Evaluation of the Corneal Endothelial Layer in Patients with Hashimoto's Thyroiditis

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Abstract

Purpose

Evaluation of corneal endothelial layer in patients with Hashimoto's thyroiditis (HT) by specular microscopy

Methods

This prospective cross-sectional study was carried out with 50 patients with Hashimoto's thyroiditis (group 1) and 44 healthy subjects (group 2) as controls. Endothelial cell density (ECD), hegzogonality (HEX), coefficient of variation (CV), central corneal thickness (CCT), minimum-maximum and mean cell volume (min-max MCV) were evaluated by noncontact specular microscope (Konan Medical Inc., Nishinomiya, Japan).

Results

The mean ages of the patients in group 1 and group 2 were 38.42 ± 9.70 and 38.18 ± 10.96 years, respectively. There was no significant difference between the two groups in terms of age and gender distribution ($p > 0.05$). HEX ratio (44.98 ± 5.86) and min-MCV values (149.82 ± 15.01) were statistically significantly lower in group 1 (p 0.05). No significant difference was observed in other parameters evaluated by specular microscopy ($p > 0.05$).

Conclusion

The detection of thyroid hormone receptors in orbital structures and corneal layers suggests that the corneal endothelial layer may be affected and that structural or functional changes may occur in this layer in patients with HT.

Introduction

Hashimoto's thyroiditis (HT) is also known as chronic autoimmune thyroiditis or autoimmune hypothyroidism. Autoimmune thyroid disease is associated with two distinct pathological mechanisms. T-cell mediated autoimmunity is involved in the aetiology of HT, whereas humoral immunity is responsible for Graves' disease. In both forms, autoantibodies to thyroid-stimulating hormone (TSH) receptors are present in the serum. These autoantibodies are serum thyroid peroxidase (TPO) and thyroglobulin (Tg) antibodies. HT presents clinically as euthyroidism or hypothyroidism. Patients are often hypothyroid and the disease is more common in women [1, 2].

Thyroid-associated ophthalmopathy (TO) is associated with thyroid autoimmunity, particularly Graves' hyperthyroidism. HT can be associated with TO, as can Graves' disease. Clinically, thyroid ophthalmopathy occurs in approximately 6% of patients with HT. Graves' disease can cause severe ocular and orbital involvement. HT, an autoimmune disease like Graves' disease, has been shown to affect orbital tissues. Classic manifestations of thyroid ophthalmopathy include lid retraction, proptosis, lagophthalmos, optic neuropathy and restrictive extraocular myopathy. In addition, the orbit and lacrimal glands are affected by lymphocyte infiltration and interstitial oedema. Abnormal immune response leads to swelling of the orbit, causing a feeling of pressure around the eye, and inflammation of the lacrimal complex causes enlarged and swollen glands and contributes to lubrication problems. The corneal endothelium has also been evaluated by specular microscopy in patients with active and inactive Graves' ophthalmopathy. It has been suggested that morphological changes in the corneal endothelium may be a quantitative marker in the evaluation of Graves' ophthalmopathy activation [3, 4, 7].

Conrad et al. demonstrated the presence of thyroxine receptors in all layers of the embryonic chick cornea [5]. In another study performed in chick cornea, changes in corneal epithelium and endothelium were histologically examined using thyroxine, a thyroid hormone, and propylthiouracil, an anti-thyroid drug, and it was shown that thyroid hormone is involved in the development of corneal layers [6]. Based on this information, the aim of the present study was to evaluate whether there are changes in the corneal endothelial layer by specular microscopy in patients with HT. There are studies in the literature on ocular surface changes and biomechanical properties of the cornea in patients with HT, however to the best of our knowledge there are no studies on corneal endothelial layer.

Methods

This prospective cross-sectional study was conducted between 22 December 2021 and 1 March 2022 with a total of 94 patients. Fifty patients with Hashimoto's thyroiditis referred from the Endocrinology outpatient clinic were selected as the study group (group 1). The control group (group 2) consisted of 44 age- and sex-matched healthy patients without any other ocular disease who were referred to the ophthalmology department for refractive error or routine eye examination.

The study adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee (protocol number:2021/211). Participants with systemic diseases other than HT such as diabetes mellitus, previous ocular surgery or laser therapy, history of any corneal disease, history of trauma, glaucoma were excluded from the study. Patients using topical medications and with previous concomitant ocular treatments were also excluded.

The diagnosis of HT was based on clinical criteria, radiological imaging and laboratory tests (TSH, free T3, free T4 levels and presence of anti- TPO, anti-Tg). The presence or absence of treatment for hypothyroidism, disease duration, and laboratory data were recorded. All patients were euthyroid, initially diagnosed as hypothyroid and treated with levothyroxine sodium. None of the patients had thyroid ophthalmopathy. Whether the diagnosis of thyroid ophthalmopathy was made on the basis of clinical

features of the disease, including at least one of the following typical orbital signs: conjunctival chemosis or diffuse redness, caruncular oedema, fluctuant eyelid oedema or erythema, exophthalmos, restrictive extraocular myopathy, eyelid retraction, and optic neuropathy. In the control group, all patients were confirmed to have normal blood levels of thyroid-stimulating hormone, free T4 and anti- TPO prior to assessment.

