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Effect of dry eye diseases on the level of Protein and Lipids in Tears of Postmenopausal Women.

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ABSTRACT
The study is aimed at investigating the effect of dry eye disease on the level of protein and lipids in tears of postmenopausal women. The study was carried out on 33 postmenopausal women with dry eye symptoms and 22 postmenopausal women that were asymptomatic. The ocular surface disease index (OSDI) questionnaire classified participants as being symptomatic or asymptomatic of dry eye. Tear stability and tear volume were assessed with Fluorescein Tear Break up Time (FBUT) and Schirmer's test respectively. Tears were analysed for protein (total protein, albumin, and globulin) and lipid (cholesterol and triglycerides). The mean total protein for Dry Eye (DE) was higher than that for Non-dry eye (NDE). Albumin and globulin were also higher for the DE group when compared to the NDE group. These were however, not statistically significant ($p > 0.05$). There was also no significant difference in triglycerides and cholesterol between DE and NDE. There was no significant correlation between clinical signs (tear volume and tear breakup time) and total protein, albumin, globulin cholesterol and triglycerides in the NDE and DE eye groups. The study shows that proteins and lipids do not change in postmenopausal women irrespective of the presences of dry eye disease.
Keywords : Dry eye, Postmenopausal women, Albumin, Globulin, Cholesterol, Triglycerides.

INTRODUCTION

Dry eye has been found to increase with age [1] and more women of ages 50 and above have been found with the disease when compared to men [2]. Studies have shown connections between menopause dry eye disease seen in aged women and this has been linked to hormonal changes such as chronic androgen deficiency [3] that occur during menopause. There has also been an association between sex hormone changes which occurs with increasing age and dry eye disease [1,4]. To diagnose these patients accurately, the combination of clinical signs and symptoms is extremely considered due to their importance. Some studies have however shown poor or weak correlation between symptoms of dry eye and the clinical signs [5, 6] and so it will be very important we understand the biochemical alterations that occur in dry eye and see if it can be used for the identification of dry eye disease.

The tear film which covers the eye is very important as it helps moist the cornea and conjunctiva, delivers oxygen to the epithelium of the cornea, removes irritants and debris, confers infection resistance to the eye and reduces friction from lids movement over the globe [7]. It is therefore, a crucial component for proper ocular surface function. The tear film consists of proteins, lipids, lipoprotein, aqueous and mucin which helps it in accomplishing these ocular functions and so, alterations in any of these components could result in dry eye [8]. Albumin and globulin are inflammatory proteins [9] which could be associated to dry eye disease as dry eye disease is an inflammatory disease [10] hence its analysis in dry eye disease is important. Also, cholesterol and triglycerides which are important non polar lipids contained in tears [11] have been found to express changes in dry eye animal model[12,13].Hence, this study is to investigate and provide information on the changes in the proteins and lipids of postmenopausal women, irrespective of their dry eye disease.

METHODS

The study protocol adhered according to the tenets of the Declaration of Helsinkin (Ethical Principles for Medical Research Involving Human Subjects). Ethical Clearance was obtained from the ethical committee of the Edo State Hospital Management Board. Informed consent was also obtained from all the subjects after proper explanation on the nature and aims of the study.

Fifty-five postmenopausal women (33 postmenopausal women with dry eye symptoms and 22 postmenopausal women that were asymptomatic) with no ocular disorders and not on hormonal replacement therapy from the outpatient section of Stella Obasanjo hospital, Benin City, Edo State were included in this study. The control group of participants were postmenopausal women with no symptoms of dry eye (did not use any artificial tears) or any other anterior segment abnormality.

The experimental group included Post–menopausal women (menses ceased more than 12 months before the start of the study) above 50 years of age, and women who have had an oculo-visual examination in the last 2 years. Exclusion criteria included women who wear contact lenses, women who had any clinically significant lid or conjunctiva abnormalities, neovascularization, or corneal opacities, women who were diabetic and have had ocular surgery in the past one year. Women in this group were excluded because these conditions could mimic dry eye disease..

