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Evaluation of the Level of Lysyle Oxidase and Some Biochemical Parameters in People with Glaucoma

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Annotation: The study sought to quantify a few biochemical factors in glaucoma patients' blood serum. A significant increase in the activity of the lysyl oxidase like -4 (LOXL-4) enzyme in patients' blood serum who have gluacoma of both genders was found statistical significance ($p \leq 0.01$) in the study, which involved 90 healthy and glaucoma patients (ages 50–83). Additionally, by assessing twelve oxidation and antioxidant variables as well as enzyme activity, it sought to compare the effects of oxidative stress on gluacoma patients to those in good condition. In contrast to those in good health, the findings showed a substantial increase in the activity of the LOXL-4 enzyme, malondialdehyde, lipoxygenase, and (TNF-a) at the p-value ($p \leq 0.01$*). Additionally, because of the increased oxidative stress, the data showed that all antioxidants (vit C, vit D, albumin, glutathione, glutathione peroxidase, ceruloplasmin , zinc) were significantly lower in glaucoma patients than in healthy individuals. Gender's impact was also examined.

Keywords: Lysyl oxidase like-4 (LOXL-4), glaucoma, oxidative stress.

Introduction:

A neurological condition called glaucoma results in permanent blindness. It mostly affects elderly people and is believed to rank as the second most common cause of permanent blindness.

The principal characteristic is the reduction in retinal ganglion cells (RGCs) in the eye and their axons in the optic nerve (Jacobi & Van Zyl, 2020). Glaucoma may be classified into two types: primary angle-closure glaucoma (PACG) and primary open-angle glaucoma (POAG).(Wang et al., 2024). The most prevalent kind of glaucoma is called primary open-angle glaucoma, or POAG. An anterior chamber angle that opens or closes, preventing fluid outflow and increasing intraocular pressure, is a characteristic of angle-closure glaucoma, a rare form of glaucoma. Impaired vision and excruciating headache pain are among the symptoms (Krzyzanowska et al., 2022). Increased risk factors for glaucoma disease development include age, genetics, ethnicity, hypertension, diabetes mellitus, cardiovascular disorders, and raised intraocular pressure (IOP) (Fujita et al., 2023). Pharmacological therapy, laser therapy, and surgical interventions are the primary treatments for gluacoma illness (Qureshi et al., 2021). The copper-dependent amine oxidase lysyl oxidase, sometimes referred to as protein lysine 6 oxidase, is essential for the biogenesis of connective tissue matrices because it crosslinks collagen, elastin, and other extracellular matrix proteins. According to NikkalaShree et al. (2023), there are five different forms of lysyl oxidase. According to Tan et al. (2021), the lysyl oxidase-like 4 (LOXL4) is the most recent member of the newly discovered family of lysyl oxidases, some of which have been demonstrated to act as copper-dependent amine oxidases that cause the creation to occur of lysine-derived cross-links in extracellular matrix proteins. In addition to the distinctive domains of the LOX family, such as the cytokine receptor-like domain and the copper-binding domain, LOXL4 also contains Cysteine-rich regions of four scavenger receptors (Wang et al., 2023). The structural and mechanical integrity of connective tissues throughout the body depend on lysyl oxidase. According to Rodriguez-Pascual et al. (2022, (It facilitates the oxidative deamination of collagen and elastin's lysine residues, which leads to reactive aldehydes that spontaneously condense to form mature cross-links.

The aims of study:

1- Investigation of Lysyl Oxidase Like 4 and a few other biochemical factors in patients with gluacoma.

2- Isolation and identification of lysyl oxidase activity in cases of transient glaucoma.

3- Comparison of its levels between glaucoma patients and healthy controls.