All patients in the study and control groups were examined by a single ophthalmologist. A complete ophthalmic examination, presence of exophthalmos with Hertel exophthalmometer and evaluation of the corneal endothelial layer with specular microscopy were performed. The right eyes of all patients and controls were examined.

Specular microscopy (Konan Medical Inc., Nishinomiya, Japan) imaging was performed by the same ophthalmologist. Each eye was measured 3 times and the average values were recorded. Corneal endothelial cell density (CD) (cells/mm^2), coefficient of variation (CV), hexagonal cell ratio (HEX), central corneal thickness (CCT), and mean cell volume (min-max MCV) values were calculated automatically using the software of the specular microscope. The obtained data were compared.

Statistical Analysis

All the statistical analyses were performed with SPSS for Windows v. 21.0 (SPSS Inc., Chicago, USA). As a result of the power analysis, the minimum sample size required per group was calculated to be 44 if the effect size for comparing changes in the corneal endothelial layer by specular microscopy between the study group and controls of the same age and sex was estimated to be 0.70 (medium) at a 95% confidence level and 90% power. The parameters used in the study were expressed as mean ± standard deviation (SD). The normal distribution of the data was tested using the Kolmogorov-Smirnov test. Student-t test and chi-squared test were used to determine the significance of differences in the results. p 0.05 was considered statistically significant.

Results

Forty-two (84%) of the patients with HT were female and 8 (16%) were male. In the control group, 36 (81.8%) were female and 8 (18.2%) were male. There was no statistically significant difference in gender distribution between the two groups (p 0.05). The mean age of the patients with HT was 38.42 ± 9.7 years, while the mean age of the control group was 38.18 ± 10.96 years (p > 0.05). There was no significant difference in intraocular pressure (IOP), best corrected visual acuity (BCVA), Hertel exophthalmometer values and age between the two groups (p 0.05) (Table 1).

Table 1
Age, IOP, Hertel Exophthalmometer and BCVA values of the groups.

	Age (year)	IOP (mm-Hg)	Hertel (mm)	BCVA (decimal)
Group 1	38,42 ± 9,7	13,06 ± 1,65	15,14 ± 2,45	0,97 ± 0,07
Group 2	38,18 ± 10,96	12,8 ± 2,29	14,5 ± 1,79	0,99 ± 0,04
p value	0,835	0,332	0,185	0,076
IOP: intraocular pressure, BCVA: best corrected visual acuity, mm: milimeter				

The mean HEX rate (44.98 ± 5.86%) and the min MCV (149.82 ± 15.01) were statistically significantly lower in the group 1 (p 0.05). No statistically significant difference was observed for other parameters, including ECD, CV, CCT and max MCV values assessed by specular microscopy (p > 0.05) (Table 2).

Table 2
Specular microscopic parameters of the groups.

	Group 1	Group 2	p value
CCT (µm)	551,9 ± 38,67	553,18 ± 50,56	0,892
ECD (mm²)	2801,92 ± 183,52	2806,39 ± 212,36	0,914
CV (%)	31,38 ± 3,26	30,82 ± 2,95	0,218
HEX (%)	44,98 ± 5,86	48,8 ± 5,11	**0,001***
AVG (µm²)	358,5 ± 24,49	358,55 ± 27,77	0,946
Min MCV (µm²)	149,82 ± 15,01	157,14 ± 19,52	**0,037***
Max MCV (µm²)	783,72 ± 144,77	765,48 ± 125,11	0,203
CCT: central corneal thickness, ECD: endothelial cell density, CV: coefficient variation of cell area, HEX: percentage of hexagonal cells, AVG = average cell area, min-max MCV: minimum-maximum mean cell volüme, * statistically significance			

Anti-TPO and anti-Tg were positive in 98% and 26% of patients with HT, respectively. The mean TSH level was 3.65 ± 3.05, the mean free-T4 level was 1.02 ± 0.37 and the mean free-T3 level was 3.38 ± 1.36. The mean disease duration of patients with HT was 57.24 ± 57.63 months. There was no correlation between disease duration and any of the specular microscopy parameters (p 0.05, correlation coefficient 0.2).

Discussion

This study showed that the HEX and min- MCV parameters evaluated by specular microscopy may be affected in patients with HT even in the absence of TO findings. No statistically significant changes were found in the other parameters evaluated.

The detection of thyroid hormone receptors in orbital tissues, lacrimal glands and corneal layers suggests that all these tissues may be affected by inflammation in diseases such as Graves' disease and HT, in which autoantibodies against these receptors are present. The effects on orbital tissues and the ocular surface have been reported in many studies, but there is a limited number of studies on corneal changes. The most comprehensive and important study on this subject was carried out by Zhou et al. Zhou et al. analysed 128 eyes of 64 patients with Graves' ophthalmopathy and reported that corneal endothelial morphology was altered in patients with active Graves' disease compared to inactive Graves' disease. The coefficient of variation of the cell area (CV) value was found to be statistically significantly higher in patients with active Graves' ophthalmopathy, and the CV value may be a quantitative marker in assessing Graves' ophthalmopathy activation according to their results [7].

