Association between levels of plasma Pentraxin 3 (PTX 3) and development and / or progression of diabetic retinopathy (DR)

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Abstract: Purpose: To evaluate the association between elevated levels of plasma pentraxin 3 (PTX3) and the development and/or progression of diabetic retinopathy (DR). **Methods:** In this case-control study, 50 diabetic patients with DR (group 1), 30 diabetic patients without DR (group 2), and 20 normal subjects (group 3) were enrolled. Log transformed values of plasma PTX3 and high-sensitivity C-reactive protein (hsCRP) concentrations were measured and used in our analysis. **Results:** Serum PTX3 level significantly increased in patients with DR more than patients without DR with cut off point 1150 pg/ml, sensitivity 93.3%and specificity 72%. Serum CRP level significantly increased in patients with DR more than patients without DR with cut off point of 760 pg/ml has sensitivity 93.3% and specificity 68%. Combined use of PTX3 and CRP decrease sensitivity to 76.7%. but combined use increase specificity to 90%. **Conclusions:** Plasma PTX3 is positively associated with DR development and progression, and may be a more accurate predictor of DR development than hsCRP.
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Key words: Diabetic retinopathy, HsCRP, Pentraxin3, diabetes mellitus.

1. Introduction

With population growth and aging, economic development, and the increasing prevalence of obesity and physical inactivity; it is estimated that the total number of people with Diabetes mellitus will be more than double from 171 million in 2000 to 366 million in 2030 ***(1).***

The extent of systemic inflammatory reaction in DM has been an important cause of microvascular complications ***(2).***

In this regard, several studies have reported that type 2 DM is associated with increased plasma concentrations of acute phase biomarkers, including C-reactive protein (CRP), which is related to the innate immune response and inflammation ***(3).***

Diabetic retinopathy (DR) is amicrovascular complication of DM, which is caused by neurovascular inflammation. The relationship between DR and inflammatory reaction has been recently examined, and elevated levels of plasma CRP, such as short pentraxin, are more frequently observed in DM and DR patients compared to patients without DM or DR. ***(4).***

Pentraxin 3 (PTX3) is an acute-phase reactant characterized by a cyclic multimeric structure. Pentraxin 3, in the form of a long pentraxin, is produced by peripheral tissues and reflects impaired vascular endothelial function. In contrast, CRP is mainly produced by hepatocytes and is predominantly under the transcriptional control of the cytokine interleukin-6 (IL- 6). ***(5).***

Pentraxin 3 inhibits angiogenesis, promotes restenosis, and increases advanced atherosclerotic lesions typically by inhibiting the fibroblast growth factor (FGF2) reaction of angiogenesis. ***(7).***

PTX 3 has been shown to be a sensitive biomarker of localized inflammatory reactions and innate immunity of cardiovascular and renal diseases ***(8).***

PTX 3 levels were independently associated with diabetic retinopathy ***(6).***

2. Methods

The study was carried out in internal medicine department, outpatient clinic of internal medicine and outpatient clinic of ophthalmology, at Ahmed Maher Teaching Hospital from June to December, 2017. The study included 80 patients with DM and 20 healthy individuals aged from 40 to 75 years, the disease duration ranged from 5 -22 years and they were classified into 3 groups:

Group I: 50 patients with diabetic retinopathy.

Group2: 30 patients with DM without diabetic retinopathy.

Group 3: 20 healthy individuals "control group".

Inclusion criteria:

- All patients had DM, and the duration of the disease ranged from 5 to 22 years.

Exclusion criteria:

1- History of cardiac disease.
2- Patients with past history of stroke.
3- Patients with malignancies.
4- Patients with current or past history of receiving any form of immune-modulating drugs.
5- Patients with end stage renal disease.
6- I.V. drug users.
7- Patients with severe eye disease and retinal detachment.

2. Methodology:

All patients were submitted to the following:

1- Full history taking including:

- Name, age, sex.
- Duration of diabetes.
- Anti diabetic treatment.
- Cardiovascular disease.
- Evidence of diabetic nephropathy, retinopathy and neuropathy.
- Hypertension and its treatment.
- Drug history.
- Smoking.

