THE RELATIONSHIP AMONG PLASMA TRANSFORMING GROWTH FACTOR BETA1, OCULAR PERFUSION PRESSURE AND GLAUCOMA IN-PATIENT ATTENDING GLAUCOMA CLINIC AT MAKKAH SPECIALIST EYE HOSPITAL, KANO, KANO STATE, NIGERIA

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ABSTRACT

This research investigated the relationship between plasma TGF-β1 and POAG among 96 participants comprising 60 glaucoma cases that served as study group and 38 apparently normal individuals that served as control group. The clinical parameters extracted from the subjects' files were intraocular pressure (IOP) and cup-disc (C/D) ratio, while Age and BP were directly obtained from subjects. Blood samples were obtained from the subjects, and subjected to chemical analysis using sandwich enzyme linked immunosorbent assay (sELISA) to assess the plasma concentration of transforming growth factor beta1 (TGF-β1) and thrombospondin-1 (TSP-1) levels. The mean arterial pressure (MAP) and mean ocular perfusion pressure (MOPP) were calculated. Data were analysed using Mann-Whitney test and Spearman's ranking methods. The plasma TGF-β1 level in glaucoma subjects was found out to be higher than normal controls (P=0.040) and a significant (P=0.009) decreased value of MOPP in glaucoma subjects than the controls. Furthermore, this study found significant positive association between TGF-β1 versus MAP (P=0.000) and MOPP (P=0.004) among subjects with POAG and significant positive association between TGF-β1 versus Age (P=0.033), MAP (P=0.006) and MOPP (P=0.024) among non-glaucoma subjects. The rise in plasma TGF-β1 together with a fall in MOPP values are believed to lead to deterioration of the optic nerve head which eventually leads to optic nerve atrophy. Therefore, there is a possibility for the use of plasma TGF-β1 as a biomarker to monitor the progression of glaucoma disease and MOPP should be evaluated in all glaucoma patients.

(Keywords: Transforming Growth Factor -Beta1, Ocular Perfusion Pressure, Kano, Nigeria)

INTRODUCTION

Glaucoma is defined as a group of disorders characterized by a progressive optic neuropathy resulting in a characteristic appearance of the optic disc and a specific pattern of irreversible visual field defects that are associated with frequently but not without raised IOP (Khurana (2007). Emphasis has shifted from elevated intraocular pressure as the only damaging factor in glaucoma to it being one of the factors in the glaucoma damage in which retinal ganglion cells (RGCs) apoptosis occurs. Agarwal *et al.,* (2009) reported that apoptosis is the primary and early cause of RGCs death in glaucoma, then necrosis occurs in the late stage of the disease process. Primary factors of glaucoma toxicity are based on two theories; mechanical theory resulting from elevated IOP and vascular insufficiency theory resulting from the decreased ocular perfusion pressure. The secondary factors include toxic effects of glutamate, oxygen free radicals and nitric oxides released during RGCs apoptosis due to primary insults (Hendrick *et al.,* 1994; Chauhan, 1995). Frequently, glaucoma is associated with a higher than normal pressure inside the eyeball (Coleman, 1999; Henson *et al.*, 2000). It has been reported that mean IOP>25.75mmHg had 13% risk of developing glaucoma. However, mean IOP>23.75 but IOP≤25.75mmHg had 10% risk of developing glaucoma, while mean IOP<23.75mmHg had 9% risk of developing glaucoma in individuals with normal central corneal thickness between 555µm to 588µm (Kanski, 2016). Glaucomatous changes have been observed in individuals with normal IOP, Chauhan (1995) suggesting a critical role of other factors in the initiation and progression of glaucomatous changes. Studies have shown an association between vascular insufficiency and glaucoma; diastolic ocular perfusion pressure (DOPP) ≤40mmHg or mean ocular perfusion pressure (MOPP) ≤50mmHg was found to be associated with 1.9 and 3.6 times increase in POAG respectively (Memarzadeh *et al.,* 2010).