Studies have shown that ocular surface changes, particularly dry eye, are observed in patients with HT. Guray et al. found a decrease in tear break-up time (TBUT) and Schirmer 1 test scores in patients with HT, but no change in Ocular Surface Disease Index (OSDI) questionnaire scores [8]. Similarly, a high rate of dry eye disease and ocular discomfort symptoms in patients with HT was reported by Ekin et al [9]. These studies have reported changes in lacrimal gland function in thyroid diseases associated with autoimmunity. In this theory, autoantibodies bind to lacrimal gland acinar cells and contribute to lacrimal gland dysfunction through abnormal signalling. However, Ekin et al suggested that the main cause of dry eye syndrome in patients with HT was excessive evaporation rather than impaired tear production [9].

Corneal endothelial cells cannot reproduce by mitosis and play major role in maintaining corneal transparency which can be affected by many systemic and local diseases. Several studies have reported that the corneal endothelial layer may be affected not only in local ocular diseases such as dry eye, glaucoma and uveitis, but also in systemic diseases such as diabetes mellitus, chronic renal failure, chronic pulmonary disease and chronic autoimmunity [10, 11]. The mechanism of corneal endothelial damage caused by local ocular inflammation and systemic inflammatory diseases is still controversial. Huang et al. found that the inflammatory cytokines interleukin (IL)-1β, IL-6, IL-8 and tumour necrosis factor α were found at high levels in the tears of patients with active Graves' disease [12]. Similar levels of pro-inflammatory cytokines have been reported in the aqueous humour of patients with uveitis. These pro-inflammatory cytokines, which are elevated in the aqueous humour, are thought to be primarily responsible for endothelial cell damage. It might be suggested that autoantibodies present in HT may cause inflammation in the cornea, where similar receptors are located, initiating an apoptotic process in the corneal endothelium and leading to structural changes.

Polymegatism is the term used to describe the increased variability in endothelial cell area. CV, a specular microscopic parameter, is an objective measure of polymegatism. Pleomorphism is a deviation from hexagonality. The objective measure of pleomorphism is the HEX value in a specular microscope. Pleomorphism and polymegatism change with age for various reasons due to endothelial cell damage. Due to ageing and environmental stress factors, a 100% perfect hexagonal ratio is not possible; however, in the endothelial layer of a healthy cornea, this ratio should be in the range of 60–70%. The most sensitive indices of corneal endothelial dysfunction are CV and HEX [13, 14]. ECD alone is not a sensitive

enough indicator to assess corneal endothelial function. This is because the corneal endothelium may be functional even if the ECD falls below 500 cells/mm [15]. Early signs that could predict a reduction in ECD were considered to be changes in CV and HEX [14]. High CV values and low HEX values indicate that the corneal endothelium is under stress [7, 16]. The statistically significant low HEX and min-MCV values in HT patients in our study indicate that the corneal endothelial layer is under stress in these patients. Similarly, Zhou et al. found a statistically high CV value in patients with active Graves' ophthalmopathy and reported that the corneal endothelium was under stress [7]. We did not find a statistically significant change in any of the other specular microscopic parameters evaluated in our study. The reduced HEX value we found in our study, together with normal other parameters, indicates that there is no damage that could lead to decompensation of the corneal endothelial layer, but that the endothelium is under stress. For example, one study has shown that if the CCT value is within the normal range, although some of the quantitative specular microscopy data are affected, this means that there is no corneal decompensation, i.e. the endothelial functions remain normal [17]. Similarly, our study found no statistically significant change in CCT, which indicates normal endothelial function.

Conclusion

In conclusion, HEX and min-MCV values were found to be statistically significantly lower in patients with HT, whereas no significant changes in other specular microscopic parameters were found. Considering the studies in the literature, this suggests that the corneal endothelial layer is affected in a way that does not cause decompensation and may be stressed in patients with HT. In addition, we believe that careful consideration of these results, particularly in patients scheduled for intraocular surgery, is important to prevent corneal complications that may ocur.

Declarations

The authors have no proprietary or financial interest in any products used in this study.

Compliance with ethical standards

Declaration of interest:*The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.*

Informed consent: *Informed consent was obtained from all participants included in the study*

Ethics Committee; *Malatya Clinical Research Ethics Committee, Reference number: 2021/211*

