Circulating Salusin-beta Levels in Various Open-Angle Glaucoma Types

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ABSTRACT

Purpose: To evaluate the levels of salusin-beta (β-Sal) in the serum in patients with open-angle glaucoma.

Materials and Methods: This pilot work was designed as an institutional controlled comparative clinical study. The serum β-Sal levels of age and sex-matched 20 healthy volunteers as controls (Group 1), 15 patients with normal-tension glaucoma (NTG) (Group 2), 18 patients with pseudoexfoliative glaucoma (PXG) (Group 3) and 26 patients with primary open-angle glaucoma (POAG) (Group 4) were measured with the enzyme-linked immunosorbent assay (ELISA) method.

Results: There was no statistically significant difference concerning age and gender among the groups (p>0. 05). The mean serum β-Sal levels in Group 1, Group 2, Group 3 and Group 4 were 4977.02±3741.44 pg/mL; 1086.57±253.41 pg/mL; 1044.98±268.80 pg/mL and 868.32±181.78 pg/mL, respectively. Although the mean β-Sal levels in all glaucoma groups seem numerically lower than the control group, there was no statistically significant difference between the serum β-Sal concentrations of the study groups (p>0.05).

Conclusion: These findings suggest that serum salusin-β levels are not different among various open-angle glaucoma types. However, theoretically, β-Sal may contribute to the pathogenesis of glaucoma and may play a neuroprotective hormone in glaucoma. Further studies with large patient population are required to say whether β-Sal plays a role in the pathogenesis of glaucomatous optic neuropathy.

Keywords: Open-angle glaucoma, Salusin beta, Serum level.

INTRODUCTION

Glaucoma is a neurodegenerative optic nerve (ON) disease characterized the loss of the retinal nerve fibers (RNFs) and excavation at the level of the ON head and visual field (VF) loss. It has been considered that the elevated intraocular pressure (IOP) is one of the important factors in initiation or progression of glaucoma.[1-2] However, recent studies have demonstrated that only lowering IOP might not prevent the progression in all glaucoma patients. Thus, neuroprotection (NP) may become an important treatment option in the glaucoma management. NP is defined as the use of therapeutic agents to prevent or reverse neuronal cell death due to neurodegenerative disease or traumatic or neurotoxic injuries.[3-6] In some central nervous system (CNS) diseases including Alzheimer's disease, Parkinson's disease, and Huntington's disease, it has been demonstrated that neuroprotective treatment might be useful.[7-11]

Salusin-alpha and salusin-beta (β-Sal) are soluble peptide hormones which may stimulate the proliferation of vascular smooth muscle cells (VSMC) and fibroblasts. It has been demonstrated that β-Sal was also found in the brain and that it has various effects such as vascular inflammation and modulation of the cardiovascular system, cytokine function and oxidative damage.[12-15] In previous studies, it has been shown that β-Sal was expressed in many tissues[16] and that it promotes inflammation by increasing activation of the I-kBa/nuclear factor kappa B (NF-kB) signaling pathway.[17]

Recent studies revealed that the inhibition of β-Sal reduced oxidative stress and inflammation in diabetic rats and that β-Sal administration increased the activity of antioxidant enzymes including superoxide dismutase and glutathione, resulting in a protective effect against acute renal failure.[12,18,19] Additionally, CNS has profuse β-Sal levels,[16]

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suggesting another function in neurons in addition to its role in the inflammatory function. However, a specific role for β-Sal in the CNS has yet to be identified. The ON is also a part of the CNS and glaucoma is a neurodegenerative disease of the ON. To the best of our knowledge, up to date, there is no previous report in the literature on the blood levels of salusin in patients with glaucoma or even any ophthalmologic disease. However, in a recent study, Chang et al reported that β-Sal has neuroprotective effects on the midbrain dopamine neurons and that it could be used as an effective treatment for Parkinson's disease because of the role of β-Sal regarding neuronal survival and apoptosis in neurodegenerative diseases.[18] Glaucoma is also a neurodegenerative disease which axonal survival, apoptosis, and neuroprotection are crucial in its pathogenesis and treatment.[1-6] At the light of this recent knowledge, we considered that β-Sal might play a role in the glaucoma pathogenesis and, in this study, we aimed to evaluate the levels of β-Sal in the serum in patients with open-angle glaucoma (OAG) and to compare with those of healthy controls.