Other risk factors of developing glaucoma in healthy subjects include old age (above 40years), the peculiar larger optic disc structure of black people, a positive family history, and vascular factors such as systemic hypertension, perfusion pressure, vasospasm, atherosclerosis and acute hypotension, so also diabetes, myopia, a history of typical migraine headaches, and thinner central corneal (Wolfs *et al.,* 1998; Omoti & Edema, 2007).

Current screening techniques have poor sensitivity and are unable to diagnose early POAG; if elevated intraocular pressure (IOP) is used to screen for POAG, more than 50% of POAG patients have an IOP that is <21 mmHg (Sommer, *et al.,* 1991). Also, screening using automated perimetry for glaucomatous visual field defects lacks the resolution to detect early POAG, as >35% of the retinal ganglion cells can be lost before any visual field defects is observed (Kerrigan-Baumrind *et al.,* 2000). Over half of individuals with POAG are undiagnosed or untreated (Shaikh *et al.,* 2014). Vision loss from glaucoma is silent, slow, progressive, irreversible, and preventable (Robert, 2008).

Transforming growth factor beta isoforms, TGF-β1, -β2, and -β3, are small (25 kDa) secreted dimeric signaling proteins (multifunctional polypeptides) that regulate many essential cellular processes in many parts of the body including liver, lungs, skin and eye structures (Tandon *et al.,* 2010; Huang *et al.,* 2014).

Transforming growth factor beta1 (TGF-β1) can serve as a nutrition factor and anti-apoptotic factor; offering a self-protection mechanism for apoptosis of RGCs (Tao *et al.,* 2011). The gradual morphogenesis leading to apoptosis can allow for neuro-protective intervention of TGF-β1 and hence salvage the RGCs. Changes in TGF-β1 secretion within the eye might be detectable as changes in the plasma concentration of TGF-β1 just as in the aqueous humor (AH)

of glaucoma patients (Kuchtey & Kuchtey, 2014). But increased TGF-β1 concentration causes chemotaxis of pro-inflammatory cells such as monocytes, lymphocytes, neutrophils. Also, it causes fibroblasts and vascular growth factors production, extracellular matrix (ECM) remodeling and angiogenesis (Padua & Massaque, 2009). The initiation of fibroblast proliferation process leads to cascade of events that leads to collagen production, amplification and decreased ECM degradation, resulting to scar formation in Trabecular meshwork (Liu, Wan, & Cao, 2004). Previous studies (Kuchtey *et al.,* 2014) did not consider the consequences of increased blood latent TGF-β1 concentration on the vessels. Therefore, assessment of plasma TGF-β1 and its relation to ocular perfusion and POAG was investigated in glaucoma patients in this study.

METHODOLOGY

The study was conducted at Makkah Specialist Eye Hospital located in Kano metropolis, Kano State. From clinic attendance register, a range of 80-100 patients are seen per clinic day; there were two glaucoma clinics and two general out patients clinics in a week giving a total glaucoma cases of 160-200 per week. With an average of 180 glaucoma patients attending the clinic per week. It was a cross sectional study of individuals attending glaucoma out patients clinic and systematic random sampling (ensuring selection of equal number males and females, within the age of 40-85years) was employed to select subjects for the study. Every subject was selected according to the inclusion and exclusion criteria from both the male and female clinics. Subjects who were diagnosed as glaucomatous (60) formed the study group while subjects who were diagnosed as non-glaucomatous (36) formed the control group. Individuals were diagnosed as glaucomatous by the consultant ophthalmologist treating them; if they had cup-disc ratio (CDR)

≥0.60, IOP >21mmHg and visual field defects while those with CDR <0.6, IOP ≤21.0mmHg and no visual field defect were regarded as non-glaucomatous. Those who had vascular diseases (hypertension, diabetes, migraine), myopia >5.00DS and neurological problems were excluded. Ethical approval was obtained from the Kano State Hospital Management Board. Participants were requested to sign the informed consent form before commencement of research after thorough explanation of the research purpose and procedures to them. The study was in line with the rules and regulations of the declaration of Helsinki 1964 as amended (World Medical Association, Brazil, 2013). After subjects signed the informed consent form, the clinical parameters extracted from the subjects files were IOP, C/D ratio while information about age and BP measurement were directly obtained from the cohorts. Some demographic data including family history were obtained from subjects directly using data captured questionnaire. Blood samples were obtained from the subjects, then subjected to chemical analysis using sELISA obtained from Bioassay Laboratory Technology (Shanghai, China). The assay method was based on the receptor binding non-acid activation of the plasma samples and had a detection sensitivity and range for TGF-β1 of 0.00511ng/ml and 0.001-4.00ng/ml and for TSP-1 of 0.00239µg/ml and 0.005-0.70µg/ml as case may be.

