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Relationship Between Serum Carbonic Anhydrase Activity and Carbonic Anhydrase Autoantibody Levels in Patients with Rheumatoid Arthritis

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Abstract

***Objective**: An organism's immune response to its own tissue antigens is called autoimmunity, and the diseases caused by such, autoimmune diseases. Carbonic anhydrase II, (CA II), the most common CA isoenzyme in humans, is considered a common target antigen, since it is abundantly synthesized in bile ducts, pancreatic ducts, renal tubular cells, and the epithelial cells of salivary glands, in addition to erythrocytes. Therefore, anti-CA measurements have become important in the diagnosis of some autoimmune diseases.*

***Method:**The effect of the carbonic anhydrase enzyme in the measurement of CA I and CA II antibodies using Enzyme-Linked Immunosorbent Assay (ELISA) was investigated using the serum of a group of rheumatoid arthritis patients.By this aim, total CA amount was found via determination of the amounts of CA I and CA II antibodies and of esterase activity by ELISA method.*

***Results:** CA II autoantibody levels in the patient group of 0.255 ±¬ 0.182 (mean + SD), and 0.137 ± 0.040 in the control group were found ($p < 0.001$). Meanwhile, CA I autoantibody level was found to be 0.153 ± 0.036 in the patient group, and the control group level was 0.129 ± 0.052; ($p = 0.965$). There was no correlation between CA I/CA II autoantibody levels and serum CA activity.*

***Conclusion**: Thus, it was concluded that the determination of CA antibodies by ELISA method is not affected by CA activity in non-hemolytic serum.*

***Keywords:** carbonic anhydrase, autoimmunity, rheumatoid arthritis, hemolysis*

1. Introduction

Carbonic anhydrase (CA), (CA, EC 4.2.1.1., carbonate hydrolyase) is an enzyme found in many species, ranging from bacteria to the most developed animals. These enzymes are metalloproteins containing Zn^{2+} ions in their active regions and are in monomer structure. Carbonic anhydrases play a role in physiological events, such as the transport of CO_2 formed in the organism by metabolic pathways, electrolyte secretion, regulation of the acid-base balance, and the formation of biosynthetic $HCO3^-$ (Menteşe et al., 2018). To date, 16 different CA isoenzymes have been identified in different cells and tissues of animals, with various cellular locations (Menteşe et al., 2017a).

Autoimmune diseases are described as the body itself produces (auto)antibodies against its own healthy tissue antigens (Menteşe et al., 2017b). In 1991, the presence of CA II autoantibodies was identified for the first time in the serum of patients with systemic lupus erythematous and Sjogren's syndrome (Inagaki et al., 1991). Thereafter, CA II autoantibodies in various autoimmune diseases were determined using western blot and Enzyme-Linked Immunosorbent Assay (ELISA) methods (Kino-Ohsaki et al 1996, .Ito et al. 1997, Gordon et al.,1995). CA II is synthesized abundantly in the epithelial cells of bile ducts, pancreatic ducts, renal tubules, and salivary gland ducts. Therefore, CA II is thought to play a role in the pathogenesis of disease complexes called autoimmune exocrinopathy and autoimmune epithelitis, which are secreted from epithelial cells of various exocrine glands (Nishimori et al. 2004). In addition to these, CA II autoantibodies have been identified in patients with systemic lupus erythematous, systemic sclerosis, dermatomyositis, polymyositis, endometriosis, autoimmune hepatitis and chronic viral

hepatitis, in end-stage renal disease, acute anterior uveitis and PCOS. However, the role of CA II autoantibodies in the pathology of the abovementioned diseases is not understood (Invernizzi et al., 1998, Akisawa et al, 1999, Alver et al. 2014, Turk et al., 2014, Menteşe et al., 2013,).

Autoimmune diseases such as endometriosis, autoimmune thyroid, rheumatoid arthritis, systemic sclerosis, idiopathic chronic pancreatitis, and Sjögren's syndrome affect about 5% of the population in Western countries (Menteşe et al., 2018). In recent years, it has been discussed whether CA II antibodies can be used as serum markers in identifying some of these autoimmune diseases and distinguishing them from diseases with similar symptoms (Aparisi et al., 2005). None of the previous studies have examined the possible effect of CA activity, which may be caused by hemolysis, on the autoantibody measurements.