MATERIALS AND METHODS

Our study was designed as an institutional controlled study according to the Helsinki Declaration and approved by the institutional ethics committee. Informed consents were obtained from the participants. This work included four group:

Group 1 (Control group) included age and sex-matched 20 healthy volunteers without history of ocular disease (except refractive error) and with normal eye examination including IOP lower than 22 mmHg, an open-angle, normal ON head appearance, RNF layer thickness (RNFLT), and VFs.

Group 2 (normal-tension glaucoma=NTG group) included 15 patients with glaucomatous cupping, an open-angle, IOP lower than 22 mmHg and defects in RNFLT and VF in at least one eye in two consecutive visits.

Group 3 (pseudoexfoliative glaucoma=PXG group) included 18 patients patients having typical pseudoexfoliative syndrome, glaucomatous cupping, an open-angle, IOP higher than 22 mmHg and defects in RNFLT and VF in at least one eye in two consecutive visits.

Group 4 (primary open-angle glaucoma=POAG group) included the 26 patients with IOP higher than 22 mmHg, an open-angle, glaucomatous cupping, and defects in RNFLT and VF in at least one eye in two consecutive visits.

All participants underwent a full ophthalmic examination including visual acuity, slit-lamp biomicroscopy, IOP measurement with applanation tonometry, gonioscopy, optic disc evaluation, and VF testing with full-threshold strategy by a Humphrey VF analyzer.

The patients with cardiac disease, renal insufficiency, diabetes mellitus, systemic hypertension, hyperlipidemia, peripheral or coronary artery disease, cerebrovascular disease, ocular inflammation, retinal vascular occlusive disease, systemic or ocular vasculitis, renal or hepatic dysfunction, morbid obesity, pregnancy, psychiatric illness, and/or chronic alcohol abuse and central nervous system (CNS) diseases as Alzheimer's disease, Parkinson's disease, and Huntington's disease were excluded from the study.

The patients which receive any antiglaucomatous drop treatment were also excluded from the study because these agents may have any effects on circulating salusin-beta levels.

Blood samples were taken from participants to measure β-Sal levels at 08.00 hours after overnight fasting in all subjects and were delivered to the laboratory within 20 min, centrifuged (2000xg for 10 min at 4 °C) and the sera aliquot is stored at −80 °C until assayed. β-Sal measurements were performed by enzyme-linked immunosorbent assay (ELISA) using with commercial β-Sal kits (Sunredbio, Baoshan, Shangai) according to the manufacturer's instructions. The minimum detectable level (sensitivity) was less than 8.756 pg / mL and the assay range was 10-3000 pg / mL. Intra- and interassay CVs were less than 10% and 12%, respectively. All samples were measured spectrophotometrically via ELx800™ Absorbance Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA) at 450 nm. The biochemist was blind to the identity of samples during processing. The results are presented as (pg/mL).

Results are given as means± standard deviation (SD) with median values with minimum and maximum levels. The Statistical Package for Social Sciences, version 11.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Individual group parameters were assessed with the one-sample Kolmogorov–Smirnov Z test and were found to be abnormally distributed ($p<0.05$). Hence, statistical comparisons between groups were performed by the non-parametric Kruskal–Wallis and the Mann–Whitney U test. Spearman's Rank order correlation coefficients were used to assess significant associations between β-Sal levels and demographic findings. For all comparisons, statistical significance was defined by $p<0.05$.

RESULTS

There was no statistically significant difference concerning age and gender among the groups (p>0.05). The mean serum

β-Sal concentrations in Group 1, Group 2, Group 3 and Group 4 were 4977.02±3741.44 pg/mL; 1086.57±253.41 pg/mL; 1044.98±268.80 pg/mL and 868.32±181.78 pg/mL, respectively. However, the differences among the serum β-Sal levels in the study groups were not statistically significant (Controls vs NTG, PXG and POAG: p=0,922; p=0,886 and p=0,191, respectively; NTG vs PXG: p=0,914; NTG vs POAG: p=0,264 and POAG vs PXG: p=0,293) (Table 1, Figure 1). The median values of serum β-Sal levels (minimum and maximum values) in Group 1, Group 2, Group 3 and Group 4 were 1039.73 pg/mL (390.24 and 27323.66), 713.60 pg/mL (530.10 and 3077.34), 724,1795 pg/mL (545,32 and 2198,21), 576,93 pg/mL (163,59 and 2224,63), respectively.