2- Clinical examination including the following items:

- Assessment of anthropometric measures (BMI and waist circumference).
- Assessment of blood pressure.
- Fundus examination.

All patients were informed about the study and written consent was taken from all patients before taking the specimens and fundus examination.

Laboratory evaluation

1. HA1C.
2. Serum SGOT, SGPT.
3. Blood urea and serum creatinine.
4-Serum lipid profile including serum triglycerides, high density lipoprotein, low density lipoprotein and total cholesterol level.
5. Serum pentraxin3.
6. hsCRP.

Blood sample were obtained at the morning after 12 hours fasting for estimation of all parameters. Blood was aspirated into three plastic tubes; the first contained k-EDTA for HbA1c measurement, the second contained no additive for serum separation. lipid profile, SGOT, SGPT, Urea, Creatinine were estimated immediately within a suitable time. Another part of serum stored at - 80 C untile assay of hs CRP and the third tube contained K-EDTA centrifuged for 15 minutes at 1000 x g within 30 minutes of collection and plasma separated and stored at - 80 C untile assay of pentraxin 3.

Statistical Methodology

Statistical analysis

Results were collected, tabulated, statistically analyzed by SPSS (statistical package for social science) version 18 on IBM personal computer. Two types of statistics were done

1- Descriptive: e.g. percentage (%), mean and standard deviation SD.

2- Analytical.

3. Results

Table (1): Shows comparison between studied groups according to demographic and clinical data.

		Control group	DM + Normal Fundus	DM + Retinopathy	One Way ANOVA test		Post Hoc analysis		
		No. = 20	No. = 30	No. = 50	F/X²*/t•	P-value	P1	P2	P3
Age (year)	Mean ± SD Range	53.3 ± 6.18 41 – 64	55.03 ± 7.44 42 – 72	60.67 ± 7.22 50 – 75	7.874	0.001	0.398	0.001	0.003
Sex	Female Male	10 (50.0%) 10 (50.0%)	14 (46.7%) 16 (53.3%)	23 (46 %) 27 (54 %)	0.218*	0.897	0.817	0.643	0.795
BMI	Mean ± SD Range	24.14 ± 1.43 21.5 – 26.4	25.55 ± 1.43 23.2 – 29	27.26 ± 1.97 23.1 – 31	21.951	0.000	0.004	0.000	0.000
Duration	Mean ± SD Range	– –	8.17 ± 1.29 6 – 10	12.63 ± 2.8 10 – 22	-7.943•	0.000	–	–	–
Smoking	No Yes	13 (65.0%) 7 (35.0%)	22 (73.3%) 8 (26.7%)	33 (66 %) 17 (34 %)	0.485*	0.785	0.529	0.903	0.573
Systolic BP	Mean ± SD Range	121.75 ± 11.27 100 – 140	128.17 ± 15.45 105 – 155	125 ± 11.45 110 – 145	1.468	0.237	0.093	0.391	0.351
Diastolic BP	Mean ± SD Range	73 ± 6.37 60 – 85	77.67 ± 9.71 60 – 100	74 ± 5.15 65 – 80	2.900	0.061	0.033	0.643	0.060

χ2=Chi-square test. **T**=independent Student t-test. Independent t-test: **P1**: control group versus DM + Normal fundus. **P2**: control group versus DM + Retinopathy. **P3**: DM + Normal fundus versus DM + Retinopathy. **No**=number. **SD**=standard deviation. **DM**: diabetes mellitus. **BMI**=body mass index. **BP**: blood pressure.

Table (1) shows:

- As regard age of the studied groups: the mean age was (53.3 ± 6.18) years in control group, (55.03 ± 7.44) years in diabetes with normal fundus group and (60.67 ± 7.22) years in diabetes with retinopathy group and these results showed no significant differences between the control group and diabetes with normal fundus group (p-value >0.05), but there is significance between control and retinopathy group (p-value=0.001) and between diabetes with normal fundus group and retinopathy group (p-value=0.003).

• As regard sex of studied groups: control group included 10males (50%) and 10 females (50%), the diabetes with normal fundus group included 16 males (53.3%) and 14 females (46.7%) and retinopathy group included 27 males (54 %) and 23 females (46 %) with no significant difference among studied groups (p=0.897).