The MOPP in all subjects were determined using the formula below (Van Keer *et al.,* 2016):

$$MOPP = \frac{2(MAP)}{3} - IOP$$

Data were presented as median and analyzed using SPSS 21.0 (SPSS Inc, Chicago, IL). Median samples were compared using Mann-Whitney test. Probability value was set at P=0.05 (P≤0.05 was considered significant). The association between TGF-β1, and other ocular physiologic parameters in the two groups were tested using Spearman's correlation coefficient.

RESULTS

Table 1: Distribution of Socio-demographic Characteristics of POAG and Non-glaucoma Subjects

Variables	POAG Total (n= 60), n (%)	Non-glaucoma Total (n= 36), n (%)	Statistic	P-value
Age group (years)				
40-49	12 (20.0)	9 (25.5)	0.133	
50-59	16 (26.7)	6 (16.7)		0.715
60-69	21 (35.0)	12 (33.3)		
70 and above	11 (18.3)	9 (25.0)		
Sex				
Male	30 (50.0)	18 (50.0)	0.000	1.000
Female	30 (50.0)	18 (50.0)		
Residence				
Urban	29 (48.3)	15 (41.7)		
Rural	23 (38.3)	15 (41.7)	0.448	0.503
Semi-urban	8 (13.3)	6 (16.6)		
Education				
Informal	15 (25.0)	8 (22.2)		
Primary (1^0)	8 (13.3)	7 (19.4)	0.115	0.735
Secondary (2^0)	15 (25.0)	10 (27.8)		
Tertiary (3^0)	22 (36.7)	11 (30.6)		
Occupation				
Workers (Private & Public)	18 (30.0)	10 (27.8)		
Petty Traders	7 (11.7)	8 (22.2)		
Retirees	11 (18.3)	2 (5.6)	0.102	0.750
House Wives	13 (21.7)	13 (36.1)		
Farmers	11 (18.3)	3 (8.3)		
Family History of Glaucoma				
Positive (+)	55(91.7)	5(13.9)	-7.581	0.000*
Negative (-)	5(8.3%)	31(86.1)		

*Statistical significance at $p \leq 0.05$

The distribution of socio-demographic characteristics of the participants is shown in table 1 above. All parameters were analysed between glaucoma and non-glaucoma groups using Kruskal Wallis test while Family history was analysed using Mann-Whitney test. Subjects comprised of 48 (50.0%) males and 48 (50.0%) females giving a total of 96 participants. Urban dwellers, tertiary level of education, workers and house wives constitute the majority of the participants in both groups. Also, majority of the subjects have positive family history of glaucoma.

Table 2: Variation of Age and Some Ocular Physiologic Parameters between POAG subjects and Non-glaucoma Subjects

Variables	**POAG**	**Non-glaucoma**		
	Median (IR)	Median (IR)	Statistic	P-value
Age (Year)	60.00(15.00)	60.00(21.00)	-0.638	0.523
MAP (mmHg)	98.50(15.00)	95.00(9.50)	-1.011	0.312
MOPP (mmHg)	43.00(13.00)	48.00(9.00)	-2.622	0.009*
IOP (mmHg)	21.00(8.00)	16.00(4.75)	-4.838	0.000*
C/D Ratio	0.85(0.10)	0.40(0.10)	-8.263	0.000*
TGF-β1 (ng/ml)	1.40(0.50)	1.20(0.40)	-2.051	0.040*
TSP-1 (μg/ml)	0.23(0.08)	0.21(0.05)	-1.035	0.301

*Statistical significance at $p<0.05$, IR= Interquartile Range, TGF-β1=Transforming Growth Factor-Beta1, TSP-1= Thrombospondin-1