Table 1. *Comparative mean β-Salusin levels in study groups.*

Group	Number	Mean β-Salusin levels±SD (pg/mL)	P value
Control	20	4977.02±3741.44	Controls vs. NTG: p=0.922
			Controls vs. PXG: p=0.886
			Controls vs. POAG: p=0.191
NTG	15	1086.57±253.41	NTG vs.PXG: p=0.914
PXG	18	1044.98±268.80	POAG vs. PXG: p=0.293
POAG	26	868.32±181.78	NTG vs. POAG: p=0.264

Abbreviations: **NTG:** normal tension glaucoma, **PXG:** pseudoexfoliative glaucoma, **POAG:** primary open-angle glaucoma, **SD:** Standard deviation.

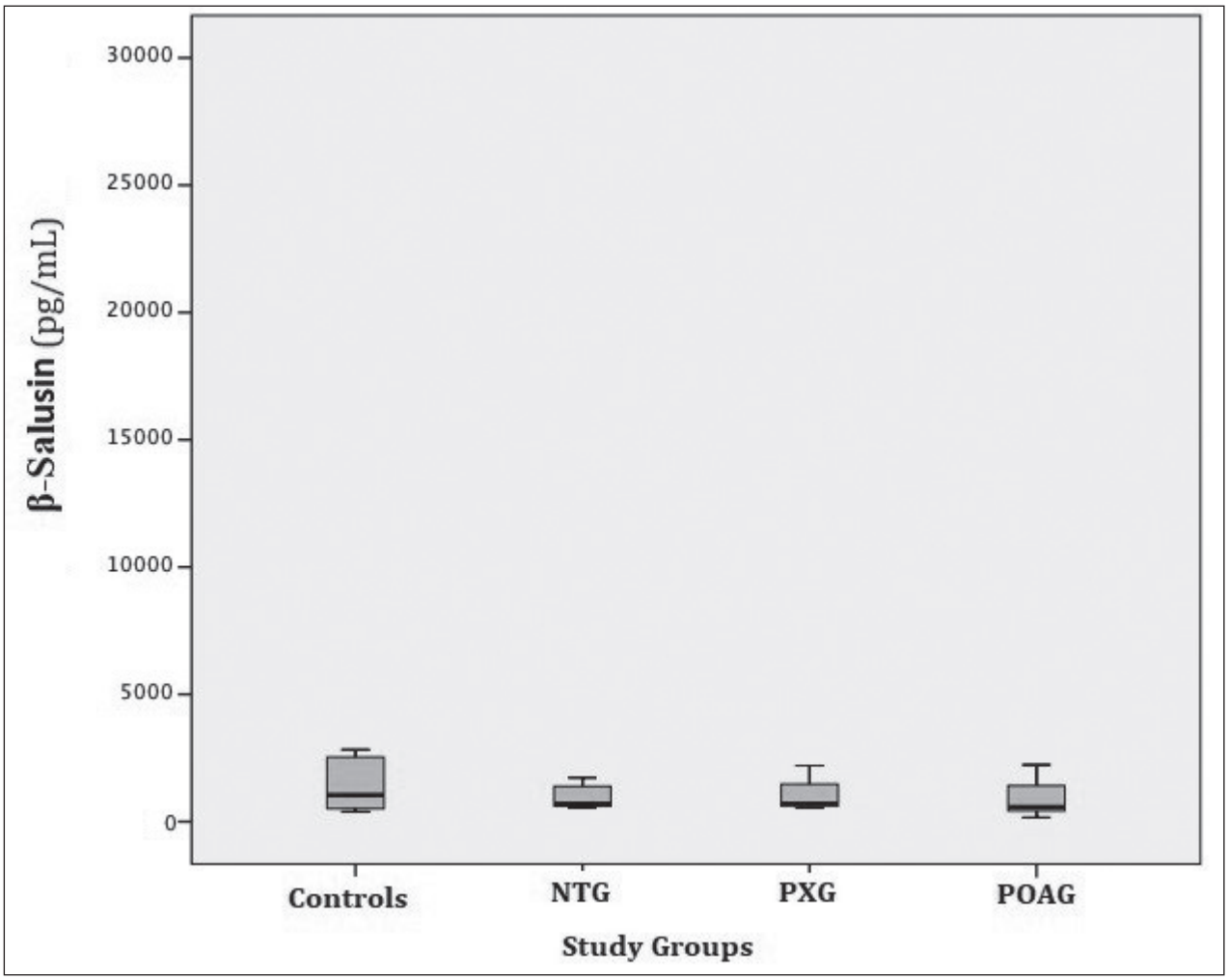

Figure 1. *Mean β-Salusin levels in study groups.*

DISCUSSION

Salusin-α and β-Sal are peptides synthesized from prosalusin by various cells such as brain cells, human VSMCs, and endothelial cells. They have anti-apoptotic effects in these cells.[12,19-23] Additionally, β-Sal stimulates VSMC proliferation and vascular fibrosis with potent mitogenic effects on human VSMCs and fibroblasts in rats and humans with the activation of immediate early response genes, *c-myc* and *fos*.[21, 24]

Circulating β-Sal levels in patients with coronary artery disease, diabetes mellitus, and cerebrovascular disease are also significantly higher levels than healthy controls while as the patients with essential hypertension have lower levels of β-Sal.[12,20,21,23,25,26] Additionally, it has been demonstrated that β-Sal is a potent hypotensive factor and its bolus injection rapidly induces profound hypotension and bradycardia by a cholinergic mechanism beside negative inotropic effects or inhibitory effect on action potentials and ion flows.[12,20,21,23,25,26] On the other hand, it has been reported that the microinjection of β-Sal into the paraventricular nucleus in the rats with renovascular hypertension caused to increase the blood pressure via the release of norepinephrine and arginine-vasopressin.[27]

Recent studies showed that β-Sal has a stimulatory effect on pro-inflammatory and oxidative stress molecules that increase inflammatory responses in human endothelial cells, and that the secretion of β-Sal was stimulated by inflammatory cytokines like tumor necrosis factor-α.[12,15,17] β-Sal induced the expression of interleukin-1β, monocyte chemoattractant protein-1, acetyl-coenzyme A acetyltransferase 1, vascular cell adhesion molecule-1, and nicotinamide adenine dinucleotide phosphate oxidase 2, a potent source of reactive oxygen species in human umbilical vein endothelial cells.[19] Additionally, it has been reported that β-Sal increased the reactive oxygene species (ROS) production attenuated by n-acetyle cysteine or nitrite oxyde syntitase (NOX)-2 siRNA.[19]

