

Cytological Screening of Conjunctiva Changes among Sudanese Patients Attending Ophthalmic Clinics

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Abstract

Introduction: The conjunctiva eye cytology is a part of the diagnostic eye cytology provides a tool of a diagnosis of many ocular diseases and conditions with using of a suitable tool of collection of sample. This kind of cytological procedures doing as screening for population, or specify the disease under study also for comparison of accuracy and usefulness of the different types of tools use in collection of samples.

Objectives: The aim of this study was to screen the conjunctival eye cytology in the population under study ,and to describe the cellular pattern in different eye condition to find out the different factors affect cellular components of the conjunctiva and subsequently the eye health status, and to evaluate the usefulness of the conjunctiva eye cytology as an important diagnostic procedures in the diagnosis of different eye condition.

Materials and Methods: This was a descriptive cross sectional hospital base study, done in university of Khartoum, faculty of medical laboratory sciences in department of histopathology and cytology ,to screen conjunctiva cytological changes of ophthalmic clinic patients using swabbing and scrapping cytology for different ocular diseases in period from January 2014 to May 2014. Conjunctiva swabbing and scrapping smears were collected from Abd Alfadeel centre for medicine and eye surgery, Noor Alauoon military eye hospital, Alneelin university eye hospital (collage of optometry) and Maka charity foundation for eye medicine (Ridhy division) ophthalmology clinic. Ninety two 92 patients participated in this study, 56 were females and 36 were males, conjunctiva smears were fixed by 95% alcohol and air then stained by Pap , Giemsa and hematoxylin stains for cytological evaluation.

Results: (89.4%) of cytological finding among adequate samples shown matching with the clinical ocular diagnosis. Scrapping method had the best role in getting adequate samples (90%), with marked increase of adequacy of sample obtained from elder age patients (34.2% from 41- 60 year, 31.5% from 61-80year) and in cases of infection (48.7%). Bacteria was the major cause of infections (86.2%).

Conclusion: Conjunctiva cytology is an impressive tool to get information about ocular tissue health status, and can be a useful complementary diagnostic method ,get its role in epidemiological surveys of endemic ocular diseases such as trachoma in our population and for better understand of diseases spreading manner.

Keywords: Conjunctiva, cytology, ocular, ophthalmic, trachoma, Pap stain, Giemsa stain.

Introduction

The conjunctival eye cytology is a part of the diagnostic eye cytology provide a tool of a diagnosis of many ocular diseases and conditions. It is a developing part of cytology in the developed countries and many studies was done on this field, other studies carryout in the most epidemic and endemic areas with infectious eye diseases such as India, Taiwan and Nepal; exhibit the cytological and histological features of conjunctiva of the patients. The diagnosis, prognosis and follow up usually involve grading of certain ocular conditions such as Dry eye syndrome, and Vitamins difeciencies [1, 2]. The cellular pattern

of the conjunctival cytological sample express a useful information about : the epithelium reaction, nature of cells, and any infested matter such as foreign bodies and micro-organisms, however the use of the tissue markers and molecular technology give more field of research and understand of causes, events of many ocular diseases [3]. The conjunctiva, cornea, vitreous, aqueous and puncum are parts of the eye which comprise the sites of cytological specimens for diagnosis of specific eye conditions such as infection, inflammation, traumas ,and tumors, choosing of type of sample depend on case under investigation and what goal of diagnosis. Samples from eyelid and eyelashes consider as skin samples [4]. Tools and methods used to get conjunctival cytology smears are: The eye spatula (scrapping), a sterile polyester tipped swab(swabbing/scrapping) [6]. eye microbrush

(brushing) [8], cellulose acetate filter paper (disc or strip) [5, 6] and Eye prim device from Opia company (France) for impression cytology method [4]. imprint method by applying a clean slide on conjunctival surface, the old method of pipetting samples in certain cases (ocular benign mucous membrane pemphigoid) (7), fine needle aspiration for eyelid lesions [12, 24].

Stains had been used for demonstration of conjunctival eye cytology are: Periodic Acid-Schiff (PAS) and counter-stained with haematoxylin and eosin, a routine Papanicolaou method Giemsa or May-Grunewald Giemsa, haematoxylin and eosin, some bioassays markers. Cells of conjunctiva epithelium obtained from cytological smears are: goblet cells, polyhedral or long columnar cells with microvilli and small cuboidal basal cells. Other cell types may be present according to the eye condition [21].

Certain ocular tumors express conjunctival cytological changes, the most common benign are hemangiomas. Samples from eyelid and eyelashes consider as skin samples [22, 23].

Materials and Methods

The data were taken from the patients by questionnaire asking them about their personal information (Age, occupation, residence area, original area and education level), sources of potential allergens in their environments, family history of diseases (ocular and non ocular diseases) and about their behaviors that may affect conjunctiva (duration of exposure to direct sun rays, hours of contact with visual media per day, their times of washing face per day, smoking and their using cosmetic creams or soaps on their faces).

Swabbing and scrapping materials was obtained by nylon tipped and cotton wound swab from conjunctiva of patient, fixed and stained and examined.

Conjunctival smears were obtained by anesthetizing the conjunctiva with topical 0.4% Benoxinate hydrochloride for about 5 minutes (this optional and not required when use cotton wound swab for collection), by using a sterile swab, the bottom eye lid conjunctiva was rubbed horizontally two times.

The swabbed and scrapped material smeared in spotted manner on clean slide and immediately fixed by 95% ethyl alcohol for 15 minute then air dried, prepared for Papanicolaou stain, slides for hematoxylin and eosin or Giemsa stain were fixed by air drying. All slides were put in slide storage box for transport to the laboratory. On arrival in the laboratory, the slide is placed in 95% ethanol prior to staining to rehydrate the sample cells.

