

# Do Chronic Alcoholism Effect to The Cornea and Retina? (Alcohol and Eye)

Abuzer Gunduz<sup>1</sup>, Tongabay Cumurcu<sup>1,\*</sup>, Birgul Elbozan Cumurcu<sup>2</sup>, Ersin Ersan Demirel<sup>1</sup>, Işıl Gögceğöz Gül<sup>2</sup> and Pempegül Fırat<sup>1</sup>

<sup>1</sup>Department of Ophthalmology, Inonu University School of Medicine, Malatya, Turkey

<sup>2</sup>Department of Psychiatry, Inonu University School of Medicine, Malatya, Turkey

**Abstract:** *Objective:* To compare quantitative anterior and posterior segment data obtained with the Pentacam and OCT in individuals who are chronic users of alcohol (ethanol).

*Methods:* Our study was a prospective, case-control study conducted on two groups. The first group (Group 1) consisted of 30 male patients who had presented at the Psychiatry outpatient department of our hospital for the treatment of alcoholism. The second group (Group 2) consisted of 30 age- and sex-matched individuals as the control group. This group included the healthy persons accompanying their relatives who had come to the Eye Department for treatment. All cases underwent an ocular examination, measurement of the axial length, Pentacam examination and Spectral Domain Optical Coherence Tomography (SD-OCT). The quantitative data from these measurements were compared between the two groups. The significance level for the tests was 0.05.

*Results:* The mean age was 45.0±8.03 (28-59) years in the group 1 and 41.83±8.5 (31-60) years in the control group. The mean alcohol use duration was 18.33±9.94 years in group 1. There was no statistically significant difference between the two groups for age or axial length. There was also no significant difference between the groups for the Pentacam K (keratometry), CCT (central corneal thickness), ACD (anterior chamber depth), ACA (anterior chamber angle), ACV (anterior chamber volume) and LT (lens thickness) data ( $p>0.05$ ). There was also no difference between the groups for the SD-OCT FCT (Foveal 1 mm central ring thickness), S-GCC (Superior-hemiretinal ganglion cells complex), I-GCC (Inferior hemiretinal ganglion cells complex), G-RNFL (Global -Retinal nerve fiber layer), I-RNFL (Inferior -Retinal nerve fiber layer), S-RNFL (Superior-Retinal nerve fiber layer), N-RNFL (Nasal-Retinal nerve fiber layer) and T-RNFL (Temporal-Retinal nerve fiber layer) data ( $p>0.05$ ).

*Conclusion:* The results of this study were not find a difference between measurements of the ocular structures of individuals with a history of chronic alcohol use and healthy individuals in this quantitative study.

**Keywords:** Chronic Alcoholism, Ocular Anatomical Structures, Pentacam, Spectral Domain Optical Coherence Tomography (SD-OCT).

## INTRODUCTION

Alcohol usage is as old as the history of mankind. The harmful effects of such usage on human health is known. The relationship with liver and pancreas cancers and also larynx and esophageal cancers has been shown [1]. Some articles report an association between alcohol and some ocular diseases [2]. For example, the incidence of ocular diseases such as age-related macular degeneration (ARMD), cataract and glaucoma has been found to be higher in alcohol users. However, there are very few scientific studies on how alcohol specifically affects ocular tissues. One experimental study has found histological thickening in the cornea and corneal epithelium [3]. Another experimental study has reported increased retinal oxidative stress with alcohol [4].

Although there is a lot of information on the harmful effects of chronic alcohol use on human health, the

data on its effect on the ocular anatomical structures are inadequate. We compared the quantitative Pentacam and OCT data from the anterior and posterior segment structures of the eye between chronic alcohol users and healthy individuals in this study.

Therefore, we used SD-OCT and Pentacam to investigate whether there was a difference in the corneal and retinal thickness of between in patients with chronic alcoholism and normal individuals. So this is important to evaluation of many ocular problems such as glaucoma, refraction and refractive surgery.

## METHODS

We obtained consent from the Inonu University local ethics committee for the study. All patients participated in the study voluntarily. We obtained information and consent form from the individuals in both the patient and volunteer control groups.

