopathy, Jahangir *et al.*[16] noted a higher frequency among females, n=270 (68.4%), compared to males n=125 (31.6%). In addition to this local study, a study done in China in the year 2019 also noted that females exhibit a significantly higher prevalence of diabetic retinopathy than males (31% vs. 29%). However, contrary results were noted in this study with 30 (33.3%) females and 60 (66.7%) males.

Mean HbA1c in controls was 5.22± 0.21% and 8.933±1.6% in cases. Among cases, the group with PDR had a lower mean HbA1c of 8.70±1.31% than the NPDR group (9.23±1.96%). Davidovic *et al.* observed a high mean HbA1c in the PDR group.[13]

In this study, the serum EPO concentration in cases was higher (8.41±1.87 IU/L) as compared to that of age-matched controls (6.50±0.91IU/L). Similar results were observed by Davidovic *et al.*[13] Those with Diabetes had a higher mean concentration of EPO in serum than controls. Chen *et al.* also observed a higher concentration of serum EPO in cases compared to controls.[18]

Among PDR and NPDR cases, serum EPO concentration noted in this study was higher in the PDR group *i.e.* 9.35±1.74 in PDR and 7.33±1.38 in the NPDR group (p <0.001). Davidovic *et al.* and Li Chen also noted a higher concentration of serum EPO in PDR cases.[13,18] Davidovic *et*

*al.* noted an average concentration of serum EPO of 9.95 mIU/ml in PDR and 7 mIU/ml in the NPDR group[13], which supports the present study. However, Chen *et al.* noted a concentration of 99.29±27.77 mIU/ml in the PDR group which is higher than that observed in this study.[18] Katsura *et al.* noted higher EPO concentration in the PDR group *vs* NPDR group in the vitreous chamber as well[9], which was not studied here. The median EPO measured by Katsura *et al.* was 366.6 IU/L (44.8-2023.1 IU/L).[9] When they focused on PDR cases, they found no significant correlation between intravitreous and serum EPO concentrations (r=0.18, p=0.18). They also observed that serum EPO concentrations did not vary much among the three subgroups of PDR.[9]

In this study, it was observed that disease severity with serum EPO concentration and noted a positive correlation with the highest serum EPO concentration in those with severe disease. There are international studies in which a positive correlation of serum EPO concentration with disease severity was noted.[4,13,18] Others also observed serum EPO concentration separately in severe PDR and NPDR and noted the highest concentrations in the severe PDR group.[13,18] This study did not observe the concentrations separately in PDR and NPDR subgroups. Apart from vascular endothelial factors, erythropoietin is one of the most important factors, and its role in neovascularisation has not been studied well yet. Besides stimulating erythrocyte production, it also acts as a growth factor. In some studies, increased erythropoietin levels were noted in the vitreous body of diabetic patients due to local hypoxia.[18] Studies have shown that patients on treatment of EPO have an earlier onset of diabetic retinopathy.

This study did not obtain vitreous body samples or included cases of EPO treatment.[19] In this study only serum levels of EPO with a type of retinopathy and clinical stages were observed.

The main limiting factor of this study was the small sample size and single-centred study. Along with these, the authors did not determine intravitreous EPO concentrations. Large multicentred studies correlating intravitreous and serum EPO levels with clinical stages of retinopathy are suggested for more generalisation of results.

## CONCLUSION

Serum EPO concentrations increase in PDR compared to NPDR, and high EPO levels correlate with disease severity. Monitoring serum EPO levels can give clinicians an idea about the severity of DR and help them adopt better and more timely treatment options.

**ETHICAL APPROVAL:**
The study was conducted after getting ethical approval from the institutional review board of AFIP, Rawalpindi (reference number FC-CHP-29/READ-IRB/21/656).

**PATIENTS' CONSENT:**
Informed consent was taken from all the participants of this study.

**COMPETING INTEREST:**
The authors declared no competing interest.

**AUTHORS' CONTRIBUTION:**
TGM: Sampling, data analysis, statistical analysis and literature review.
ZHH: Study design and data review.
MY, MUM: Literature search.
MAM: Sampling, data analysis and result analysis.
MA: Statistical analysis.

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