Neuroprotection is crucial in the treatment of glaucoma and other neurodegenerative or apoptosis-associated diseases.[6-8,10] Recently, it was reported that endogenous neuropeptide β-Sal has neuroprotective effects on the midbrain dopamine neurons and it can be used as an effective treatment for Parkinson's disease.[18] Additionally, it has been demonstrated that β-Sal antagonizes the apoptotic death of cardiomyocytes.[28] It is well-known that β-Sal is widely expressed within the CNS. Additionally, ON is a part of the CNS. A previous animal study has demonstrated that central β-Sal was involved in sympathetic activation and hypertension while peripheral β-Sal contributed to vascular remodeling associated with hypertension by promoting human VSMC proliferation.[24] So, the central and peripheral β-Sal may have distinct effects in different tissues such as blood and nerves. Additionally, the levels of β-Sal in vitreous or aqueous samples may be lower or higher in glaucomatous patients compared to serum samples.

The limitations of this study are the lacks of the measurement of β-Sal levels in the vitreus and the evaluation of the subgroups classified according to glaucoma severity.

To the best of our knowledge, this is the first report investigating the relation of serum β-Sal level in glaucoma. In the set of our study, we hypothesized that the levels of β-Sal in the serum in patients with OAG might be lower than those of healthy controls. At the end of the study, we observed that the mean β-Sal levels in OAG groups were found numerically lower than that of the control group. However, we analysed that this difference between the serum β-Sal levels of the study groups was not statistically significant. Our study showed that serum β-Sal levels might not change in patients with glaucoma. The insignificant results concerning with the levels of serum β-Sal in our study may be due to local neurodegenerative disease in the ON. But yet, based on recent evidence on neuroprotective properties of β-Sal, it may play a role in the pathogenesis of glaucoma. Thus, further researches are needed to have more information on the effects of β-Sal, and to measure free and bound levels of β-Sal in both vitreous and aqueous humor samples in glaucoma patients with and without treatment to determine the exact role of β-Sal in the pathogenesis of glaucoma.

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