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Figures

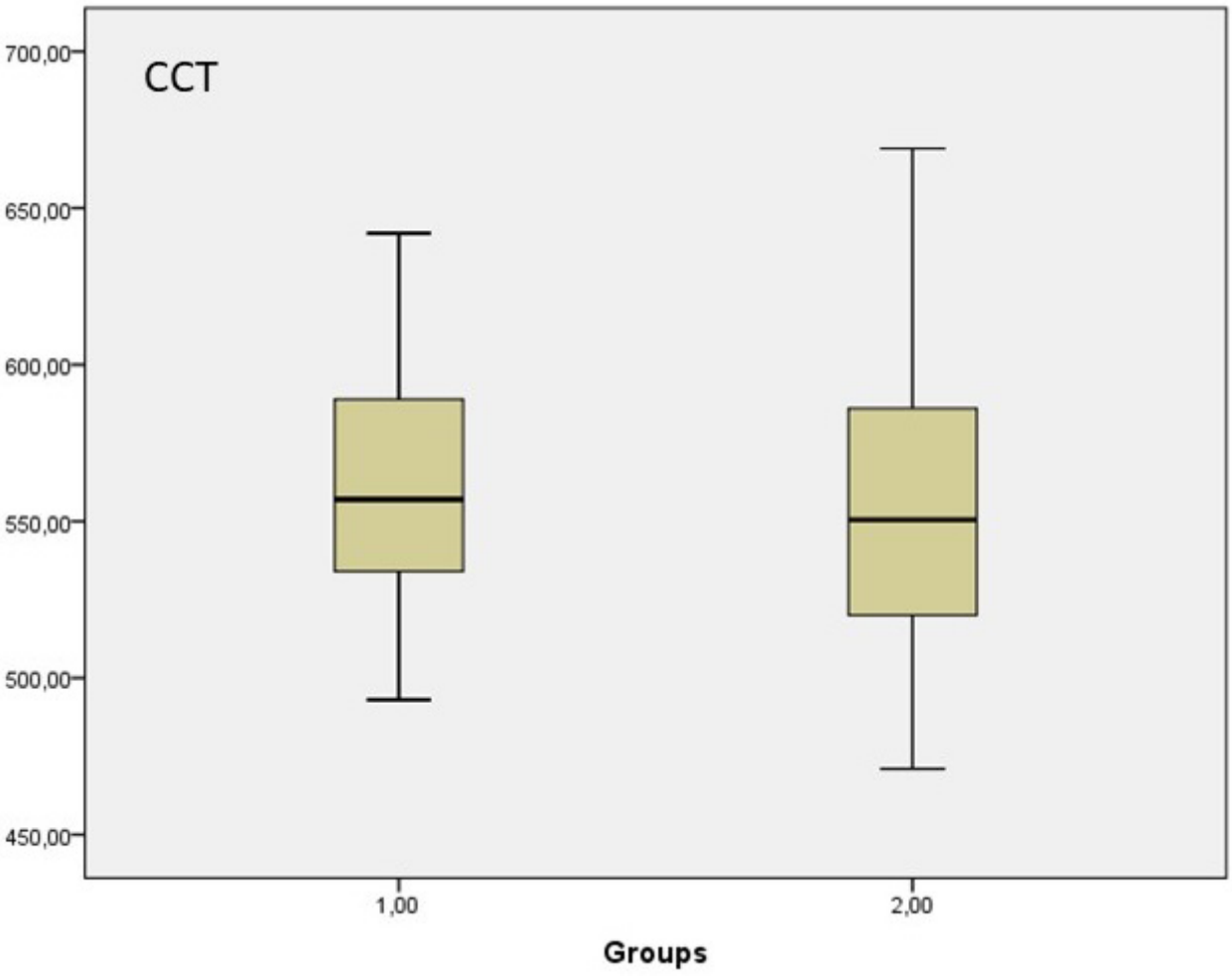

Figure 1

Graph shows the distribution of central corneal thickness (CCT) in Group 1 and 2

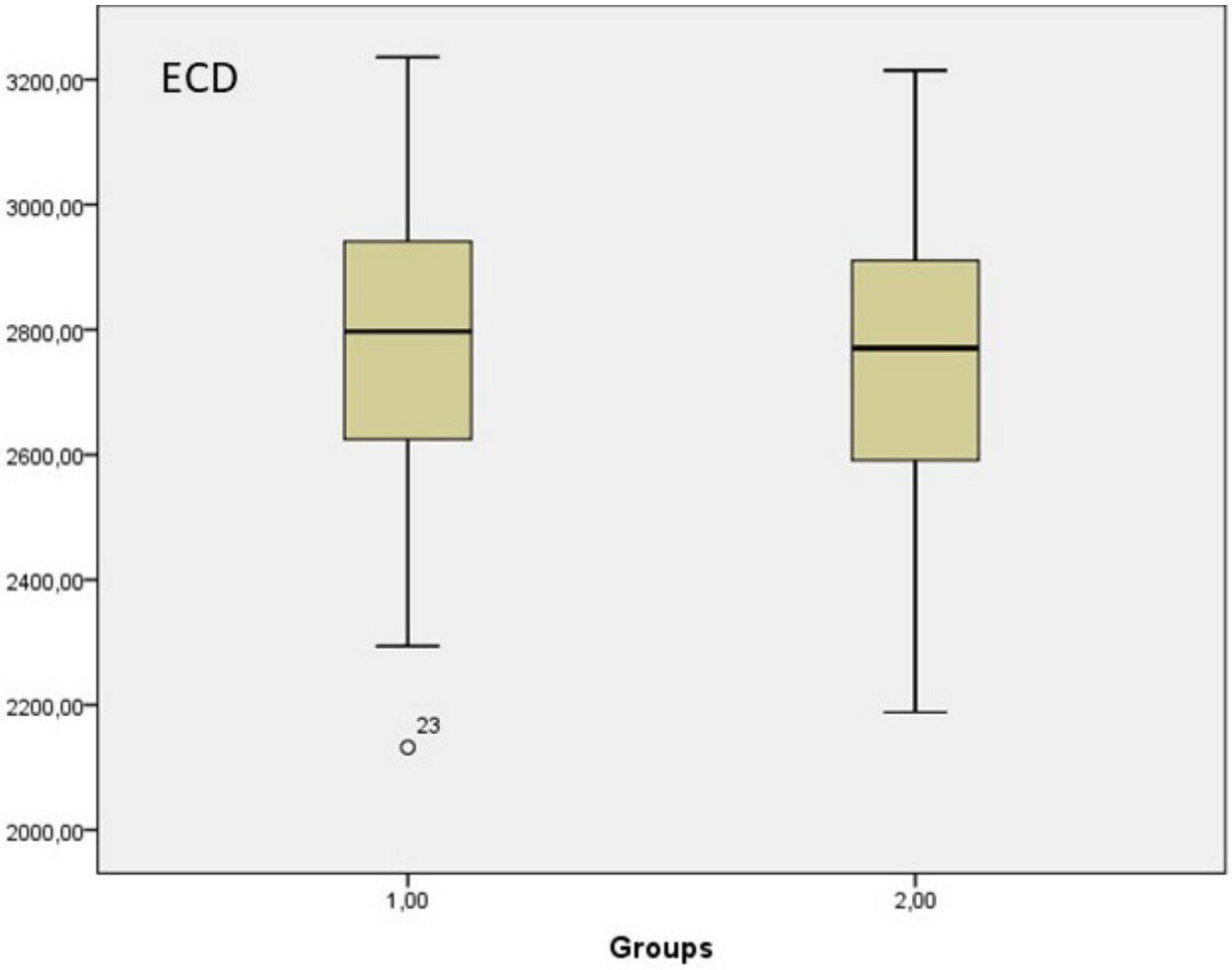