Although blood collection methods are standardized, there is carbonic anhydrase activity present in serum and carbonic anhydrase activity from erythrocytes resulting from minimal hemolysis. Aim of this study is to reveal the quantitative relationship between total CA activity and CA I and II autoantibody measurements in rheumatoid arthritis patients' sera and whether these values correlate with total CA activity originating from hemolysis.

2. Materials and Methods

Sample Collection

For the study, permission was obtained from the clinical research ethics committee of Karadeniz Technical University KTU) with a meeting number of 04/2007 and decision

number 2007/8, and the patients were informed with an informed consent form. Sera used in this study were obtained from rheumatoid arthritis patients (n = 32, 25 female/7 male) who applied to the KTU Faculty of Medicine Physical Medicine and Rehabilitation outpatient clinic, and the blood used for the control group was taken from volunteer individuals (n = 24, 9 female/15 male). Collected blood samples were centrifuged at 3000 rpm for 10 minutes, and the sera obtained were stored at -20°C.

Enzyme-Linked Immunosorbent Assay

Determination of the CA I and CA II autoantibody levels was performed using the ELISA method developed before, with minor modifications (Hosoda et al., 2004). Each sample was assayed in duplicate and the specific binding of serum antibody to CA I or II was calculated with the following formula :

Specific binding = ODcoated – ODcontrol.

Determination of Carbonic Anhydrase Activity

Determination of CA activity in serum was performed using the esterase activity measurement method developed by Armstrong et al. (Verpoorte et al., 1967). The method is based on the hydrolysis of p-nitro phenyl acetate to *p*-nitro phenol and acetate by carbonic anhydrase. Carbonic anhydrase activity against p-nitro phenyl acetate was determined by measuring the amount of released p-nitro phenol spectrophotometrically at 348 nm.

To determine CA activity in serum samples, at the first glance, total esterase activity was determined in each serum. For this purpose, 1.3 mL Tris-SO_4 buffer was well mixed with 0.1 mL serum or 0.1 mL buffer solution at room temperature. Then, on top of this mixture,

1.5 mL of *p*-nitro phenyl acetate solution was added, and its absorbance was read at 348 nm immediately. After 3 min of incubation time, the absorbance was read again for the same samples. The difference between the absorbance values in the first and second read was calculated as esterase activity. With this experiment, the overall activities of total CA and other ester hydrolysis enzymes in serum were measured.

In the second part, CA in serum was completely inhibited using 0.1 M acetazolamide solution. 1.3 mL Tris-sulfate buffer added to test tubes with 0.1 mL serum and 0.1 mL inhibitor solution. This mixture was incubated for ten minutes to ensure full enzyme inhibitory interaction. Then, 1.5 mL of substrate solution was added to it, and absorbance was read at 348 nm immediately. Following this, samples were incubated for three minutes, absorbance was read again, and differences between two time points was calculated. By subtracting total esterase activity found in the first experiment from the value in the second part of the experiment, the CA activity within the total esterase activity was determined. Absorbance values were used for statistical analysis.

Statistics

Fitness of the data obtained from the patient and control groups to normal distribution was determined by the Kolmogorov-Smirnov test. Student's t test was used for those that conformed to the normal distribution, and Mann-Whitney U test was used for those that did not fit the normal distribution. Significance was considered at the level of $p < 0.05$.

3. Results

ELISA Results for CA I Autoantibodies

The levels of CA I autoantibody were analyzed using ELISA method in serum collected from

32 rheumatoid arthritis patients and 24 healthy individuals. Individuals with an average absorbance value greater than 0.233 were considered positive (cut off value). This value was calculated by adding 2SD (standard deviation) value (0.052) to the average absorbance value (0.129) in the control group. While none of the 32 patients with rheumatoid arthritis had positive results, one positive result was found in the control group (4.2%). (Figure 1)