Material and method

A cohort of glaucoma patients was included in this study. 45 cases were diagnosed by hospital specialists and underwent laboratory testing in external laboratories between December 1, 2024 and June 1, 2025. Their ages ranged from 50 to 83 years. Each case was surveyed using a questionnaire prepared for this purpose. The control group included 45 healthy men and women, aged between 50 and 83 years. Blood samples were tested for lysyl oxidase and other biochemical parameters

Assessment of serum biochemical variables:

1-Determination of the gluacoma patient's serum level of Lysyl Oxidase Like-4 (LOXL-4): The method used by the researchers allowed them to determine the efficacy of the enzyme Lysyl oxidase like -4.

2. Sirtuin 3 (SIRT3) level determination:

The researchers have established the SIRT3 concentration.

3. The researchers have ascertained the concentration of tumor necrosis factor-α (TNF-α)

by TNF-α levels in blood serum were measured using a sandwich ELISA technique.

4. Assessment of serum lipoxygenase activity

The researchers' methodology allowed them to assess the lipoxygenase (LOX) enzyme's efficacy.

5. Albumin content in blood serum measurement:

Using a prefabricated analysis kit, the albumin was measured using the Bromocresol Green Method, in accordance with the procedure employed by Tietz (1982).

6. The researchers have calculated the efficiency of the enzyme glutathione peroxidase (GPX) concentration (Sunderman and Nomoto, 1970).

7. Serum Ceruloplasmin (Cp) determination:

The researchers have calculated the Cp concentration (Sunderman and Nomoto, 1970).

8. Assessing the level of reduced glutathione (GSH)

The researchers employed a modified method to measure serum glutathione (Sedlak & Lindsay, 1968).

9. Measurement of malondialdehyde (MDA), an oxidant marker

The researcher's method was employed to calculate the quantity of malondialdehyde in blood serum (Buege and Aust, 1973).

10-Determination of Vitamin D3:

The researchers have calculated the concentration of vitamin D3 (Heijboer et al., 2012).

11- measurement of the blood serum's copper (Cu) content:

The researcher has ascertained the concentration of copper (Cu).

12. tenacity of zinc (Zn) level in blood serum:

The researchers have established the concentration of zinc (Zn).

Statistical analysis:

Software called SPSS been put to use to examine the information . Previously, The Duncan tests and the T-test were used to evaluate parameters based on occupancy between the entire control group and patients at $p \leq 0.01$, along with the Pearson correlation test coefficient (Bewick et al., 2004).

Result

The study included, as in Table (1), the effects of oxidative stress on gluacoma patients and comparing them to healthy ones by evaluating antioxidants and oxidation variables, as well as enzyme activity. This was done on 90 samples of patients and healthy individuals, whose 50 to 83 years old was the age range.

The findings supported Table (1) and demonstrated a significant increase the amount of lysyl oxidase-like 4 present in the glaucoma patient population at the probability level ($P \leq 0.01$). These conclusions are in line with those of Boestein et al. (2014). When compared to healthy persons, the blood serum levels of SIRT3, vit D, (Cp, vit C, (GPx) , (GSH), zinc, and copper were considerably reduced ($P \leq 0.01$) in patients with gluacoma illness. MDA , TNF-alpha, and lipoxygenase levels were considerably greater in gluacoma patients than in healthy people. Patients' concentrations dropped as they aged.

Table: 1 Variable Levels in the Blood Serum of glaucoma Patients group as Compared to the Control Group