Our study was designed a cross sectional and case-controlled. The first group (Group 1) consisted of

\*Address correspondence to this author at the Inonu University, School of Medicine, Malatya, Turkey; Tel: 90-422-342 06 60/4002; Fax: 90-422-3410728; E-mail: abuzergunduz@hotmail.com

chronic alcohol users. This group included 30 male patients who had presented at the Psychiatry Department of our hospital for alcoholism treatment. Of these 30 patients, 12 smoked 20 cigarettes (1 pack) a day. We defined heavy drinking as  $\geq 4$  drinks per day accordance with the suggestions of Blue Montains Eye Study (BMES) and Beaver Dam Eye Study [5,6]. In addition, taking the amount of alcohol in a double raki as 20 gr, the amount of alcohol consumption was  $> 80$  g/day in Group 1 patients [7].

The second group (Group 2) consisted of 30 age- and sex-matched individuals. This group included the healthy persons accompanying their relatives who had come to the Eye Department for treatment. We also included 12 individuals who smoked 20 cigarettes (one pack) daily in the control group to make the groups similar. The years of smoking was also matched.

All patients in both groups underwent an eye examination by an ophthalmologist. This examination included refraction, visual acuity check, cornea and anterior segment evaluation by biomicroscopy, fundus examination and intraocular pressure check. Only eyes that had not amblyopia and did not suffer from any ocular disease were included in the study.

One eye of each case was included in the study. Both groups consisted of individuals with no systemic disease who had not used any systemic or topical drugs in the last three months. Eyes found to be emmetropic were included in the study after all subjects underwent a standard ocular examination. Eyes with an axial length (ALX) less than 22.0 mm or more than 26.0 mm were also excluded from the study. The other exclusion criteria were glaucoma, amblyopia, history of ocular trauma, corneal problems (e.g. scar, edema, dry eye), history of refractive surgery.

The right eye of each patient was evaluated for the statistical analysis. All eyes included in the study underwent Pentacam, SD- OCT and ALX measurements, in this order.

### **SD-OCT Measurements**

High speed and resolution Spectral Domain Optical Coherence Tomography (SD-OCT) is a non-invasive device that enables imaging of the retina and other tissues at micron-level resolution. It is possible to perform retinal evaluations such as retinal nerve fiber thickness, optic head analysis, ganglion cell complex measurement in more detail with this device. A motion artifact is not created thanks to the high speed of the device. This enables viewing the surface contours of

retinal layers without disturbance. The feature leads to more accurate and reliable measurements [8].

The thickness of the RNFL (Retinal nerve fiber layer), macula, and GCC (ganglion cells complex) was measured with the RS-3000 OCT RetinaScan (Nidek Inc., CA), which is a high-speed SD-OCT/confocal ophthalmoscope system. Real-time, high-contrast, and wide-view ( $40^\circ \times 30^\circ$ ) confocal scanning laser ophthalmoscope (SLO) imaging ensures the accuracy of OCT scanning of the pathological target. The technique provides 53,000 A-scans/sec and a  $4 \mu\text{m}$  OCT axial resolution, showing the discrete retinal layers. Mapping a wide area ( $9 \text{ mm} \times 9 \text{ mm}$ ) enables the GCC status to be observed, even in peripheral regions. The OCT scanning position is precisely matched with the SLO fundus image.

The macula map x-y, disc map x-y scanning protocols were performed for all subjects in this study. Superior, and inferior hemiretinal GCC, fovea thickness (1 mm diameter ring) and global, inferior, superior, nasal, and temporal RNFL values were included for the analysis. All of the SD-OCT measurements were obtained by the same clinician. Submitted scans were assessed for signal strength index, image centration and color cross section. A signal strength index greater than 50 was included. Scans that were decentered or had poor color cross-sections were excluded.

### **Pentacam Measurements**

For imaging the anterior segment of the eye the Pentacam Scheimpflug was designed which is a non-contact optical system. Its easy use, high reliability, repeatability and independence from the operator are the advantages of the pentacam scheimpflug system [9]. Quantitative information and qualitative imaging of the cornea, anterior chamber depth, anterior chamber angle, iris and lens are obtained by the Pentacam rotating Scheimpflug camera [10].