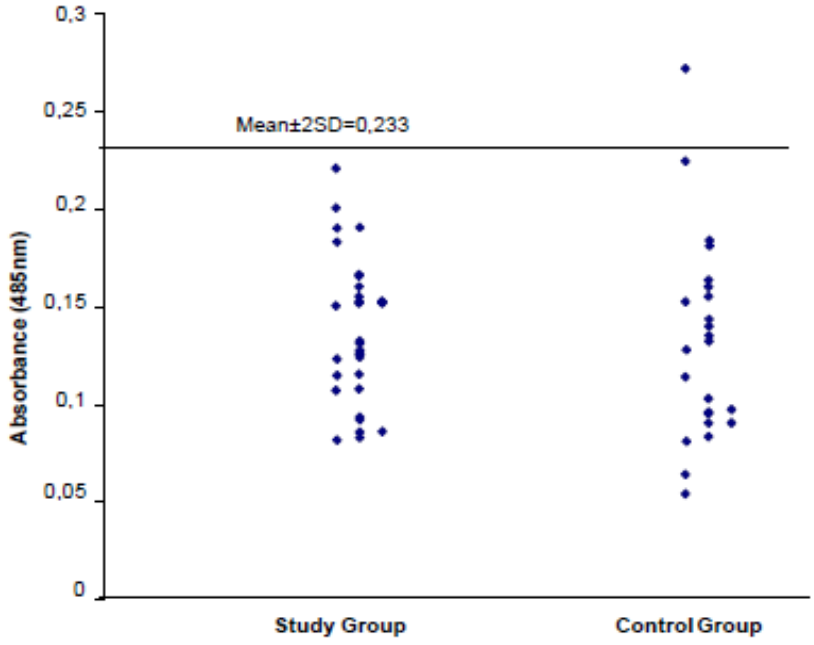

Figure 1. ELISA Results for CA I Autoantibodies

ELISA Results for CA II Autoantibodies

The sera of 32 patients with rheumatoid arthritis and 24 healthy individuals were evaluated for CA II autoantibody levels by ELISA method. Individuals with absorbance values greater than 0.217 were considered positive. This value was calculated by adding 2SD (0.040) to the average absorbance level of the control group. On 17 of the patients

with rheumatoid arthritis, the sera was found positive (53%). No positive results were found in the control group. (Figure 2)

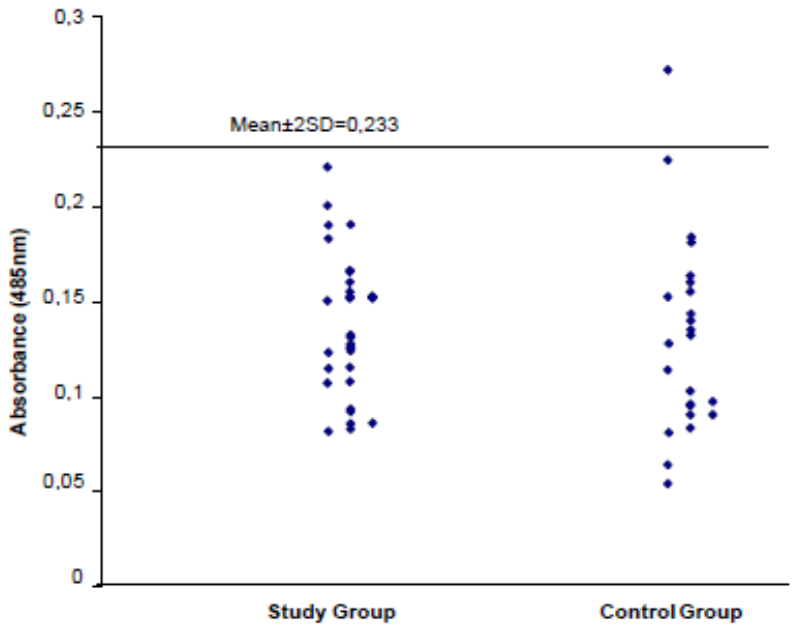

Figure 2. ELISA Results for CA II Autoantibodies

Statistical Results

CA II autoantibody level in the patient group was 0.255±0.128 (mean+SD) and 0.137±0.040 in the control group, and there was a statistically significant difference between them ($p<0.001$).CA I autoantibody level in the patient group was 0.135 ±0.036, and 0.129 in the control group.It was found ±0.052, and there was no statistically significant difference between them ($p=0.965$).(Table 1)

Table 1. Statistical analysis of ELISA for CA I and CA II antibodies

	Patient (n = 32)	Control (n = 24)
CAI autoantibody	0.135 ± 0.036	0.129 ± 0.052
CAII autoantibody	0.255 ± 0.128*	0.137 ± 0.040

** Mann Whitney U test, p<0.001*

Pearson correlation test was used to determine whether there was a relationship between CA I and CA II autoantibody levels and esterase activity. Here, the activity of CA is taken as absorbance and not as enzyme unit, and these values are also not given in tabular form. There was a very low positive correlation between CA I autoantibody level and esterase activity in the patient group (r=0.016), but it was not statistically significant (p=0.927). There was a low negative correlation between CA I autoantibody level and esterase activity in the control group (r=-0.157), but it was not found to be statistically significant (p=0.435). A low and insignificant negative correlation (r=-0.269, p=0.107) was obtained between CA II autoantibody values and esterase activities in the patient group. In the control group, a low negative correlation (r=-0.026) and a statistically insignificant (p=0.898) correlation was found between CA II autoantibody level and esterase activity.