Figure 2

Graph shows the distribution of endothelial cell density (ECD) in Group 1 and 2.

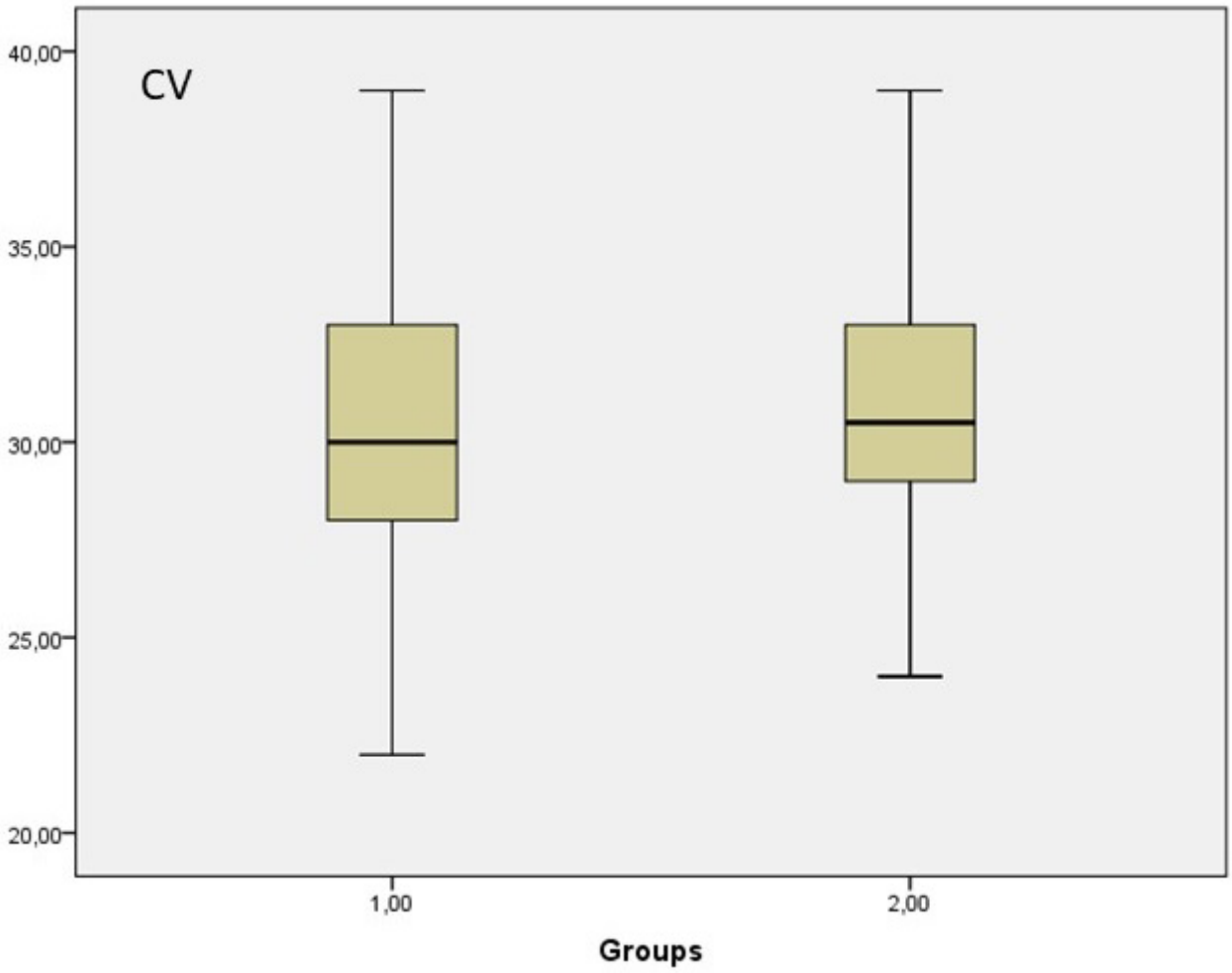

Figure 3

Graph shows the distribution of coefficient of variation (CV) in Group 1 and 2.