- As regard BMI of studied groups: control group (24.14 ± 1.43), mean BMI in the diabetes with normal fundus group (25.55 ± 1.43) and mean BMI in retinopathy group (27.26 ± 1.97). There's significance between control group and diabetes with normal fundus group (p-value=0.004), high significance between control and retinopathy group (p-value=0.000), also high significance between diabetes with normal fundus group and retinopathy group (p-value= 0.000).
- As regard duration of diabetes, it is ranged from 6 to 10 years with mean (8.17 ± 1.29) in diabetes with normal fundus group and from 10 to 22 years with mean (12.63 ± 2.8) in retinopathy group and shows high significance (p-value=0.000).
- As regard smoking control group include 7 smokers 13 non-smokers, in diabetes with normal fundus group 8 smokers 22 non-smokers and in retinopathy group 17 smokers 33 non-smokers with no significance (p-value=0.785).
- Systolic blood pressure ranged from 100 to 155 mmHg in all groups with no significance (p-value=0.237).
- Diastolic blood pressure ranged from 60 to 100 mmHg in all groups with no significance (p-value=0.061).

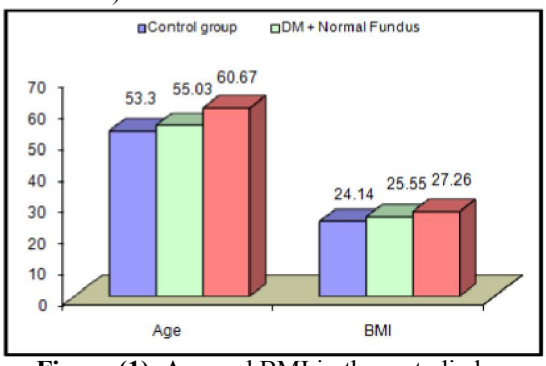

Figure (1). Age and BMI in three studied groups.

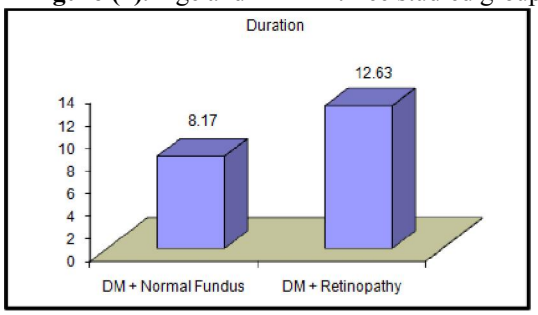

Figure (2). Diabetes duration in patient groups.

Table (2): Shows comparison between studied groups according to biochemical investigations

		Control group	DM + Normal Fundus	DM + Retinopathy	One Way ANOVA test		Post Hoc analysis		
		No. = 20	No. = 30	No. = 50	F	P-value	P1	P2	P3
AST (U/L)	Mean ± SD Range	33.35 ± 20.7 5 – 77	24.37 ± 14.93 5 – 64	27.87 ± 17.82 9 – 77	1.566	0.215	0.081	0.283	0.443
ALT (U/L)	Mean ± SD Range	31.15 ± 14.01 13 – 53	26 ± 11.85 13 – 60	30.23 ± 16.29 8 – 74	1.010	0.369	0.212	0.824	0.251
Creatinine (mg/dl)	Mean ± SD Range	0.75 ± 0.22 0.32 – 1.1	0.89 ± 0.29 0.31 – 1.76	1.08 ± 0.39 0.43 – 1.9	6.844	0.002	0.117	0.001	0.025
Cholesterol (mg/dl)	Mean ± SD Range	180.4 ± 56.14 96 – 299	198.87 ± 44.31 140 – 325	206.33 ± 52.93 120 – 301	1.599	0.209	0.211	0.080	0.570
Triglycerides (mg/dl)	Mean ± SD Range	118.55 ± 36.58 72 – 180	147.27 ± 29.79 88 – 211	138.73 ± 39.96 89 – 270	3.973	0.023	0.007	0.053	0.356
HDL (mg /dl)	Mean ± SD Range	39.35 ± 9.14 25 – 55	45.27 ± 12.11 28 – 78	43.93 ± 12.97 22 – 75	1.580	0.213	0.086	0.182	0.663
LDL (mg /dl)	Mean ± SD Range	110.9 ± 49.41 44 – 231	125.83 ± 43.08 60 – 242	135.63 ± 46.48 62 – 215	1.735	0.183	0.264	0.066	0.412
HA1C (%)	Mean ± SD Range	5.18 ± 0.14 5 – 5.4	8.71 ± 1.86 6.5 – 12.6	8.76 ± 1.72 6.7 – 12	39.093	0.000	0.000	0.000	0.914