All eyes were evaluated with the Pentacam (Oculus Optikgerate GmbH, Wetzlar, Germany). All measurements were obtained under standard dim light conditions and without dilation. The instrument automatically starts the measurement when correct alignment with the corneal apex and focus is achieved. One measurement with the Scheimpflug system takes approximately 2 seconds, during which time the Scheimpflug camera captures 25 images by rotating 360 degrees around the optical axis of the eye. The qualities of the 3 images were checked, and the examination with the best quality was recorded for

each eye. After completing a scan, the Pentacam software constructs a 3-dimensional image of the anterior segment and calculates the anterior chamber parameters [11]. This imaging provides measurements of anterior chamber depth (ACD), anterior chamber volume (ACV), anterior chamber angle (ACA) width, lens thickness (LT) and pupil size. The Pentacam system measures ACD from the endothelium to the anterior lens surface without the corneal thickness, while the ACV is from the posterior cornea to the anterior lens surface [12].

### Axial Length Measurements

Proparacaine HCl 0.5% drops (Alcain, Alcon Couvreur, Puurs, Belgium) were used for topical corneal anesthesia before the Alx measurement. The Alx measurements were determined by ultrasound (A-Scan, Biovision V Plus). A scan examination was carried out with an 8 MHz linear probe. The average of 10 Alx measurements was used.

### Statistical Analysis

The SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The

Shapiro-Wilk test was used to determine normal distribution. The t-test and Mann-Whitney U tests were used to compare the variables between groups. The significance level for the tests was 0.05.

### RESULTS

The participants comprised 30 male cases who were aged  $45.0 \pm 8.03$  years range: 28-59 years and 30 controls who were aged  $41.83 \pm 8.5$  years range 31-60 years. The mean alcohol use duration was  $18.33 \pm 9.94$  years in group 1. There was no significant difference between the groups in terms of age and sex ( $p > 0.05$ ). The Alx data were  $23.86 \pm 0.51$  in group 1 and  $23.92 \pm 0.61$  in group 2 respectively. There was not find a statistically difference when the two groups were compared for axial length ( $p = 0.128$ ) (Table 1).

There was not find a significant difference between the groups for the Pentacam K, CCT, ACD, ACA, ACV and LT data in the study (Table 2).

We also did not find a significant difference between the two groups for the SD-OCT FCT, S-GCC, I-GCC, G-RNFL, I-RNFL, S-RNFL, N-RNFL and T-RNFL data (Table 3).

**Table 1: Age, Gender, Axial Length of the Patients Included in the Study and the Mean Alcohol Use Duration for Group 1 Cases**

Patient	Group 1 (n=30)	Group 2 (n=30)	P value
Age (years)			
Mean	45.0±8.03	41.83±8.50	0.094
Range	28-59	31-60	
Gender			
Male	30	30	0.128
Female	-	-	
ALX* (mm)	23.86±0.51	23.92±0.61	
Alcohol use duration (years)	18.33±9.94		

\*Alx, axial length.

**Table 2: Comparison of the Study Groups for Pentacam Data**

Pentacam Data	Group 1 (n=30) (Mean±SD)	Group 2 (n=30) (Mean±SD)	P value
Mean K	42.83±1.62	42.48±1.25	0.352
CCT(μm)	542.40±31.91	543.43±31.91	0.958
ACD (mm)	2.91±0.418	3.43±0.289	0.192
ACA(degree)	32.92±5.25	34.80±4.64	0.168
ACV(mm <sup>3</sup> )	161.46±45.06	178.83±29.02	0.081
LT(mm)	3.37±0.17	3.74±0.16	0.820
Pupil size(mm)	2.28±0.49	2.96±0.41	0.510

K, keratometry; CCT, central corneal thickness; ACD, anterior chamber depth; ACA, anterior chamber angle; ACV, anterior chamber volume; LT, lens thickness.

**Table 3: Comparison of the Study Groups for SD-OCT Data**

OCT Data	Group 1(n=30) (Mean±SD/ $\mu\text{m}$ )	Group 2 (n=30) (Mean±SD/ $\mu\text{m}$ )	P value
FCT	232.26±24.28	230.10±17.67	0.988
S-GCC	101.00±6.64	100.03±7.09	0.370
I-GCC	107.83±6.71	107.40±7.60	0.772
G-RNFL	112.16±22.43	105.30±19.45	0.293
I-RNFL	138.20±15.41	138.25±14.43	0.375
S-RNFL	122.20±22.23	140.26±17.95	0.352
N-RNFL	81.13±21.38	68.80±10.56	0.110
T-RNFL	70.80±16.00	87.90±17.98	0.353

FCT, Foveal 1 mm central ring thickness; S-GCC, Superior-hemiretinal ganglion cells complex; I-GCC, Inferior hemiretinal ganglion cells complex; G-RNFL, Global - Retinal nerve fiber layer; I-RNFL, Inferior -Retinal nerve fiber layer; S-RNFL, Superior-Retinal nerve fiber layer; N-RNFL, Nasal-Retinal nerve fiber layer; T-RNFL, Temporal-Retinal nerve fiber layer.