Description of Procedure

A brief case history which involved an ophthalmic and systemic history of each subject was conducted. External eye examination was done to examine the external ocular and anterior segment structures. Fundoscopy using the direct ophthalmoscope was also carried out. Subjects who met the selection criteria were recruited for the study. Participants were then assisted to complete the ocular surface disease index (OSDI) questionnaires. The OSDI questionnaire was used to classify participants as being symptomatic or asymptomatic dry eye subjects. Tear stability and tear volume were assessed with fluorescein tear break up time (FBUT) and Schirmer's test respectively.

Ocular Surface Disease Index (OSDI).

The dry eye OSDI questionnaire was used to calculate the ocular surface index score after it was properly filled. The response given by each subject helped in assessing the severity of the symptoms. OSDI scoring was based on a 0-100 scale, the higher the score, the greater severity. The OSDI is a good tool in differentiating between subjects that are normal and those with dry eye disease.

Measurement of Tear Volume: Tear volume was assessed using the Schirmer's I test. The lower lid was pulled down gently, and the folded 3mm portion of the strip was placed on the palpebral conjunctiva at the specified position. Each eye was tested with the eyes open for 5minutes. During the test the patients were instructed to look straight ahead and blink normally. After 5minutes, the lower lid was gently pulled down, and the strip gently removed with an upward motion. Care was taken to pull the eyelid down before removal of the strip to avoid discomfort. The level of wetness was measured in millimeters from the very tip, regardless of the fold. No topical anesthetic was used. A stop watch was used to measure the time [14].

Tear Break Up Time (TBUT): The end of a fluorescein strip was moistened with one drop of distilled water and applied to the subject's lower temporal bulbar conjunctiva. The subjects were asked to blink several times to spread the dye over the cornea and conjunctiva surfaces, and instructed to keep eyes open while looking straight ahead. The cobalt blue filter was used to scan the entire cornea looking for dry areas which appeared as dark spots or streaks. The time in seconds between the last blink and the first appearance of a dry spot was recorded using a stopwatch as TBUT. The mean of three consecutive TBUT was taken. A tear break up time of less than 10 seconds was indicative of an unstable tear film. A break period of 20 minutes was given between the tear volume measurement and tear break up time test [15].

Tear collection using capillary tube: Participants were asked to sit on a chair. A drop of sterile water was instilled in to the eye. Participants were asked to incline their head towards the tear collector, and asked to look up away from the tear collector. Disposable graduated sterile, fine micro point 75mm glass capillary tubes was used to collect tear samples. This sample was transferred carefully to a micro plain tube for the estimation of protein and lipid. The plain tubes were placed on ice and transferred to storage at -80°C [16].

Total Protein analysis

Total protein was analyzed using the Biuret method [17]. Reagent blank was prepared by pipetting 0.02mls of distilled and 1ml of Biuret

reagent mixed in an EDTA tube. The standard was prepared by pipetting 0.02mls standard (CAL) and 1ml of Biuret reagent mixed in an EDTA tube. The tear sample was prepared by pipetting 0.02ml of tears and 1ml of Biuret reagent in an EDTA tube. They were then incubated for 30mins at 20 to 25^{0}C. After which measurement for concentration of protein was done using the spectrophotometer at wavelength of 546nm.

Albumin analysis

Albumin was analysed using the Bromocresol Green method [18]. Reagent blank was prepared by pipetting 0.01ml distilled water and 3ml of BCG reagent mixed in an EDTA tube. The standard was prepared by pipetting 0.01ml standard (CAL) and 3ml of BCG reagent mixed in an EDTA tube. The tear sample was prepared by pipetting 0.01ml of tears and 3ml of BCG reagent in an EDTA tube. They were then incubated for 5mins at 20^{0}C to 25^{0}C.After which measurement for concentration of albumin was done using the spectrophotometer at wavelength of 578nm.