Table 2 shows the Mann-Whitney test result for age and some ocular physiologic parameters between POAG subjects and non-glaucoma subjects. It was shown that the median concentration of TGF-β1 (ng/ml) among POAG subjects (1.40ng/ml) was higher than that of non-glaucoma subjects (1.20 ng/ml) which was statistically significant (P=0.040). On the other hand, the median of mean ocular perfusion pressure (MOPP) was lower in POAG subjects (43.00mmHg) than in non-glaucoma subjects (48.00mmHg) which was also statistically significant (P=0.009). Besides, median IOP and C/D ratio were higher in POAG subjects (21, 00 and 0.85, respectively) than in non-glaucoma (16.00 and 0.4, respectively).

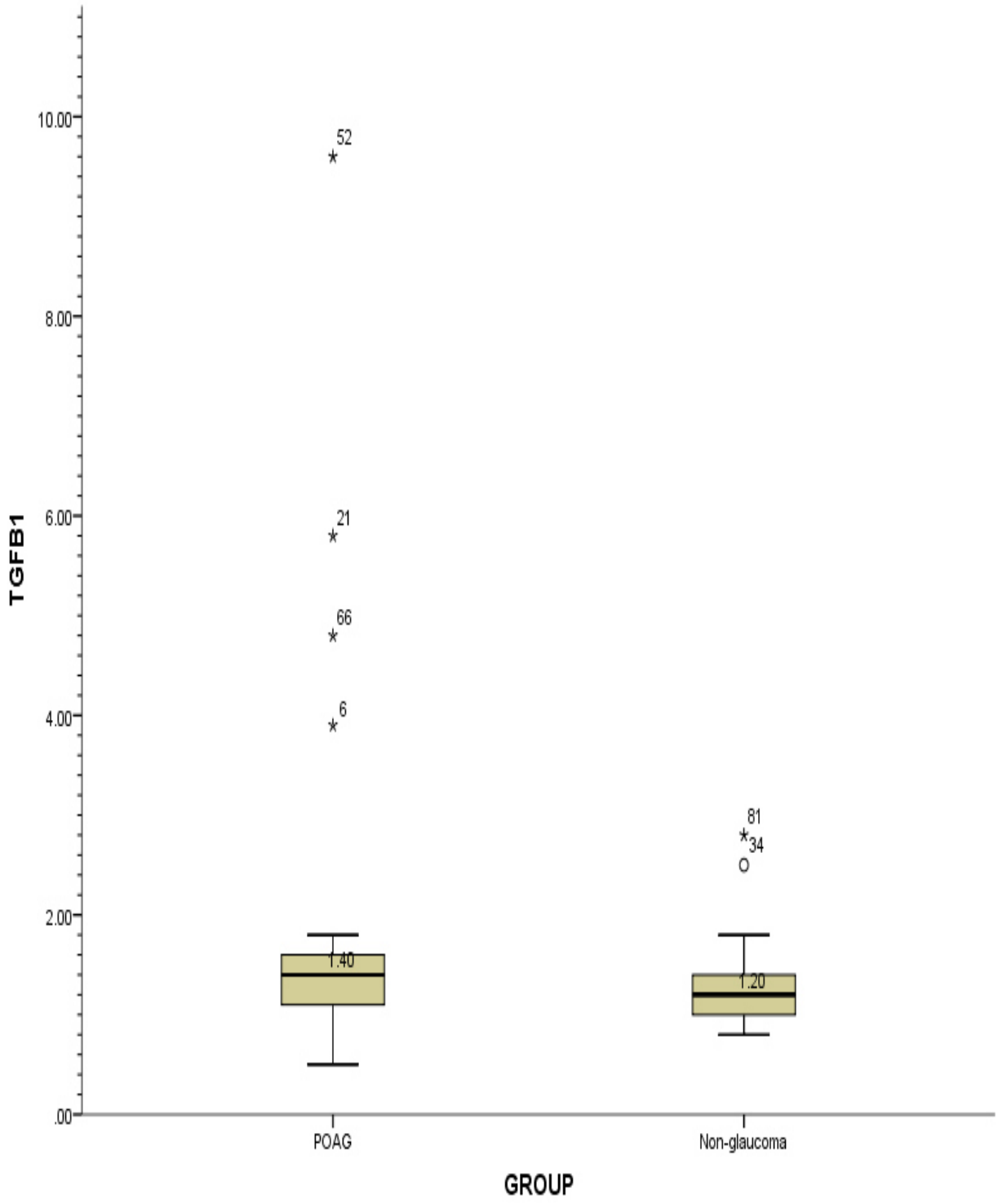

Figure 1: A Plot of Median Interquartile Range of TGF-β1 (ng/ml) between POAG and Non-glaucoma Subjects