## DISCUSSION

Many organs, such as liver, brain, kidney, and heart affect from alcohol consumption. Because of its low molecular weight, solubility in both water and lipids alcohol penetrates all body tissues. Free radicals generated from alcohol metabolism causes cell structure and function damage. Alcohol consumption increases the production of reactive oxygen species and these can damage or cause complete degradation of essential complex molecules in the cells, including protein, fat molecules and DNA [13,14].

Chronic alcohol (ethanol) consumption increased lipid peroxidation products such as malondialdehyde (MDA) and decreases antioxidant substances such as glutathione (GHS), thus leading to the apoptotic death of the cell [15].

Alcohol usage can also damage the visual system. Visual loss can occur as a result of tobacco-alcohol amblyopia and the vitamin deficiencies secondary to the nutritional deficiencies caused by alcohol [4].

Alcohol usage has also been shown to have effects on the anatomical structures of the eye. For example, an experimental study has found thickening of the cornea and corneal epithelium with alcohol usage [3]. However, we did not find a significant difference regarding CCT values.

Some studies have found no significant relationship between alcohol usage and glaucoma [16,17]. However, an association between alcohol use and increased intraocular pressure has been found in males [18]. Schuze *et al.* [19] have reported that imaging the GCC with OCT is helpful for early diagnosis of glaucoma. We did not find a significant difference between the two groups for ACA, ACD and

pupil diameters, parameters we had evaluated regarding glaucoma. We also did not find a difference between the two groups for superior and inferior GCC parameters, a posterior segment findings that could have been one of the earliest signs of glaucoma.

There are various studies on cataract and alcohol use in the literature. It has not been possible to show a definite association between cataract and alcohol usage in most of these studies. Munoz *et al.* [20] have found an increased risk of posterior subcapsular cataract in persons who drink more than 7 units of alcohol daily. Another study found a decreased prevalence of cataract with alcohol usage [21]. Our study consisted of individuals with a clear lens and we did not find a significant difference between the groups in lens thickness.

Alcohol affects nerve tissue by increasing oxidative stress [22]. It's effect on the retina is with a direct oxidative toxic effect [4]. Losses in the retinal nerve fiber layer of the papillomacular bundle have been reported in chronic alcoholics who also smoke [23]. We did not find a significant difference between the two groups regarding RNFL layer parameters.

There are also various reports on the association between alcohol use and age-related macular degeneration (AMD). Obisesan *et al.* [24] have reported that drinking a moderate of wine protects individuals from AMD. Another study has found an increased prevalence of AMD with alcohol usage [25]. A 10-year prospective study on alcoholic patients drinking 4 units a day found a higher incidence of late type AMD development than in individuals who did not use alcohol [26]. We did not find a difference between the groups regarding the measurements in the foveal 1 mm ring which be the first affected by AMD.

As mentioned above, many studies have shown that alcohol use causes various ocular disorders. However, some studies have not found any relationship between alcohol use and significant ocular changes. For example, Klein *et al.* [27] found no marked relation between alcohol use and cataract. Another study found a significant relationship between alcohol use and AMD in females but not in males [28]. We did not find a significant difference between the two groups for ocular anatomical structure measurements. Our study shows no significant effect of alcohol on ocular anatomical structures. However, we believe more specific studies are needed to show the effect of alcohol on pathophysiological structures.

In addition, one limitation of our study is the lack of a female subject. The reason is the almost complete absence of chronic alcohol use in women due to the sociocultural and religious beliefs of our region. We also had a small number of alcoholic patients who did not smoke so twelve cases were individuals who both smoked and used alcohol. We therefore included the same number of smoking individuals who had smoked for the same duration in the control group. This negated our disadvantage.