F=ANOVA TEST. **SD**=standard deviation. Independent t-test: **P1**: control group versus DM + Normal fundus. **P2**: control group versus DM + Retinopathy. **P3**: DM + Normal fundus versus DM + Retinopathy. **DM**: diabetes mellitus. **No**=Number, **AST**=Aspartate aminotransferase, **ALT**=Alanine aminotransferase. **HDL**=high density lipoprotein. **LDL**=low density lipoprotein
HA1C=glycated hemoglobin.

- There was no significant difference between diabetes with normal fundus group, retinopathy group and controls as regard their AST level (p-value= 0.215).
- There was no significant difference between diabetes with normal fundus group, retinopathy group and controls as regard their AST level (p-value=0.369).
- Creatinine level in retinopathy group was significantly higher than controls (p=0.001), no significant difference between retinopathy group and diabetes with normal fundus group (p=0.025) and no significant difference between diabetes with normal fundus group and controls (p=0.117).
- There was no significant difference between diabetes with normal fundus group, retinopathy group and controls as regard their cholesterol level (p-value=0.209).
- Triglycerides in diabetes with normal fundus group was significantly higher than controls (p=0.007), No significant difference between retinopathy group and controls (p=0.053) and no significant difference between diabetes with normal fundus group and retinopathy group (p=0.356).
- There was no significant difference between diabetes with normal fundus group, retinopathy group and controls as regard their HDL level (p=0.213).
- There was no significant difference between diabetes with normal fundus group, retinopathy group and controls as regard their LDL level (p=0.183).
- HA1C is highly significant in diabetes with normal fundus group than controls (p=0.000), also HA1C is highly significant in retinopathy group than controls (p=0.000) and no significance between retinopathy group and diabetes with normal fundus group (p=0.914).

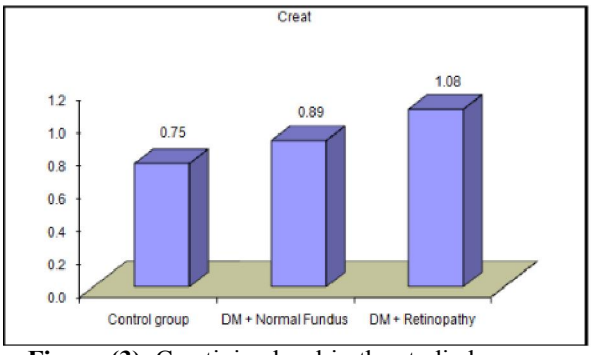

Figure (3). Creatinine level in the studied groups.

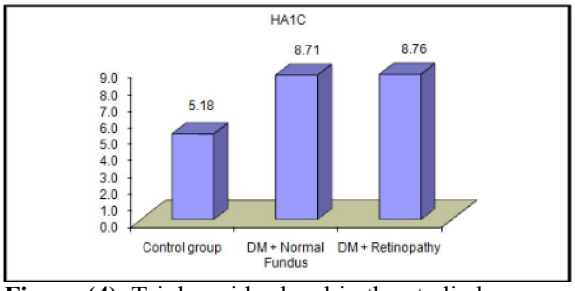

Figure (4). Triglycerides level in the studied groups.

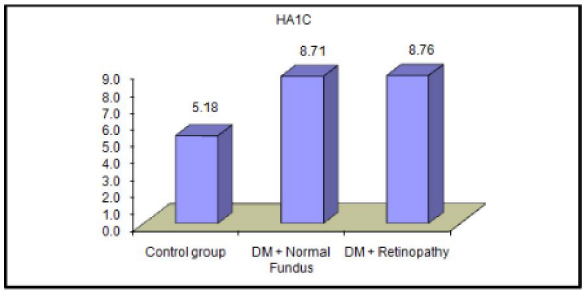

Figure (5). Glycated hemoglobin in the studied groups.