Figure 1 above shows a boxplot of the median interquartile range of TGF-β1 (ng/ml) concentration among POAG was from 0.90ng/ml to 1.90ng/ml which was higher than of the non-glaucoma subjects, being from 0.80ng/ml to 1.60ng/ml. Also, the graph shows some outliers among POAG subjects.

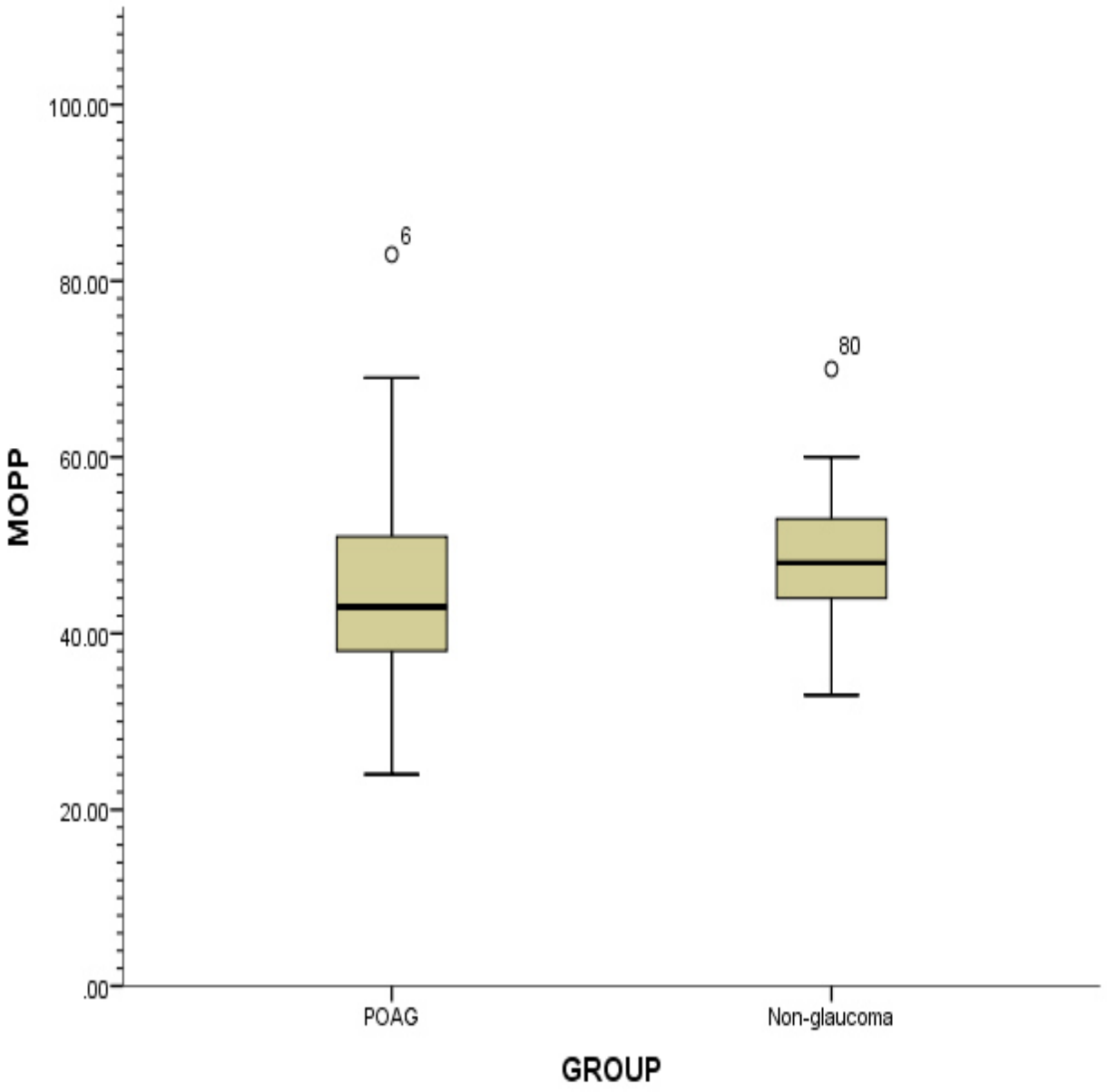

Figure 2: A Plot of Median Interquartile Range of MOPP (mmHg) between POAG and Non-glaucoma Subjects

Figure 2 above shows a boxplot of the median interquartile range of MOPP (mmHg) among POAG was from 30.00mmHg to 56.00mmHg, though median value was lower but it had higher than that of the non-glaucoma subjects; being from 39.00mmHg to 57.00mmHg. Being higher means that more subjects were within the median of the POAG than non-glaucoma; showing that more of subjects had lower MOPP. Also, the graph shows very few outliers among POAG and non-glaucoma subjects.

Table 3: Spearman's Correlation between TGF-β1 (ng/ml) and Some Ocular Physiologic Parameters among POAG Subjects

Variables	**r_s**	**P-value**
Age (year)	0.126	0.337
MAP (mmHg)	0.503	0.000*
MOPP(mmHg)	0.364	0.000*
IOP (mmHg)	0.002	0.990
C/D ratio	-0.118	0.368
TSP-1(μg/ml)	-0.108	0.411

*Statistical significance at $p<0.05$

As shown in table 3 above, the Spearman's correlation test of association between TGF-β1 (ng/ml) concentration in the plasma and age, and some ocular physiologic parameters demonstrated a significant association with MAP (P=0.000) and MOPP (P=0.000).

Table 4: Spearman's Correlation between TGF-β1 (ng/ml) and Some Ocular Physiologic Parameters among Non-glaucoma Subjects

Variables	r_s	P-value
Age (year)	0.353	0.033*
MAP (mmHg)	0.447	0.006*
MOPP(mmHg)	0.375	0.024*
IOP (mmHg)	-0.269	0.113
C/D ratio	-0.016	0.928
TSP-1 (μg/ml)	-0.016	0.928