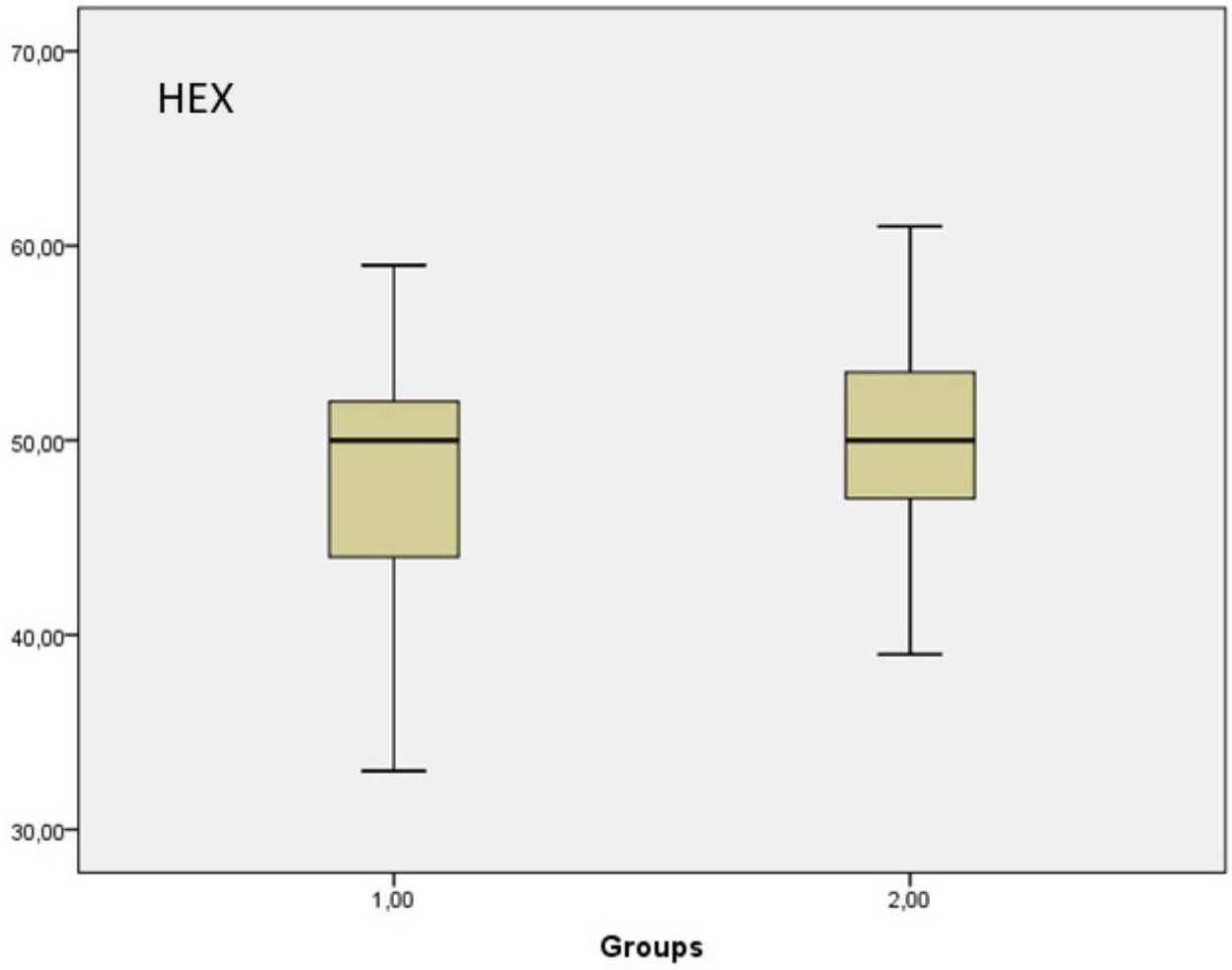

Figure 4

Graph shows the distribution of hexagonal cell ratio (HEX) in Group 1 and 2.