Table (3): Shows comparison between studied groups according to log hsCRP and log PTX3.

		Control group	DM + Normal Fundus	DM + Retinopathy	One Way ANOVA test		Post Hoc analysis		
		No. = 20	No. = 30	No. = 50	F	P-value	P1	P2	P3
log hsCRP (pg/ml)	Mean ± SD	2.6 ± 0.35	2.76 ± 0.33	3.09 ± 0.2	18.537	0.000	0.062	0.000	0.000
	Range	1.88 – 2.99	1.94 – 3.12	2.43 – 3.42					
log PTX3 (pg/ml)	Mean ± SD	2.89 ± 0.17	2.91 ± 0.32	3.22 ± 0.15	18.284	0.000	0.751	0.000	0.000
	Range	2.6 – 3.13	2.23 – 3.26	2.75 – 3.45					

F=Anova test **no**=number **SD**=standard deviation Independent t-test: **P1**: control group versus DM + Normal fundus. **P2**: control group versus DM + Retinopathy. **P3**: DM + Normal fundus versus DM + Retinopathy. **Log hsCRP**=log transformed value of high-sensitivity C-reactive protein. **Log PTX3**= log transformed value of Pentraxin 3.

Table (3) shows:

- As regard log hsCRP there is no significance between diabetes with normal fundus group and controls (p=0.062), log hsCRP is highly significant in retinopathy group than controls (p=0.000) and highly significance in retinopathy group and diabetes with normal fundus group (p=0.000).
- As regard log PTX3 there is no significance between diabetes with normal fundus group and controls (p=0.751), log PTX3 is highly significant in retinopathy group than controls (p=0.000) and highly significance in retinopathy group and diabetes with normal fundus group (p=0.000).

Table (4): Shows correlation between log PTX3 and other measured laboratory parameters.

log PTX3	All Patients		DM + Normal Fundus		DM + Retinopathy		NPDR		PDR	
	r	P-value	R	P-value	r	P-value	R	P-value	r	P-value
log hsCRP	.751**	0.000	.778**	0.000	0.603**	0.000	.661**	0.002	.661*	0.038
Age	.767**	0.000	.902**	0.000	0.618**	0.000	.759**	0.000	0.607	0.063
BMI	.306*	0.018	0.050	0.795	-0.035	0.853	0.122	0.607	-0.523	0.121
Duration	.486**	0.000	0.008	0.968	-0.118	0.535	-0.299	0.201	0.291	0.415
Systolic BP	0.216	0.097	.577**	0.001	0.078	0.681	0.061	0.798	0.124	0.732
Diastolic BP	0.105	0.423	0.321	0.084	0.215	0.253	0.244	0.301	0.113	0.757
AST	0.089	0.498	-0.066	0.727	0.076	0.691	0.191	0.421	-0.622	0.055
ALT	0.091	0.490	-0.045	0.811	-0.011	0.955	0.058	0.808	-0.433	0.211
Creatinine	0.196	0.132	-0.213	0.259	0.331	0.074	0.319	0.170	0.527	0.117
Cholesterol	0.006	0.966	-0.036	0.851	0.000	0.999	0.182	0.442	-.699-*	0.024
Triglycerides	-0.236	0.070	-0.134	0.479	-0.126	0.506	0.067	0.779	-0.401	0.250
HDL	-0.100	0.446	-0.171	0.368	-0.027	0.888	0.030	0.899	-0.232	0.519
LDL	0.044	0.737	0.059	0.758	0.042	0.824	0.217	0.359	-.657-*	0.039
HA1C	0.184	0.160	0.304	0.102	0.156	0.412	0.220	0.351	0.043	0.907

BMI: body mass index, **no**=number, **AST**=Aspartate aminotransferase, **ALT**=Alanine aminotransferase
HbA1C: glycated hemoglobin, **HDL**: high density lipoprotein,
LDL: low density lipoprotein**, r**: Pearson correlation coefficient.
BP=blood pressure
NPDR= non-proliferative diabetic retinopathy, **PDR**= proliferative diabetic retinopathy.
Log hsCRP=log transformed value of high-sensitivity C-reactive protein.
Log PTX3= log transformed value of Pentraxin 3.