Globulin: Globulin was got from the formula: Globulin = Total protein minus Albumin [19]

Total Cholesterol Analysis

Total cholesterol was analysed using Enzymatic Endpoint method [20]. Reagent blank was prepared by pipetting 10µl distilled water and 1000µl of R1 reagent mixed in an EDTA tube. The standard was prepared by pipetting 10µl standard (CAL) and 1000µls of R1 reagent mixed in an EDTA tube. The tear sample was prepared by pipetting 10µl of tears and 1000µl of R1 reagent. They were then incubated for 10mins at 20^{0}C to 25^{0}C. After which measurement for concentration of total cholesterol was done using the spectrophotometer at wavelength of 546nm.

Triglycerides Analysis

Triglycerides was analysed using the enzymatic method [21]. Reagent blank was prepared by pipetting 10µl distilled water and 1000µl of enzyme reagent mixed in an EDTA tube. The standard was prepared by pipetting 10µl standard and 1000µl of enzyme reagent mixed in an EDTA tube. The tear sample was prepared by pipetting 10µl of tears and 1000µl of enzyme reagent in an EDTA tube. They were then incubated for 10mins at 20^{0}C to 25^{0}C. After which measurement for concentration of triglycerides was done using the spectrophotometer at wavelength of 546nm.

RESULTS

The mean age (mean ± SD) of the non-dry eye subjects (n=22) was 57.91 ± 4.42 years and dry eye subjects (n=33) was 61.36 ± 7.94years. The total OSDI score was significantly different between the Non-Dry Eye (DE) = 9.90 ± 12.75 and dry eye (DE) = 30.71± 16.45 ($p<0.05$). The Dry Eye (DE) group exhibited a shorter tear breakup time and lower tear volume. There was also a significant difference between NDE and DE subjects when tear breakup time and tear volume were compared for the two groups ($p<0.05$).

Table 1: Age, Clinical parameters and Symptom score for the classification of the dry eye and non-dry eye postmenopausal women. (Data are mean ± standard deviation $p<0.05$)

GROUP	AGE (years)	OSDI Score	p-value
NDE n = 22	57.91 ±4.42	9.90 ±12.75	.000
DE n = 33	61.36 ±7.94	30.71 ±16.45	

Table 2: Tear volume and tear break up time of the dry eye and non-dry eye postmenopausal women. (Data are mean ± standard deviation, $p<0.05$).

GROUP	Tear vol (mm/5min)	p-value	Tear Breakup Time (s)	p-value
NDE n = 22	20.18 ± 11.22	.042	11.36 ± 4.12	**.**000
DE n= 33	14.06 ± 10.67		7.67 ± 2.07	

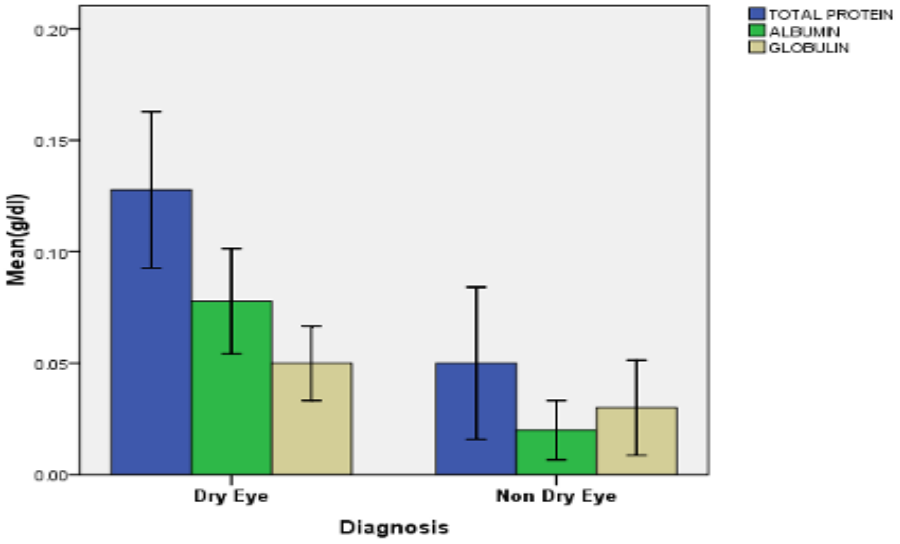

Figure1: Protein concentration (mg/dl) in dry eye subjects and non-dry eye subjects. Bars represent standard error bar (SE).