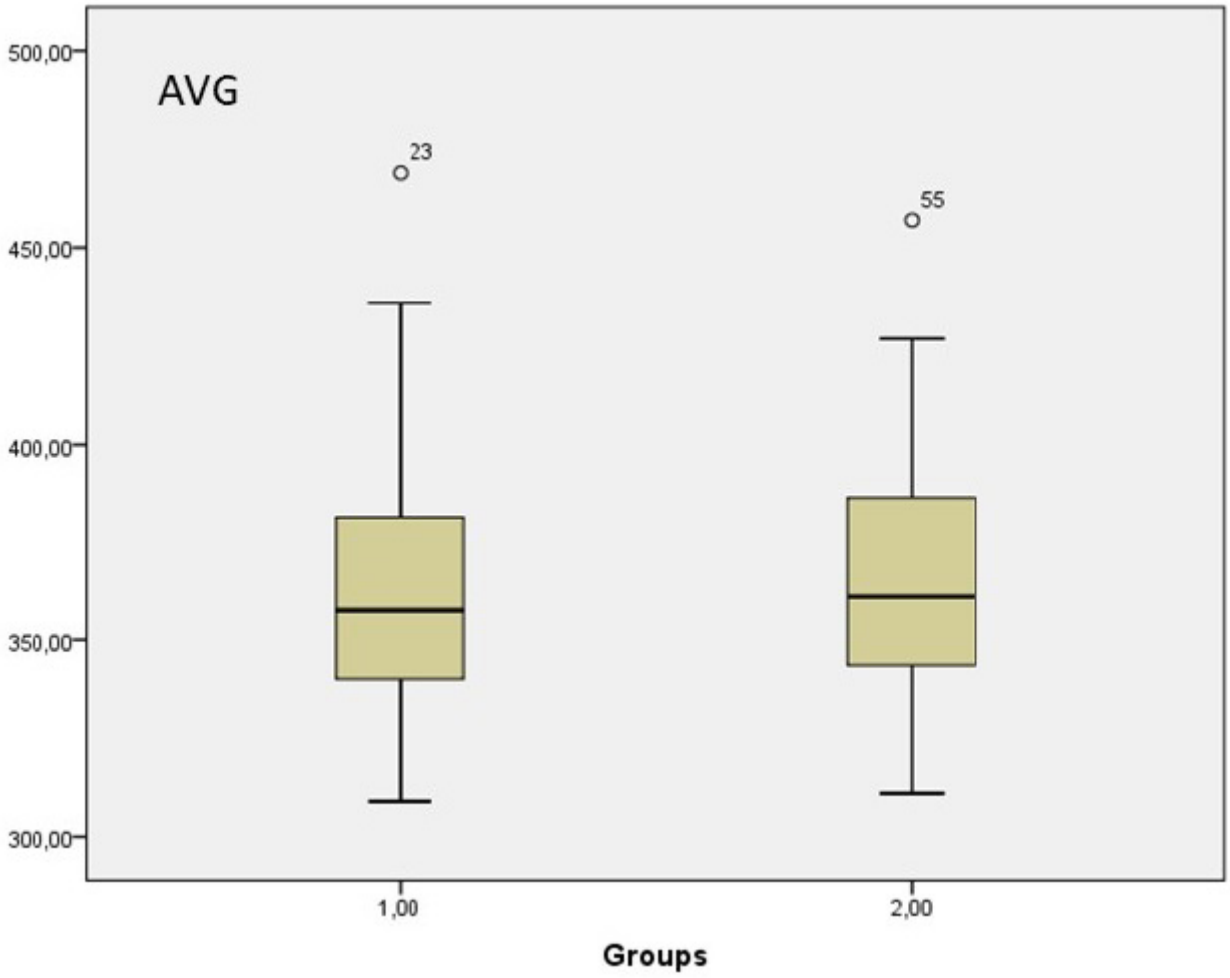

Figure 5

Graph shows the distribution of average cell area (AVG) in Group 1 and 2.