*Statistical significance at $p<0.05$

Spearman's correlation test shown in table 4 above revealed significant positive association of TGF-β1 (ng/ml) concentration in the plasma with Age (P=0.033), MAP (P=0.006) and MOPP (P=0.024).

DISCUSSION

This study was done to evaluate relationship among plasma TGF-β1, ocular perfusion pressure and primary open angle glaucoma (POAG). Among recruited 96 subjects cohorts comprising of 60 glaucoma subjects as study group and 38 non-glaucoma subjects that served as control group.

The significant association (P=0.000) between the family history and glaucoma as found in this study shows that those with glaucoma had more positive family history than those without glaucoma. This demonstrates that subjects who had their fathers, mothers or siblings with glaucoma have higher chances of developing glaucoma than those without family history of glaucoma. Hence, the finding of more glaucoma subjects with positive family history provides more evidence in support of positive family history as a risk factor for POAG as documented by other studies (Wolfs *et al.,* 1998; Omoti & Edema, 2007; Kyari *et al.,* 2015).

There was no significant difference (P=0.523) in the median age of subjects between the study and control groups indicating that there was no difference in the age of subjects in both the study and control groups making the groups to be comparable but not contrastable. On the other hand, there was a significant difference in the median C/D ratio (P=0.000) and median IOP (P=0.000) of subjects between the study and control groups, that demonstrated that both groups were actually independent of each other because those were variables that qualified subjects placement into each group.