In conclusion, we did not find a difference between the ocular structures of chronic alcohol users and healthy individuals in this quantitative study. The number of cases in our study was limited. It may be possible to obtain more accurate results if such studies are performed on larger series.

## REFERENCES

- [1] Room R, Babor T, Rehm J. Alcohol and public health. *The Lancet* 2005; 365: 519-30.
- [2] Wang S, Wang JJ, Wong TY. Alcohol and Eye Diseases. *Survey Ophthalmol* 2008; 53(5): 512-25. <http://dx.doi.org/10.1016/j.survophthal.2008.06.003>
- [3] Emre S, Yilmaz Z, Oztürk F, Emre MH. Propolis prevent the effects of chronic alcohol intake on ocular tissues. *Ophthalmic Res* 2009; 42(3): 147-51. <http://dx.doi.org/10.1159/000229029>
- [4] Sancho-Tello M, Muriach M, Barcia J, Bosch-Morell F, Genovés JM, Johnsen-Soriano S, *et al.* Chronic alcohol feeding induces biochemical, histological, and functional alterations in rat retina. *Alcohol Alcohol* 2008; 43(3): 254-60. <http://dx.doi.org/10.1093/alcalc/agn006>
- [5] Barkan Y, Gerber Y, Elbaz U, Schwartz S, Ken-Dror G, Avni I, *et al.* Central corneal thickness measurement with the Pentacam Scheimpflug system, optical low-coherence reflectometry pachymeter, and ultrasound pachymetry. *J Cataract Refract Surg* 2005; 31(9): 1729-35. <http://dx.doi.org/10.1016/j.jcrs.2005.03.058>
- [6] Knudtson MD, Klein R, Klein BE. Alcohol consumption and the 15-year cumulative incidence of age-related macular degeneration. *Am J Ophthalmol* 2007; 143: 1026-29. <http://dx.doi.org/10.1016/j.ajo.2007.01.036>
- [7] Oncu F, Ogel K, Cakmak D. Alcohol and Culture: 2. Culture of Drink and Drink in The Literature. *Bagimlilik Dergisi* 2002; 3: 31-36.
- [8] Nassif N, Cense B, Park B, Pierce M, Yun S, Bouma B, *et al.* *In vivo* high-resolution video-rate spectral-domain optical coherence tomography of the human retina and optic nerve. *Opt Express* 2004; 12(3): 367-76. <http://dx.doi.org/10.1364/OPEX.12.000367>
- [9] Barkan Y, Gerber Y, Elbaz U, Schwartz S, Ken-Dror G, Avni I, *et al.* Central corneal thickness measurement with the Pentacam Scheimpflug system, optical low-coherence reflectometry pachymeter, and ultrasound pachymetry. *J Cataract Refract Surg* 2005; 31(9): 1729-35. <http://dx.doi.org/10.1016/j.jcrs.2005.03.058>
- [10] Rabsilber TM, Khoramnia R, Auffarth GU. Anterior chamber measurements using Pentacam rotating Scheimpflug camera. *J Cataract Refract Surg* 2006; 32: 456-59. <http://dx.doi.org/10.1016/j.jcrs.2005.12.103>
- [11] Doganay S, Bozgul Firat P, Emre S, Yologlu S. Evaluation of anterior segment parameter changes using the Pentacam after uneventful phacoemulsification. *Acta Ophthalmol* 2010; 88(5): 601-606. <http://dx.doi.org/10.1111/j.1755-3768.2008.01446.x>
- [12] Uçakhan OO, Ozkan M, Kanpolat A. Anterior chamber parameters measured by the Pentacam CES after uneventful phacoemulsification in normotensive eyes. *Acta Ophthalmol* 2009; 87(5): 544-48. <http://dx.doi.org/10.1111/j.1755-3768.2008.01305.x>
- [13] Wu D, Cederbaum AI. Alcohol, oxidative stress and free radical damage. *Alcohol Res Health* 2003; 27: 277-83.
- [14] Pushpakiran G, Mahalakshmi K, Anuradha V. Taurine restores ethanol-induced depletion of antioxidants and attenuates oxidative stress in rat tissue. *Amino Acids* 2004; 27: 91-96. <http://dx.doi.org/10.1007/s00726-004-0066-8>
- [15] Albano, E. Alcohol, oxidative stress and free radical damage. *Proc Nutr Soc* 2006; 65: 278-90. <http://dx.doi.org/10.1079/PNS2006496>
- [16] Leske MC, Warheit-Roberts L, Wu SY. Open-angle glaucoma and ocular hypertension: the Long Island Glaucoma Casecontrol Study. *Ophthalmic Epidemiol* 1996; 3: 85-96. <http://dx.doi.org/10.3109/09286589609080113>
- [17] Klein BE, Klein R, Ritter LL. Relationship of drinking alcohol and smoking to prevalence of open-angle glaucoma. The Beaver Dam Eye Study. *Ophthalmology* 1993; 100: 1609-13.
- [18] Yoshida M, Ishikawa M, Kokaze A, Sekine Y, Matsunga N, Uchiha Y, *et al.* Association of lifestyle with intraocular pressure in middle-aged and older Japanese residents. *Jpn J Ophthalmol* 2003; 47: 191-98. [http://dx.doi.org/10.1016/S0021-5155\(02\)00666-4](http://dx.doi.org/10.1016/S0021-5155(02)00666-4)
- [19] Schuze A, Lamparter J, Pfeiffer N, Berisha F, Schmidtmann I, Hoffmann EM. Diagnostic ability of retinal ganglion cell complex, retinal nerve fiber layer, and optic nerve head measurements by Fourier-domain optical coherence tomography. *Graefes Arch Clin Exp Ophthalmol* 2011; 249(7): 1039-45. <http://dx.doi.org/10.1007/s00417-010-1585-5>
- [20] Munoz B, Tajchman U, Bochow T, West S. Alcohol use and risk of posterior subcapsular opacities. *Arch Ophthalmol* 1993; 111: 110-12. <http://dx.doi.org/10.1001/archophth.1993.01090010114036>
- [21] Cumming RG, Mitchell P. Alcohol, smoking, and cataracts: the Blue Mountains Eye Study. *Arch Ophthalmol* 1997; 115: 1296-303. <http://dx.doi.org/10.1001/archophth.1997.01100160466015>
- [22] Sun AY, Ingelman M, Neve E, Matsumoto H, Nishitani Y, Minowa Y, *et al.* Ethanol and oxidative stress. *Alcohol Clin Exp Res* 2001; 25(5 Suppl ISBRA): 237S-43S.