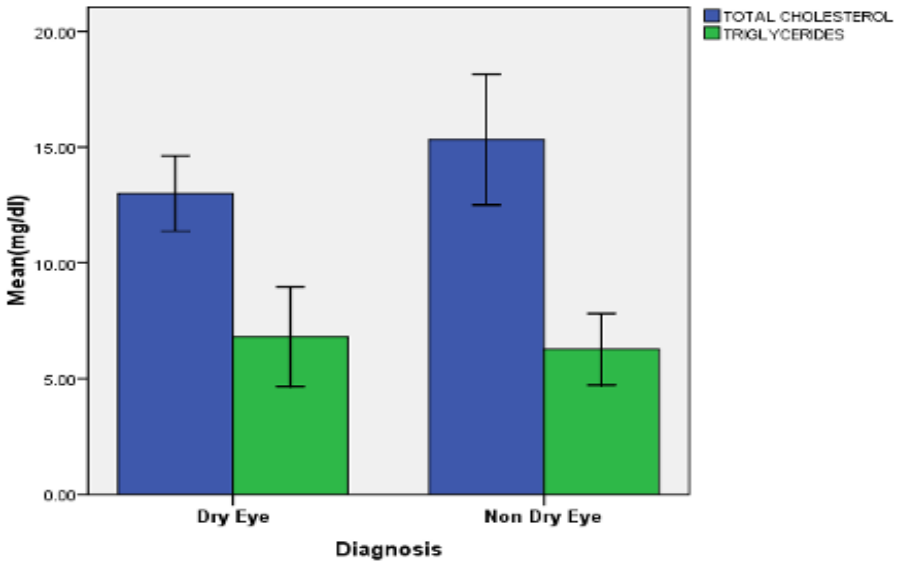

Figure 2: Lipid concentration (mg/dl) in dry eye subjects and non-dry eye subjects.

The mean total protein was 0.13 ± 0.15 for DE and 0.04 ± 0.09 for NDE, albumin was 0.07 ± 0.1 for DE and 0.02 ± 0.04 for the NDE, and globulin was 0.1 ± 0.07 for the DE and 0.05 ± 0.08 for NDE. There was no significant difference in total protein concentration when DE group was compared to the NDE ($p > 0.05$). Also, for the lipids, the mean total cholesterol was 13.00 ± 7.62 for DE and 15.33 ± 10.95 for NDE and triglycerides 6.81 ± 10.09 for DE and 6.27 ± 5.98 for NDE. There was also no significant difference in triglycerides and total cholesterol between DE and NDE ($p > 0.05$). There was weak correlation between clinical signs and total protein, cholesterol and triglycerides in the NDE and DE eye group.

Table 3: Correlation between clinical signs (tear volume, tear breakup time measurement) and biomarkers (total protein, albumin, globulin, cholesterol and triglycerides).

Biomaker	Tear Stability	P Value	Tear Volume	P Value
TOTAL PROTEIN	-0.136	0.46	-0.219	0.48
ALBUMIN	-0.158	0.39	-0.217	0.23
GLOBULIN	-0.075	0.69	0.016	0.93
CHOLESTEROL	0.291	0.08	-0.14	0.41
TRIGLYCEROL	0.165	0.33	-0.16	0.34

No significant correlation was found between the clinical signs (tear volume and tear breakup time) and biomarkers (total protein, albumin, globulin, cholesterol and triglycerides).