4. Discussion

After Inagaki et al. showed the presence of carbonic anhydrase enzyme in systemic lupus erythematous and Sjogren's syndrome, CA II autoantibodies were detected in different autoimmune diseases using western blot and ELISA methods (Kino-Ohsaki et al 1996, .Ito et al. 1997, Gordon et al.,1995). CA II is synthesized abundantly in the epithelial cells of bile ducts, pancreatic ducts, renal tubules, and salivary gland ducts. Therefore, CA II is

thought to play a role in the pathogenesis of disease complexes called autoimmune exocrinopathy and autoimmune epithelitis as a common target antigen secreted from epithelial cells of various exocrine glands (Nishimori et al., 2004).

The main task of the immune system is to recognize foreign antigens and create an immune response against them. B and T lymphocytes and macrophages are responsible for the initiation and control of immune response (Menteşe et al., 2017b). Under physiologic conditions, organisms do not produce immunity against their own antigens. However, as a result of some pathologic conditions, malfunction of receptors and/or changed/delayed activities of immune system members, the organism can exhibit autoimmunity.

In the diagnosis of autoimmune diseases with unknown mechanism, the determination of cellular type of immune responses and of autoantibodies against the organism's own tissues is used.

Autoimmune diseases, such as endometriosis, autoimmune thyroiditis, rheumatoid arthritis, systemic sclerosis, idiopathic chronic pancreatitis, and Sjögren's syndrome affect about 5% of the population in western countries (Menteşe et al., 2018). Rheumatoid arthritis manifests as a nonspecific and generally symmetrical inflammation in peripheral joints. It is a chronic inflammatory disease that is likely to result in progressive destruction in and around the joints. In recent years, the use of CA II autoantibodies as a serum marker has been discussed in determining and identifying some of these autoimmune diseases (Akisawa et al.,1999, Aparisi et al., 2005,). Previously, in our department, studies were performed based on determination of CA II antibodies by ELISA method. (Alver et al., 2014,

Turk et al., 2014, Menteşe et al., 2013, Menteşe et al., 2015, Menteşe et al,. 2017c). Effect of hemolysishad not been investigated so our results will contribute the literature. For this purpose, in the serum of 32 patients with rheumatoid arthritis, together with 24 controls, total CA activities and CA I and II antibodies were measured, and the relationship between them was quantitatively examined.

As a result of the ELISA tests, while all the data for anti-CA I measurement was negative, 53% of anti-CA II measurements were positive. In ELISA measurements made in different disease groups, anti-CA I levels were found to be greater and more positive than anti-CA II (Kino-Ohsaki et al.,1996, Bartolome et al.,2002, Frulloni et al.,2000). However, our measurements gave totally opposite results, and there was no significant difference between the control and patient groups. As a result, it can be claimed that anti-CA I levels do not change in rheumatoid arthritis.

Carbonic anhydrase enzymes can be found in serum as a result of hemolysis of erythrocytes. With this study, we wanted to demonstrate whether the carbonic anhydrase enzymes affect the ELISA measurements of anti-CAs in hemolytic situations. For this purpose, we performed a correlation test between total CA and anti-CA activities measured in patient and control groups. As a result, in the patient group, a low positive ($r = 0.016$) and statistically insignificant ($p = 0.927$) correlation between CA I autoantibody level and esterase activity was calculated. In the control group, a negative ($r = -0.157$) and statistically insignificant ($p = 0.435$) correlation between CA I autoantibody level and esterase activity was found. Similarly, a negative ($r = -0.269$) and statistically insignificant ($p = 0.107$) correlation was observed between CA II autoantibody level and esterase

activity in the patient group. In the control group, a negative (r =-0.026) and statistically insignificant (p = 0.889) correlation was found between CA II autoantibody level and esterase activity. It was concluded that unless there is an easily noticeable excessive hemolysis, it is not necessary to consider serum CA activity in serum ELISA measurements.

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Conflict of Interest

The authors stated that there were no conflicts of interest.