Biochemical variable	Patient group (No=45)		Control group (No =45)		P – value
	Mean	Std. Deviation	Mean	Std. Deviation	
LOXL-4(Pg /ml(	178.92	44.881	57.15	12.071	0.01**
SIRT 3 (ng /ml)	2.40	0.79	14.87	2.40	0.01**
TNF – α (Pg /ml)	290.68	41.85	8.49	1.37	0.01**
Lipoxygenase (U/L)	115.52	62.38	57.65	5.70	0.01**
Albumin (g/l)	27.90	6.20	59.71	4.98	0.01**
Glutathione peroxidase (U/L)	65.31	11.51	134.15	23.36	0.01**
Ceruloplasmin (g/dl)	0.44	0.10	1.02	0.48	0.01**
Glutathione (µmol/L)	9.07	1.72	44.65	8.14	0.01**
Malondialdehyde (µmol/L)	2.27	0.56	0.19	0.15	0.01**
Vitamin C (mg/dl)	0.06	0.02	0.28	0.14	0.01**
Vitamin D (nmol/L)	13.24	1.54	24.73	1.60	0.01**
Zinc (µg/dl)	79.17	6.87	107.84	5.65	0.01**
Copper (µg/dl)	139.46	15.92	228.25	25.49	0.01**

Several factors influencing biochemical factors measured in gluacoma patients' blood serum were examined in relation to a control group.

1. The Effect of Gender:

The impact of gender on the group of gluacoma patients and the control group was investigated, as was the impact of gender on all biochemical variables assessed in the patient and control groups. Hormonal variations between men and females create variances in gluacoma levels, with estrogen protecting females against gluacoma while androgens increase the risk (Wagner et al., 2008).

Tables 2, 3, and 4 provide the biochemical test results for both men and women glaucoma patient groups. At the probability level, the sex factor was shown to have a significant effect on the majority of the chemical components and the LOXL-4 enzyme's amount that were analyzed (sirt3, TNF-α, LOP, CP, GSH, vitamin C, vitamin D, Zn, and Cu) ($P \leq 0.01$).

Glutathione peroxidase, malonaldehyde, and albumin concentrations did not vary substantially by sex at The degree of probability ($P \leq 0.05$); nevertheless, the average concentrations of lipoxygenase, albumin, TNF -a, and GSH were greatest in the female group (Patel et al., 2018).

SIRT3 levels in the male and female patient groups were significantly lower ($P \leq 0.01$) than in the control group, as indicated by Tables (2), (3), and (4). The decline is brought on by a higher inadequacy in the removal and defense against free radicals generated by oxidative processes.

There were no appreciable variations in albumin levels.

In comparison to the control group, MDA and lipoxygenase levels rose significantly ($P < 0.01$) in patients, both male and female , with the MDA increase being greater in males. The findings showed that the levels of Zn, Cu, and vitamin D were significantly lower ($P \leq 0.01$) in the patient group (males and females) than in the control group (males and females), and that the level was lower in the female patients than in the male patients. Lack of zinc, copper, and vitamin D in glaucoma patients is typically brought on by a complicated interplay of chronic inflammation, oxidative stress, and deficits in food or absorption. This deficiency could lead to or be the cause of the onset of sickness (Zarei-Ghanavati et al., 2020).

Table (2): The levels of chemical variables in men with glaucoma disease were compared to those in men in good health.

Variable	Patient group (No=23) Males		Control group (No =23) Males		p-value
	Mean	Std. Deviation	Mean	Std. Deviation	
LOXL4	222.81	7.54	46.25	4.34	0.01**
SIRT 3	3.14	0.199	17.11	0.673	0.01**
TNF -α	250.51	3.666	7.34	0.650	0.01**
Lipoxygenase	55.25	2.979	52.35	1.860	0.01**
Albumin	27.87	7.220	57.93	4.662	0.01**
Glutathione peroxidase	76.01	3.292	154.64	3.633	0.01**
CP	0.43	0.120	0.84	0.396	0.01**
GSH	8.49	1.320	47.40	5.476	0.01**
MDA	2.34	0.528	0.19	0.117	0.01**
Vitamin D	14.69	0.371	26.12	0.766	0.01**
Zinc	85.76	0.824	113.22	1.043	0.01**
Copper	154.82	1.251	252.89	1.209	0.01**