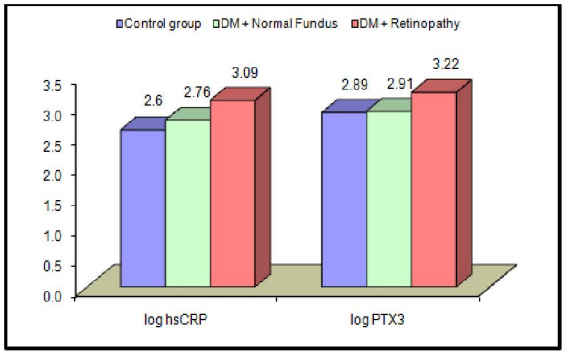

Figure (6). Comparison between log PTX3 and log hsCRP in the studied groups.

Table (4) shows:

- In all patients log PTX3 is highly significant with (log hsCRP, age, duration), significant with BMI and not significant with (AST, ALT, Creatinine, Cholesterol, triglycerides, blood pressure, LDL, HDL and HA1C).
- In diabetes with normal fundus group log PTX3 is highly significant with (log hsCRP and age), significant with systolic blood pressure and no significance with (duration, AST, ALT, Creatinine, Cholesterol, blood pressure, LDL, HDL and HA1C).
- In retinopathy group log PTX3 is highly significant with (log hsCRP and age) and no significance with (duration, blood pressure, AST, ALT, Creatinine, Cholesterol, LDL, HDL and HA1C).
- In NPDR group log PTX3 is highly significant with age, significant correlation with log hsCRP and no significant correlation with (duration, blood pressure, AST, ALT, Creatinine, Cholesterol, blood pressure, LDL, HDL and HA1C).
- In PDR group log PTX3 is significant with (log hsCRP, cholesterol, LDL) and no significant correlation with (age, duration, blood pressure, AST, ALT, Creatinine, Cholesterol, blood pressure,, HDL and HA1C).

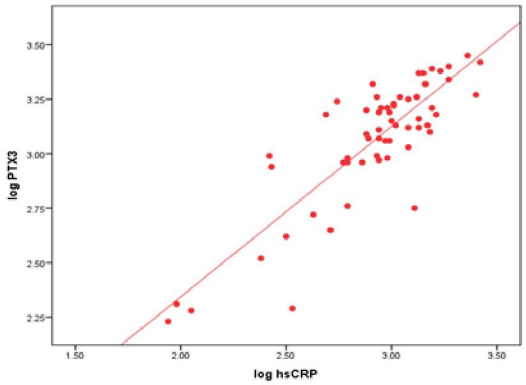

Figure (7). Correlation between log hsCRP and log PTX3 in all patients.

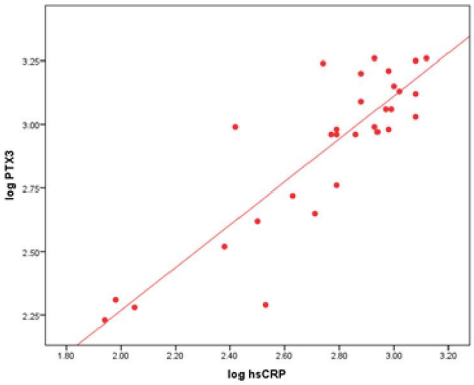

Figure (8). Correlation between log hsCRP and log PTX3 in diabetes with normal fundus group.

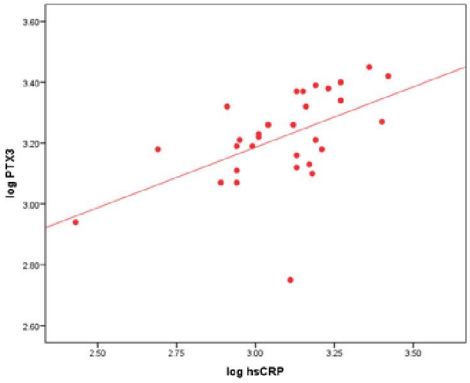

Figure (9). Correlation between log hsCRP and log PTX3 in diabetes with retinopathy.