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Oftalmolojik Rahatsızlıklarda Yeni Bir Tanım: İhmal Edilen Göz Sendromu(İES-22)

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ÖZ

Amaç: Fizyolojik sebepli ortaya çıkan lagoftalmi problemine yeni bir tanımlama getirerek, erken tanı ve doğru tedavi konusuna pozitif katkı sağlamaktır.

Tasarım: IES-22, dışlama ve tanı kriterleri belirlenirken diğer lagoftalmi sebepleri ile karşılaştırıldı ve tamamen ayrı bir tanımlama yapıldı. IES-22 kesin tanısı için dışlama kriterlerinin olmaması, majör kriterlerden en az 1, minör kriterlerinden en az 2 bulgu olması yeterli görüldü.

Metot: IES-22 dışlama kriterleri:Gözler, göz kapakları ve orbitada, lagoftalmiye neden olan herhangi bir patolojik durumun olması şeklinde belirlendi. IES-22 tanı kriterleri majör ve minör olarak ikiye ayrıldı. Majör kriterler primer, minör kriterler ise sekonder sebepler olarak kabul edildi. Majör kriterler: Göz kapakları vertikal küçüklüğü, konjenital exoftalmisi olmak, alt-üst göz kapaklarının limbusu örtmemesi, alt orbita kenarının normalden daha aşağıda alması ve iri gözlü olmak (buftalmus). Minör kriterler: Aile öyküsü olmak, ışık açıkken uyuyamamak, sabah ilk uyandığında gözde yabancı cisim hissi, göz ağrısı bulunması ve gözde sulanma görülmesi, olarak belirlendi. Ayrıca, IES-22 derecelendirmesi; Grade I (hafif), Grade II (orta) ve Grade III (ağır) şeklinde yapıldı.

Sonuç: IES-22, göz açık uyuma vakalarında yeni bir tanımlama olabilir. Altta yatan herhangi bir hastalık olmaması ve uzun süre günlük yaşantımızı etkilememesi sebebiyle göz ardı edilme özelliğine sahiptir. Ayrıca IES-22 majör kriterleri içerisinde konjenital etkenlerin olması, genetik zeminli klinik araştırmalar yapılmasını da uygun kılmaktadır.

Anahtar Kelimeler: IES-22, görmezlikten gelme, göz açık uyuma, göz bozuklukları

A New Definition in Ophthalmological Disorders: Ignored Eye Syndrome (IES-22)

Abstract

Objective: To provide a positive contribution to early diagnosis and correct treatment by bringing a new definition to the problem of lagophthalmos that occurs due to physiological reasons.

Design: IES-22 was compared with other causes of lagophthalmos when determining exclusion and diagnostic criteria, and a completely separate definition was made. For the definitive diagnosis of IES-22, in the absence of exclusion criteria, at least 1 of the major criteria and at least 2 of the minor criteria were considered sufficient.

Method: IES-22 exclusion criteria were defined as the presence of any pathological condition in the eyes, eyelids, and orbit causing lagophthalmos. The IES-22 diagnostic criteria were divided into major and minor. Major criteria were accepted as primary and minor criteria were considered as secondary causes. Major criteria: Vertical small eyelids, congenital exophthalmos, lower and upper eyelids not covering the limbus, lower orbital rim lower than normal, and large eyes (buphthalmus). Minor criteria: Having a family history, not being able to sleep when the light is on, foreign body sensation in the eye when first waking up in the morning, eye pain and watering in the eye were determined. Also, IES-22 rating; It was performed as Grade I (mild), Grade II (moderate) and Grade III (severe).

Conclusion:IES-22 may be a new definition in cases of sleeping with eyes open. Since there is no underlying disease and it does not affect our daily life for a long time, it has the feature of being ignored. In addition, the presence of congenital factors within the IES-22 major criteria makes it appropriate to conduct clinical studies based on genetics.

Keywords: IES-22, ignored, lagophthalmia, ophthalmological disorders

1. Introduction

When people sleep, they sleep with their eyelids completely closed like a normal physiological phenomenon. This event prevents the eye from drying out and being damaged by external factors. On the other hand, while sleeping, the anterior surface of the eye (corneal epithelium and conjunctiva) is fed by the vessels under the lid, and fluid and oxygen exchanges are made. In this way, our eyes are both moist and nourished. Thus, when people wake up, their eyes are rested and fed, and they are ready for daytime work. While this should be the case in normal individuals with healthy eyes, sometimes we may be going through a troublesome process without realizing it. Moreover, the secret hero of this process may be sleeping with eyes open (lagophthalmia). Since there is no underlying pathological condition, we may not have noticed it for a long time and ignored the minor discomforts (tearing, feeling of a foreign body in the eye, etc.). Our idea of defining IES-22 has emerged in the light of these detections.