Table: 3 Variable Levels in the Blood Serum of Female gluacoma Patients Compared to Females in Control

variable	Patient group (No=22) Females		Control group (No =22) Females		P-value
	Mean	Std. Deviation	Mean	Std. Deviation	
LOXL4	135.04	5.649	68.54	4.428	0.01**
Sirtuin 3	1.61	0.165	12.53	0.637	0.01**
Tumor necrosis factor – α)	332.26	6.476	9.69	0.752	0.01**
Lipoxygenase	178.53	2.884	63.19	1.270	0.01**
Albumin	27.93	5.101	61.57	4.712	0.01**
Glutathione peroxidase	54.13	3.210	112.73	13.775	0.01**
CP	0.44	0.090	1.20	0.514	0.01**
Glutathione	9.67	1.914	41.77	9.517	0.01**
MDA (µmol/L)	2.19	0.603	0.18	0.185	0.01**
Vitamin D3	11.72	0.380	23.29	0.697	0.01**
Zinc	72.29	1.027	102.21	0.949	0.01**
Copper	123.40	1.054	202.49	0.804	0.01**

Table (4): Variable Levels in the Blood Serum of Females Diagnosed with gluacoma Compared to Males with the Same Disease

variable	Patient group (No=23) males		Patient group (No =22) females		P-value
	Mean	Std. Deviation	Mean	Std. Deviation	
LOXL4	222.81 a	7.543	135.04 b	5.649	0.01**
SIRT 3	3.14 c	0.199	1.61 d	0.165	0.01**
TNF – α	250.51 b	3.666	332.68 a	6.476	0.01**
Lipoxygenase	55.25 c	2.979	178.53	2.884	0.01**
Albumin	27.87 c	7.220	27.93 c	5.101	0.01**

GPx	76.01 c	3.292	54.13 d	3.210	0.02*
CP	0.43 c	0.120	0.44 c	0.090	0.01**
Glutathione	8.49 c	1.320	9.67 c	1.914	0.01**
MDA	2.34 a	0.528	2.19 a	0.603	0.039*
Vitamin D	14.69 c	0.370	11.72 d	0.380	0.01**
Zinc	85.76 c	0.824	72.29 d	1.027	0.01**
Copper	154.82 c	1.251	123.41 d	1.054	0.01**

2-The Effect of Smoking:

Because smoking exposes smokers to high levels of oxidative stress from free radicals, which harms the visual nerve and is linked to glaucoma, smoking increases the likelihood of developing glaucoma (Jain et al., 2017). According to certain research, gluacoma in smokers is frequently more intrusive than in non-smokers (Pérez-de-Arcelus et al., 2017). According to the report, smokers now had an 88% higher risk of developing glaucoma than non-smokers. However, the findings presented in Tables (5), (6), and (7) demonstrated that smoking did not significantly alter the mean values of the biochemical parameters examined in gluacoma patients . The smokers' group recorded the highest averages for these variables (LOXL-4, MDA, LOP, and TNF-α) In contrast to the control group, and the smokers' group recorded the highest averages for these variables (LOXL-4, Sirtuin3, GPx, CP, MDA, Zn, and Cu), reaching 185.15, 2.49, 67.18, 0.43, 2.38, 80.11, and 141.62, respectively, compared to the non-smokers (Tran et al., 2023). Malondialdehyde concentrations were considerably greater in smokers than in non-smokers. vit C in glaucoma patients' serum was negatively correlated with smoking. Additionally, smoking lowers blood flow to the optic nerve, which raises intraocular pressure and blood pressure and increases the risk of glaucoma (Isik et al., 2007).