- [23] Chia EM, Mitchell P, Rochtchina E, Lee AJ, Maroun R, Wang JJ. Prevalence and associations of dry eye syndrome in an older population: the Blue Mountains Eye Study. *Clin Experiment Ophthalmol* 2003; 31: 229-32.  
<http://dx.doi.org/10.1046/j.1442-9071.2003.00634.x>
- [24] Obisesan TO, Hirsch R, Kosoko O, Carlson L, Parrott M. Moderate wine consumption is associated with decreased odds of developing age-related macular degeneration in NHANES-1. *J Am Geriatr Soc* 1998; 46: 1-7.
- [25] Smith W, Mitchell P. Alcohol intake and age-related maculopathy. *Am J Ophthalmol* 1996; 122: 743-45.
- [26] Klein R, Klein BE, Tomany SC, Moss SE. Ten-year incidence of age-related maculopathy and smoking and drinking: the Beaver Dam Eye Study. *Am J Epidemiol* 2002; 156(7): 589-98.  
<http://dx.doi.org/10.1093/aje/kwf092>
- [27] Klein BE, Klein R, Lee KE, Meuer SM. Socioeconomic and lifestyle factors and the 10-year incidence of age-related cataracts. *Am J Ophthalmol* 2003; 136(3): 506-12.  
[http://dx.doi.org/10.1016/S0002-9394\(03\)00290-3](http://dx.doi.org/10.1016/S0002-9394(03)00290-3)
- [28] Cho E, Hankinson SE, Willett WC, Stampfer MJ, Spiegelman D, Speizer FE, *et al.* Prospective study of alcohol consumption and the risk of age-related macular degeneration. *Arch Ophthalmol* 2000; 118(5): 681-88.  
<http://dx.doi.org/10.1001/archophth.118.5.681>

---

Received on 08-04-2013

Accepted on 20-06-2013

Published on 16-08-2013

DOI: <http://dx.doi.org/10.12974/2309-6136.2013.01.01.2>

© 2013 Gunduz *et al.*; Licensee Savvy Science Publisher.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.