A New Definition: Ignored Eye Syndrome-22 (IES-22)

"Luo et al. (1999); Zhang et al. (2014); According to Yip et al. (2005)" among the causes of lagophthalmos: surgical interventions to the eye, paralytic causes or endocrinological diseases such as Graves' disease are shown. They have been the subject of research for a long time. "Korula et al. (1995); Valdéz-de la Torre et al. (1999); According to Kievit et al. (2018)" in addition, lagophthalmos can be seen in some rare genetically transmitted syndromes. Our aim is not to repeat an already known and researched symptom. We aim to define lagophthalmos as a syndrome that has been admitted to our ophthalmology clinic in recent years and has been ignored for a long time because there is no underlying disease. In the definition process of Ignored Eye Syndrome-22 (IES-22), the word "ignored" was particularly preferred. Because, in IES-22, lagophthalmos is generally ignored because it is not a result of any disease. That is until complications arise and adversely affect daily life. In this definition, the number 22 represents the year it was defined (2022).

Design

IES-22: In lagophthalmia without any disease (facial paralysis, ptosis, stroke, graves

disease, orbital tumor, cranial tumors and eye trauma, and others) that causes eye open sleep; it is a syndrome that occurs due to the eyelids remaining slightly, partially or entirely open. Since IES-22 does not affect daily life at first and is not an underlying disease, it is often ignored and goes unnoticed. If there is no one to follow the patient while he is asleep, this situation continues. That is until unexplained eye complaints occur. On the other hand, the diagnosis of IES-22 can be easily made by an experienced physician with a detailed anamnesis and physical examination. In IES-22, Although it is not a disease, the eyelids cannot be closed while falling into a deep sleep and remain partially or entirely open, most of the time bilaterally. This openness is not noticed for a long time and is ignored because it does not cause serious disturbances. However, as time progresses, the variety and severity of complaints increase. Among these complications, dry eye, foreign body sensation in the eye, redness in the eyes, blurred vision (especially after waking up), not being able to sleep in a light environment, and feeling tired despite sleeping for hours are among the leading ones.

Could IES-22 be a syndrome?

As it is known, the syndrome; is the whole of the findings that seem to be unrelated to each other, but when they come together, they show themselves as a single phenomenon. "Robin (2000)" is a disorder that may include ocular manifestations such as Stickler Syndrome, myopia, cataracts, and retinal detachment. The diagnosis of Stickler syndrome is clinically based, just like IES-22. There are currently no consensus minimal clinical diagnostic criteria for Stickler syndrome.

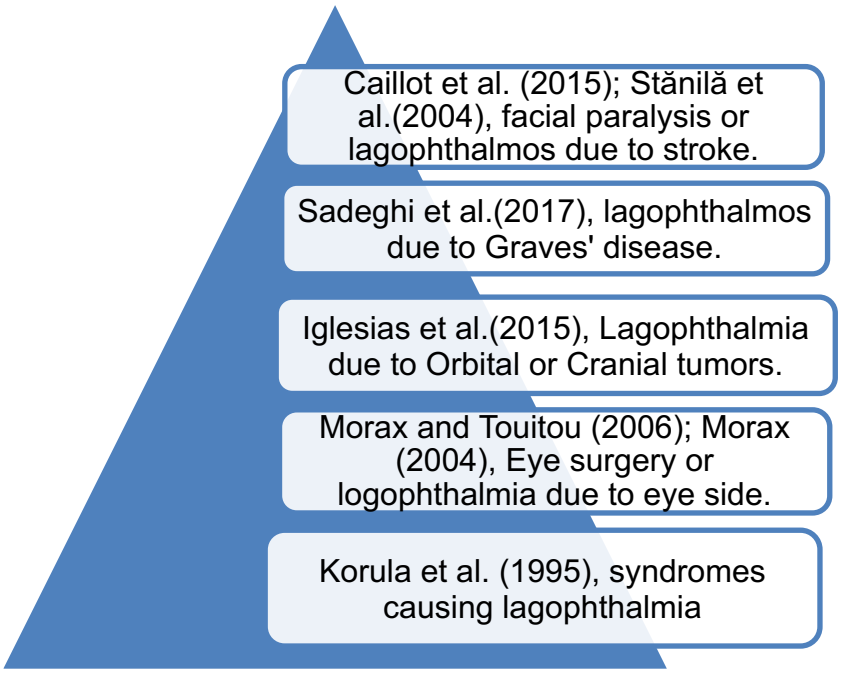
Another reason why IES-22 is called a syndrome is that it causes multi-systemic complications in an insidious way as a physiological condition that is ignored for a long time and goes unnoticed. Severe stress management disorders, distractibility, and intellectual-academic deficiencies can also be seen in lagophthalmos patients with IES-22. In our opinion, in this respect, it is likely that IES-22 patients will be the subject of extensive research in the future. Arora et al.(2021), because research on sleep quality disorders confirms us. In addition, the poor sleep quality of IES-22 patients may disrupt the hormonal balance by negatively affecting the circadian rhythm during the night. "Erland