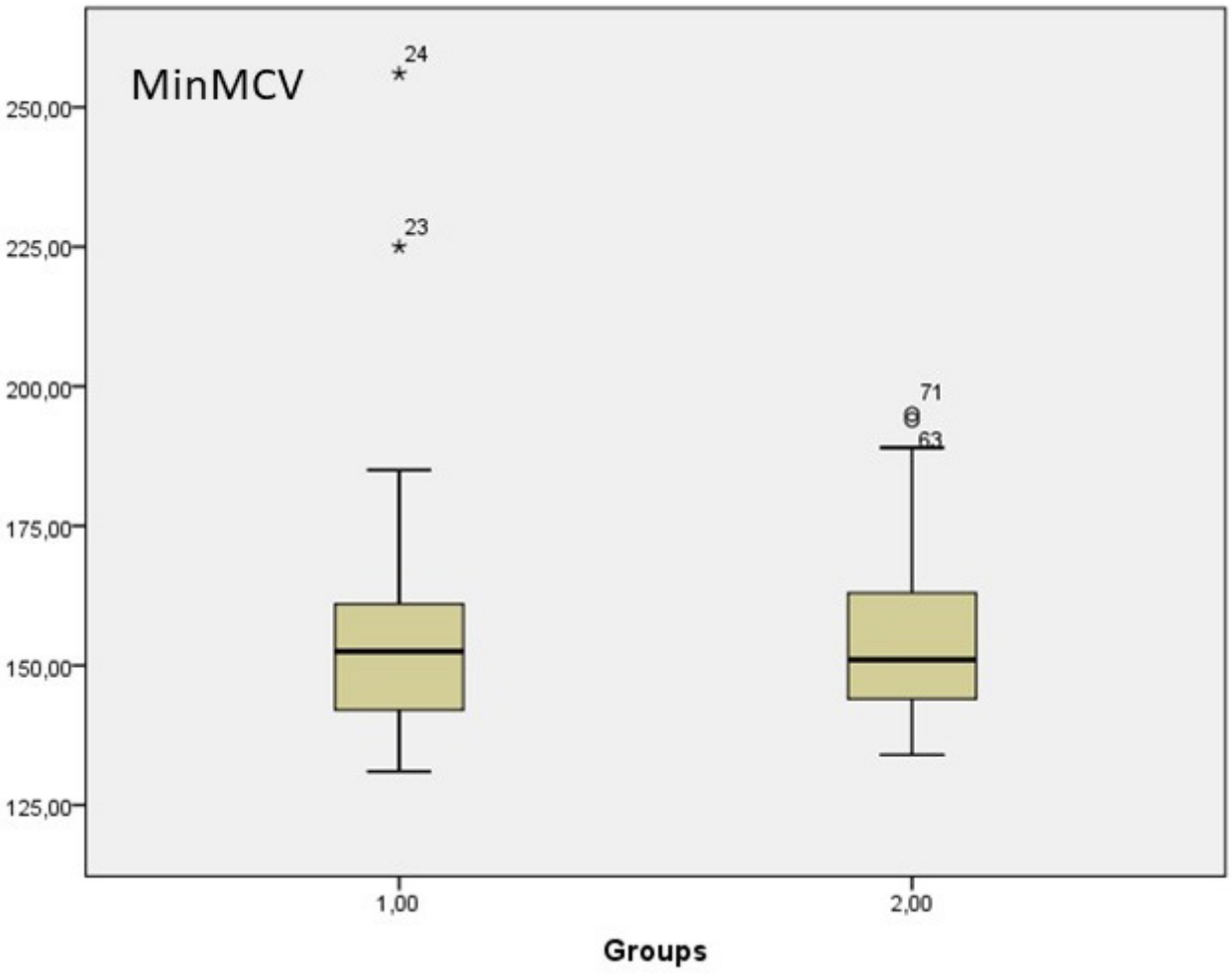

Figure 6

Graph shows the distribution of minimum mean cell volüme (Min MCV)in Group 1 and 2.

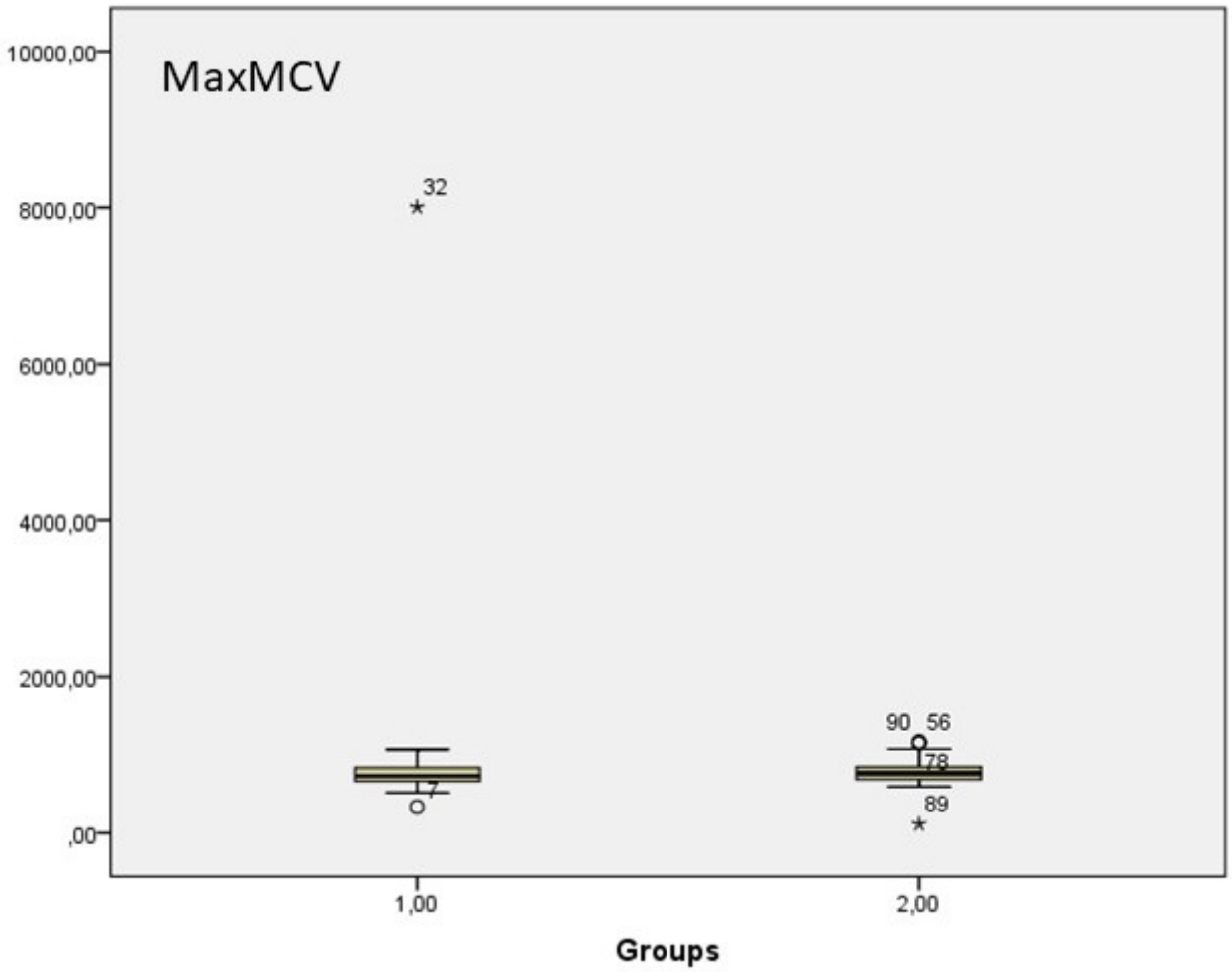

Figure 7

Graph shows the distribution of maximum mean cell volüme (Max MCV)in Group 1 and 2.