According to Wakefield *et al.* (1995) normal human subjects had mean TGF-β1 of 4.1 ± 2.0 ng/ml in acid activated plasma, but in non-acid activated plasma (with sodium citrate or EDTA as anticoagulant) assays reported TGF-β1 concentrations well <0.1ng/ml or not detectable at all (Kropf *et al.,* (1997). In studies using sELISA receptor binding non-acid activation of plasma method to quantify the TGF-β1 concentration, normal plasma mean TGF-β1 concentrations of 0.121±0.007ng/ml was found (Jiang *et al,* 2013). In addition to receptor binding, acid activation of plasma method reported a higher normal plasma active plus latent (total) concentration of mean TGF-β1 to be 2.10ng/ml and 2.46ng/ml in two different studies (Kuchtey *et al.,* 2014; Zhang *et al.,* 2016). Also, a normal median plasma concentration of TGF-β1 was reported to be 2.5ng/ml using acid activation method (Mia *et al.,* 2019). The possibility of detecting active plasma TGF-β1concentration in glaucoma using non-acid activation method was reported by Kuchtey *et al.,* (2014).

This study found a significant (P=0.040) elevation of plasma TGF-β1 (0.2ng/ml) concentration in POAG subjects as compared to non-glaucoma subjects, but a higher plasma concentration of TGF-β1 in POAG subjects than controls was reported in the study of Kuchtey *et al.,* (2014)

because they measured total plasma TGF-β1 concentration. It is obvious from this finding that people with POAG had elevated plasma TGF-β1 level. It is believed that dysregulated activation of latent TGF-β1 increased the expression of small latent TGF-β1 complex by the ECM of lamina cribosa of the optic nerve head, and supporting cells of RGCs and rendering it available in the blood vessels where they are being activated. This is in line with other studies that found elevated blood TGF-β1 level in patients with glaucoma (Burgoyne, 2011; Quigley, 2011; Kuchtey *et al.,* 2014). It was documented that as the period of stress prolonged TGF-β1 use for signaling decreases due to abnormal inhibition, remodeling of the microfibrils or microfibrils defects producing inactivated latent TGF-β1 that were not utilized for signaling (Agapova, 2001; Kuchtey & Kuchtey, 2014) leading to elevated plasma TGF-β1 and then apoptosis of RGCs takes lead as reported by Tao *et al.,* (2011). Also, this is consistent with the studies that found increased TGF-β1 concentration with rise in IOP indicating that mechanical stretch induced optic nerve head astrocytes and trabecula meshwork cells expression of high level of TGF-β1 (Kirwan *et al.,* 2004; Kuchtey & Kuchtey, 2014).

Likewise, this study found a significant decrease (P=0.009) in median ocular perfusion pressure (MOPP) in POAG subjects than the non-glaucoma subjects which is in line with other studies that reported lower MOPP values in subjects with glaucoma (Tsai, 2009; Memarzadeh, 2010; Cherecheanu, 2012; Budenz *et al.,* 2018). This finding provides more evidence in support of vascular insufficiency theory in the pathogenesis of glaucoma as documented by Agarwal *et al.,* (2009).

Platelet TGF-β1 intervening to the plasma TGF-β1 was determined by quantifying platelet TGF-β1 activator Thrombospondin-1 (TSP-1) and it was used as a marker of platelet activation in

other studies (McGillicuddy *et al.,* 2006). In this study there was no significant difference (P=0.301) in the level of TSP-1 between glaucoma subjects and controls and with no correlation between TGF-β1 and TSP1 in both POAG (P=0.411) and non-glaucoma subjects (P=0.928), indicating that platelets do not contribute much to the plasma TGF-β1 which is consistent with previous study that platelets do not contribute to the TGF-β1 level in citrated plasma (Kropf *et al.,* 1997). However, other study that found otherwise may be because of degranulation of platelets (Kuchtey *et al.*, 2014). Platelets which contain high amount of TGF-β1 in their α-granules when released during platelet degranulation constitute intervening molecules to the normal plasma TGF-β1 variable. This could occur during blood collection, using serum TGF-β1, or acid activation of plasma TGF-β1 but it was significantly minimized in this study by precautions taken; minimized injury during venous puncture was ensured, sample transferred to EDTA coated tubes immediately, and centrifuged within two hours of sample collection. TSP-1 is an activator of TGF-β1; expression of TGF-β1 in the trabecular meshwork of glaucoma patients has been shown to be induced by TSP-1 and TSP-1 mRNA expression is induced by stretch activation of cultured lamina cribrosa cells, indicating that level of activated TGF-β1 may be increased in POAG leading to elevated blood TSP-1 and activated TGF-β1 (Flügel-Koch *et al.,* 2004; Kuchtey *et al.*, 2014), but it was not investigated in this study.