et al. (2017)", as a matter of fact, studies have shown the interactions of sleep and hormonal balance.

2. Materials and Method

IES-22 exclusion criteria were defined as the presence of any pathology causing lagophthalmos in the eyes and eyelids orbit. These pathologies; include facial paralysis, stroke, graves disease, orbital tumor, cranial tumors, surgical procedures, and eye trauma. In addition, some rare syndromes were also considered among the causes of pathological lagophthalmos (Figure 1).

Figure 1. IES-22 exclusion criteria



Model application

The IES-22 diagnostic criteria were analyzed under two headings major and minor.

Major criteria: Small eyelids, congenital exophthalmos, protrusion of the lower and upper eyelids together or separately outside the limbus, lower orbital border than normal, and large eyes.

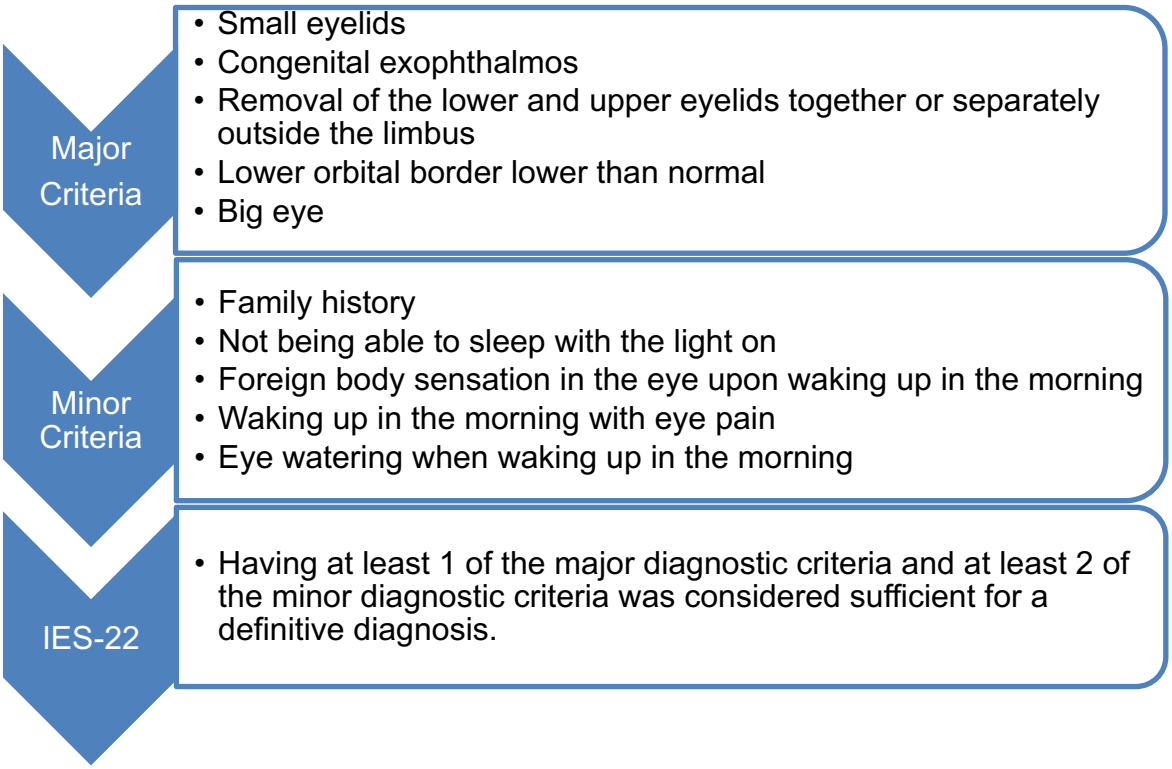
Minor criteria:

Having a family history, not being able to sleep with the light on, waking up first in the

morning, foreign body sensation in the eye, presence of eye pain, and watering in the eye was determined.