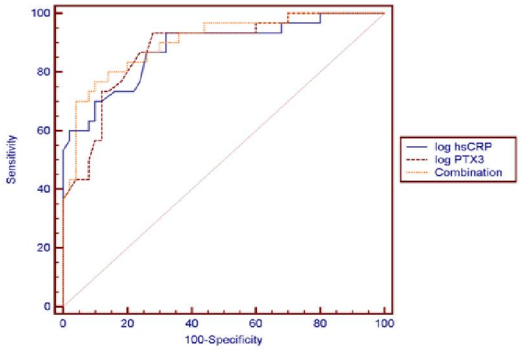

Figure (10): ROC curve for detection of the best cutoff point of serum PTX3 and hsCRP in case of diabetic retinopathy.

- At cut off point of 760 pg/ml for hsCRP, the sensitivity is 93.3%, specificity 68%, positive predictive value 63.6% and negative predictive value 94.4%.
- At cut off point of 1150 pg/ml forPTX3, the sensitivity is 93.3%, specificity 72%, positive predictive value 66.6% and negative predictive value 94.7%.
- With both hsCRP and PTX3 the sensitivity is 76.7%, specificity 90%, positive predictive value 82.1% and negative predictive value 86.5%.

Table (5) shows cut off point, sensitivity and specificity of log_hsCRP and log_PTX3

	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
log_hsCRP, pg/ml	> 2.88 = 760 pg/ml	0.882	93.3	68	63.6	94.4
log_PTX3, pg/ml	> 3.06 = 1150 pg/ml	0.88	93.3	72	66.7	94.7
Combination	-	0.902	76.7	90	82.1	86.5

Conclusion

1. Serum PTX3 level significantly increased in patients with DR more than patients without DR with cut off point 1150 pg/ml, sensitivity 93.3%and specificity 72%.
2. Serum CRP level significantly increased in patients with DR more than patients without DR with

cut off point of 760 pg/ml has sensitivity 93.3% and specificity 68%.

3. Combined use of PTX3 and CRP decrease sensitivity to 76.7%. but combined use increase specificity to 90%.

4. Significant relation between diabetes duration and progression of DR.

Significant relation between poor glycemic control and development of DR and its severity.

Reference:

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–1053.
2. Goksen D, Levent E, Kar S, Ozen S, Darcan S. Serum adiponectin and hsCRP levels and non-invasive radiological methods in the early diagnosis of cardiovascular system complications in children and adolescents with type 1 diabetes mellitus. J Clin Res Pediatr Endocrinol. 2013;5: 174–181.
3. Mugabo Y, Li L, Renier G. The connection between C-reactive protein (CRP) and diabetic vasculopathy. Focus on preclinical findings. Curr Diabetes Rev. 2010;6:27–34.
4. Muni RH, Kohly RP, Lee EQ, Manson JE, Semba RD, Schaumberg DA. Prospective study of inflammatory biomarkers and risk of diabetic retinopathy in the diabetes control and complications trial. JAMA Ophthalmol. 2013;131:514–521.
5. Mantovani A, Garlanda C, Doni A, Bottazzi B. Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3. J ClinImmunol. 2008;28:1–13.
6. Yang HS, Woo JE, Lee SJ, Park SH, Woo JM. Elevated plasma pentraxin 3 levels are associated with development and progression of diabetic retinopathy in Korean patients with type 2 diabetes mellitus. Invest Ophthalmol Vis Sci. 2014;55:5989–5997. DOI:10.1167/iovs.14-14864.
7. Shindo A, Tanemura H, Yata K, et al. Inflammatory biomarkers in atherosclerosis: pentraxin 3 can become a novel marker of plaque vulnerability. PLoS One. 2014; 9:e100045.
8. Zhou Y, Ni Z, Zhang J, et al. Plasma pentraxin 3 may be a better marker of peripheral artery disease in hemodialysis patients than C-reactive protein. Vasc Med. 2013;18:85–91.

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