The association between TGF-β1 and other ocular physiologic parameters was tested among subjects in both study and Control groups. This study found statistically significant positive association between TGF-β1 and MAP (P=0.000), TGF-β1 and MOPP (P=0.004) in glaucoma subjects. Also, significant positive associations were found between TGF-β1 and MAP (P=0.006), TGF-β1 and MOPP (P=0.024) in non-glaucoma subjects. These same associations of TGF-β1 and MAP or MOPP found in both study and control groups shown that there was no

possible vascular contribution to the increased plasma TGF-β1 in this study. It is believed that the increased level of TGF-β1 upregulates vascular endothelial growth factor (VEGF) which has its consequent scaring effect on vascular endothelium as reported in other study (Sundberg & Rubin, 1996; Ferrari *et al.,* 2009). Furthermore, other studies found increased TGF-β1 with upregulated VEGF and consequent scarring of the vascular endothelium and angiogenesis (Liu *et al.,* 2004; Chang & Wu, 2009; Padua & Massaque, 2009) leading to decreased ocular perfusion among POAG patients. Then, the consequent scaring effect on endothelium will lead to decreased ocular perfusion. Likewise, some studies found that stress increased the expression of TGF-β1 and VEGF inhibited production of matrix metalloproteinase (MMP) and stimulated tissue inhibitors of MMP leading to scaring of lamina cribrosa of optic nerve head (ONH) and trabecular meshwork (TM) stenosis (Sundberg & Rubin, 1996; Chang & Wu, 2009).

In addition to associations of TGF-β1 and ocular physiologic parameters found among non-glaucoma subjects, this study found an association between TGF-β1 and age which was statistically significant (P=0.033) and is consistent with other studies that reported prevalence of about 2% of cases in middle age and 3.85% in above 40years of age (Klein *et al.,* 1992; Coffey *et al.,* 1993; Kyari *et al.,* 2015). This finding provides more evidence in support of advanced age as a risk factor for POAG as documented by Omoti & Edema, (2007); Bowling, (2016) and possibility of elevated plasma TGF-β1 as a risk factor for POAG with advanced age.

The study by Imanishi *et al.,* (2000) concluded that a number of growth factors and cytokines participate in the regulation of ONH astrocytes/ganglion cell proliferation and apoptosis as it occurs in the maintenance of corneal transparency. Other studies had shown that down regulation of TGF-β1 through inhibition of VEGF directly by binding to receptors (e.g Avastatin) or

indirectly by reducing receptor expression (e.g Fluvastatin) can prevent angiogenesis (Ferrari *et al.,* 2009; Robinson *et al.,* 2011). Also, recent study had shown that N-acetylcysteine (NAC) or Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (e.g NOX1/4) inhibitor may be useful in blocking fibrotic effects of TGF-β1 on fibroblastic cells while keeping its level upregulated, thus acting as antifibroblastic agent (Murphy-Marshman *et al.,* 2017).

CONCLUSION

The rise in plasma TGF-β1 and reduced MOPP values in glaucoma subjects are believed to be as a result of its dysregulated activation of TGF-β1 in lamina cribrosa of the optic nerve head which eventually leads to optic nerve atrophy in glaucoma as seen as increased C/D ratio and reduced MOPP irrespective of IOP level. Therefore, TGF-β1 level modulation can help prevent RGCs loss, optic nerve atrophy and there is possibility of plasma TGF-β1 to be used as a biomarker to monitor the progression of glaucoma disease.

LIMITATIONS OF STUDY

This study may have been limited in the small value of plasma TGF-β1 concentration and difference in the TGF-β1 concentration between the POAG subjects and controls because the sELISA method used did not measured total plasma TGF-β1 concentration.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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