DISSCUSSION

Lipids and proteins are present in tear fluids [22] and are known to play important role in defenses of the eye [7]. Studies have suggested that lipids and proteins found in tear film can be used in identification of different ocular disorders such as dry eye disease [23].Tsuji and Kawazu [23] suggested that a good strategy for assessment and diagnosis of ocular diseases would be combination of potential ocular-associated lipids, lipid/protein, and protein biomarkers. In our study, we analysed the concentration of total proteins, albumin, globulin, cholesterol and triglycerides in postmenopausal women symptomatic of dry eye to ascertain the effect of dry eye diseases on the level of protein and lipids in tears of postmenopausal women.

Our result showed a statistically significant higher OSDI scores for the dry eye group (DE) when compared to the non-dry eye group (NDE). This is consistent with works done by other researchers on dry eye. Li *et al*. [24] in their study also showed a significantly higher OSDI score among the dry eye patients than in the control group. The study is also in line with the work by Schiffmann *et al*. [25] where their OSDI scores were higher in the DE and subgroup than the NDE, further buttressing the importance of OSDI questionnaire in the assessment of dry eye symptoms and therefore making it useful in differentiating symptomatic dry eye patients from asymptomatic dry eye patient.

Our study showed the tear breakup time and the tear volume were significantly lower in the dry eye group when compared to the non-dry eye group. This is significant with the work done by Bekibele *et al* [26] where they observed a lower tear breakup time and tear volume in patients suggestive of dry eye when compared to their healthy control. It is also in line with Careba [27] study on postmenopausal women where tear volume was significantly lower and tears breakup time significantly shorter in dry eye subjects when compared to non-dry eye subject. Tear film stability and reduced tear volume are therefore important diagnostic test in dry eye disease [11].

We analysed albumin and globulin which are inflammatory related proteins. Most studies on dry eye have analysed antimicrobial related protein, some found no significant difference between dry eye and non-dry group [7,27] others have found a decrease in the dry eye group [9,28,29] when compared to the non-dry eye group. Our study showed a higher mean total protein, albumin and globulin content in symptomatic dry eye subjects compared to asymptomatic dry eye subjects, the increase in our study was however not significant when compared with the non-dry eye group. Although increases in albumin have been found in dry eye disease as reported by some studies [9,30], it cannot be used to differentiate between

Sjogren's syndrome dry eye disease patient and Sjogren's syndrome non-dry eye patient [31]. Versura *et al* [9] study reported a decrease in total protein and an increase in albumin in early dry eye patients when compared to non-dry eye patients. We studied a different population (postmenopausal women) and in this population, it has been found that menopause and aging alters metabolism of total protein and albumin[32,33,34].

The study showed no significant differences in total cholesterol in tears of dry eye postmenopausal women compared to non-dry eye postmenopausal women indicating that the concentration in dry eye postmenopausal women and non-dry eye postmenopausal women are same. Cholesterol is a non-polar lipid which is important in the stability of tears [35]. Abnormal proportion of cholesterol and cholesterol esters in the composition of the tear film may lead to tear film instability [36]. This in turn can lead to symptoms associated with dry eye. However the study failed to show a significant difference in total cholesterol between the dry and non-dry eye groups. The differences in the concentration of triglycerides between the two groups were very minimal and not significant as well. Not much work has been done on total cholesterol and triglycerides in tears of dry eye patients. However studies [37,38] done on total cholesterol and triglycerides in serum of dry eye and non-dry eye subjects showed no statistically significant difference between dry eye and non-dry eye group.

No significant correlation was found between biomarkers (protein and lipids) with clinical signs (tear breakup time and tear volume). This is consistent with other studies, where they reported no significant correlation between signs and tear protein [27,29] concentrations.

Although our study showed postmenopausal women symptomatic of dry eye had decreased tear stability and tear volume, tears produced by this group of subjects were same biochemically in respect to protein (total protein, albumin and globulin) and lipids (total cholesterol and triglycerides) content.

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