Table: 5 Variables were Measured in gluacoma patients who were Not Smokers and in Patients Who were Smokers

variable	Patient group (No=28) Smokers		Patient group (No=17) Non smokers		P-value
	Mean	Std. Deviation	Mean	Std. Deviation	
LOXL4	185.15 a	44.244	169.03 a	45.421	0.01**
SIRT 3	2.49 a	0.757	2.24 a	0.849	0.31^{NS}
TNF - α	285.78 a	41.213	298.75 a	42.908	0.31^{NS}
LIP	107.64 a	61.804	128.51 a	63.009	0.28^{NS}
Albumin	27.70 b	5.977	28.237 b	6.735	0.80^{NS}
GPx	67.18 b	11.881	62.231 b	10.504	0.16^{NS}
CP	0.43 b	0.116	0.45 b	0.087	0.23^{NS}
Glutathione	8.80 b	1.747	9.517 b	1.640	0.18^{NS}
MDA	2.38 a	0.556	2.08 b	0.542	0.08^{NS}
Vit C	0.060 b	0.022	0.061 b	0.023	0.97^{NS}
Vit D	13.36 b	1.555	13.05 b	1.557	0.37^{NS}
Zinc	80.11 b	6.838	77.63 b	6.844	0.18^{NS}
Copper	141.62 b	15.836	135.90 b	15.884	0.24^{NS}

Table: 6 Levels in the Blood Serum of Non-Smokers with gluacoma Disease were Compared to those of a Control Group Who Did not Smoke

variable	Patient group (No=17) (non-smokers)		Control group (No =23) (non- smokers)		P-value
	Mean	Std. Deviation	Mean	Std. Deviation	
LOXL4	169.03	45.421	58.82	11.898	0.01**
SIRT3	2.24	0.849	14.53	2.491	0.01**
TNF-A	298.75	42.908	8.611	1.567	0.01**
LIP	128.51	63.009	58.54	5.828	0.01**
Albumin	28.23	6.735	59.09	6.107	0.01**
Glutathione peroxidase	62.23	10.504	130.32	25.888	0.01**
CP	0.45	0.087	0.97	0.403	0.01**
Glutathione	9.51	1.640	45.50	9.050	0.01**
MDA	2.08	0.542	0.17	0.103	0.01**
Vit C	0.06	0.023	0.27	0.129	0.01**
Vitamin D	13.05	1.557	24.51	1.763	0.01**
Zinc	77.63	6.844	106.98	5.716	0.01**
Copper	135.90	15.884	224.54	25.604	0.01**

Table (7): Blood Serum Levels of Biochemical Variables in Smokers with gluacoma Disease were Compared to those of a Control Group

variable	Control group (No=22) smoker		Patient group (No= 28) smoker		P-value
	Mean	Std. Deviation	Mean	Std. Deviation	
LOXL4	55.40	12.278	185.15	44.244	0.01**
SIRT 3	15.23	2.314	2.49	0.757	0.01**
TNF – α	8.37	1.161	285.78	41.213	0.01**
Lipoxygenase	56.733	5.550	107.64	61.804	0.01**
Albumin	60.35	3.485	27.70	5.977	0.01**
GPx	138.16	20.218	67.18	11.881	0.01**
CP	1.07	0.568	0.43	0.116	0.01**
GSH	43.76	7.175	8.80	1.747	0.01**
MDA	0.21	0.192	2.38	0.556	0.01**
Vit C	0.28	0.164	0.06	0.022	0.01**
Vit D	24.96	1.423	13.36	1.555	0.01**
Zinc	108.73	5.567	80.11	6.838	0.01**
Copper	232.13	25.389	141.62	15.836	0.01**

Conclusion

The significance of lysyl oxidase (LOX) and its isoforms in the pathogenesis of glaucoma, a major cause of irreversible blindness globally, is highlighted by the study's findings. LOX is a crucial facilitator of extracellular matrix (ECM) remodeling in ocular structures, specifically the trabecular meshwork and lamina cribrosa, in addition to its well-established enzymatic role in collagen and elastin cross-linking. Aqueous humor outflow is impeded, intraocular pressure (IOP) is raised, and optic nerve head deformation is encouraged—all of which are important processes that lead to glaucomatous neurodegeneration—by the pathological stiffening of these tissues caused by aberrant LOX activity.

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