Minor criteria were created from secondary symptoms because these symptoms can also be seen in other causes of lagophthalmos other than IES-22. For a definitive diagnosis of IES-22, at least 1 of the major criteria and at least 2 of the minor criteria were considered sufficient (Figure 2).

Figure 2. The IES-22 diagnostic criteria are represented schematically.



IES-22 lagophthalmos grades

Grade grading of eye aperture in patients diagnosed with IES-22 can be easily done by an ophthalmologist. Grade classification was made in three groups according to the degree of eye opening. These; Grade I (mild): if the lower quadrant remains open until the limbus is visible while the patient is asleep (6 o'clock limbus), Grade II (middle): if the lower edge of the pupil is visible while the patient is asleep, and Grade III (severe): part of the pupil or if all remains open (Figure 3).

Figure 3.The IES-22 lagophthalmos grades

Grade I Mild	Grade II Middle	Grade III Severe
• Keeping the eye open enough to see the limbus in the lower quadrant during sleep (6 o'clock limbus)	• Keeping the eye open enough to expose the lower edge of the pupil during sleep	• Keeping the eye open during sleep with the pupil partially or completely open

Treatment recommendations in patients with lagophthalmia caused by IES-22.

Our primary goal in the treatment of lagophthalmos is to prevent keratitis or secondary dryness and to eliminate the loss of eyelid function. It is equally essential for the patient to have a cosmetically acceptable appearance. "Pereira et al. (2010)" recommends moisturizing drops and ointments for the clinical treatment of nocturnal lagophthalmos. Our proposal for IES-22 is similarly qualified. The procedures for correcting eyelid misposition suggested by Latkany et al. (2006) also partially overlap with our treatments. However, the treatment procedures we propose for IES-22 are versatile and offer more opportunities for accurate and timely diagnosis, relief of patient suffering, and prevention of severe ocular surface pathology. We can collect our IES-22 treatment recommendations under five headings.

Conservative treatments can be grouped under five main headings:

1. Application of classical keratitis or eye dryness treatment according to the patient's eye condition. For this, It is appropriate to apply local antibiotics (drops, cream), artificial tears (drops, gels), and light non-steroidal drops.

2. Use a sleeping strip while sleeping. The aim is to apply pressure to the lids and prevent eye dryness and light discomfort by bringing the lower and upper lids closer together.

3. Using therapeutic contact lenses when needed.

4. Performing the exercise of pulling the lower lid up by frequently blinking and squeezing the eyelids.

5. To increase the quality of tears; It is important to massage around the lid (increase blood circulation), regularly consume sufficient water, and use iron and vitamins (vitamin A, beta

carotene).

In addition to the conservative treatments applied to IES-22 patients, it is essential to use sunglasses regularly, protect them from light and wind, avoid direct contact with air conditioners, and prefer more humid environments.

Eye photographs of some of our cases diagnosed with IES-22

Photographing and use in academic studies were carried out with the consent of the patients.

3. Conclusion

Suitability to Clinical Practice

The term lagophthalmos is the first thing that comes to mind when sleeping with one's eyes open. “Serrat et al., 1998; Stănilă et al., 2004; Araújo et al., 2019” still, it should be noted that the etiology of lagophthalmos is mostly facial nerve palsy and orbicular muscle dysfunction. However, there is no disease in the etiology of IES-22, which we defined for the first time in the literature. Although IES-22 patients continue their daily lives, they live under severe stress. This situation negatively affects their work life and family life. Even so, if IES-22 is diagnosed early in ophthalmology clinics, many complications can be prevented before they even begin. Therefore, IES-22 is an easily preventable cause of lagophthalmos with a high degree of relevance to clinical practice.

As a result, IES-22 is a new definition in lagophthalmia cases and has the feature of being ignored since there is no underlying disease and it does not affect our daily life for a long time. On the other hand, IES-22; deserves serious research on it with its early diagnosis and accessible treatment features. In addition, IES-22 should also be evaluated in terms of genetics since there are hereditary factors among its major criteria.

*Conflict of Interest*Conflict of Interest

No conflict of interest has been declared by the author.

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