Note: Trachoma smeared slides were fixed by 95% and air dry ones, two slides each patient for Pap and Giemsa stains. The staining were assessed by an experience cytologist.

In Pap stained smears: Nuclei are stained blue while cytoplasm displays varying shades of pink, orange, gray and green. In hematoxylin stained smears nuclei are stained blue while cytoplasm stain red. In Giemsa stained smears nuclei are stained dark blue while cytoplasm stain gray.

The adequacy of sample is reported, the type of cells, presence or absence of inflammatory process, other materials on smear like bacteria, crystals, mucous were reported.

Result

The matching of conjunctiva cytological finding with the ophthalmology clinical diagnosis within adequate samples shows high specificity (89.40%).

Ninety two (92) patients were screened using conjunctival cytology (swabbing and scrapping), 56 (61%) were female and 36 (39%) were male. Their ages were ranged from 1 to 80 years with the age mean 43 years. The study population were classified into age groups, large number of them were within the age group 41 to 60 years age (34%) and least number fill in group range 1 to 20 years. The most frequent groups according to occupation/ job were house wives 29 (31.5%), then students 17 (18.5%), the workers 10 (10.9%) and the drivers 7 (7.6%), show the population according to types of job activities.

They distributed according to resident area as 16 (17.4%) from out of state and the rest of them distributed in the Khartoum state in the three cities (Khartoum 44 (47.8%), Omdurman 12 (13.1%) and Bahri 20 (21.7%).

The most frequent patients from the middle states (31.5%) and the northern states (29.3%) in origin. Most of patients had a primary level of education (40.2%). The most eye involved was the right one (70.7%). Infectious conjunctivitis (19.6%) and infection with Chlamydia trachomatis (trachoma) (13%) show marked frequency as diagnosis. In infection cases the most common cause was bacteria (86.2%) include cases of Chlamydia trachomatis infection. According to the study typing of diagnosis the category of (other) show highest frequency of occurrence (32.6%), which include (refractive errors, cataract, glaucoma and who diagnosed as normal), and vitamin deficiency category had least percentage of cases (1.1%) as type of diagnosis. (and the infections Show the highest percentages among house wives (40%) and students (35%), while the category named (other) shows the highest percentages among drivers (40%) and workers (50%) groups. The most common cause of traumatic conjunctiva cytological smear among patients were surgery (80%), while (20%) from an accident. According to washing occasion involve face per day population distributed as (64.1%) in range of -more than five times- involved (Wado), (30.4%) in range- 3 to 5- times occasion and least group fill in range of -less than three times- events. The association between frequency of infection and different ranges of number of washing times occasion involve face per day shows in .According to exposure to direct sunrays (39.1%) exposed to sunrays in a range more than 9 hours per day, (33.7%) in a range of (5 to 9) hours per day and (27.2%) had less than 5 hours per day. Population distribution according to duration of contact with visual media shows in (78.3%) of population answered (yes) and (21.7%) answered (no) about their using cosmetic creams /soaps on their faces. Smoking behavior distributed within population as (12%) were smokers while (88%) were non smokers. (66.3%) of population answered (yes) about their wearing glasses and (33.7%) answered (no). According to general medical status of patients (80.4%) answered they were healthy. (52.2%) of population had no family history of eye diseases and (21.7%) had a family history of cataract.

Distribution of family history of non ocular diseases within population shows that (56.5%) had no family history of diseases while blood hypertension (15.2%) and diabetes (16.3%).

The types of potential allergen among population under study shows in. The hot air and dust had highest percentages of distribution within

different jobs environments with minimum variation, house wives (50.9%) hot air (23.5%) dust, students (52.3%) hot air (28.5%) dust, workers (43.75%) hot air (25%) dust and drivers (66.6%) hot air (33.3%) dust.

The house wives group had the highest number of the involved potential allergens in their environments (five factors).

Cotton wound swab was used as a tool of collection of sample in (51.1%) of population and show adequacy (19.5%), Nylon fiber tip flocced swab in (38%) (swabbing) had adequacy (50%) and in (10.9%) as scrapping tool of sampling and show the highest adequacy percentage (90%).

The frequency of getting adequate samples in different types of diagnosis shows the highest percentage (48.70%) in infections diagnosed patients.

Also the elder age groups appear to get more adequate samples and exfoliated cells, (41-60) group had highest percentage (34.20%) and (61-80) group had (31.5%) of adequate samples in cytology smears preparations. (**Figure 1**)

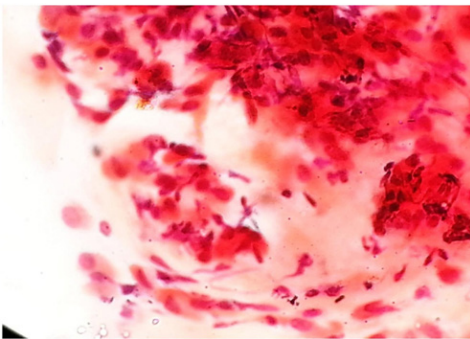


Figure 1: inflammatory cells in eye cytology smear.

Discussion

This was a descriptive study which aimed to screen the conjunctiva cytological changes of ophthalmic clinic patients using swabbing and scrapping cytology.

Somnath M *et al* (2013) assessed the health of ocular surface in a defined urban population, conjunctival goblet cell density and degree of surface squamous metaplasia were utilized as study tools. Two thousand of those aged between 20 and 79 years were initially selected, environmental factors like the method of cooking and occupational variables such as outdoor activity, prolonged period of computer use were modify the health of the ocular surface [2]. In our study the age group included wider range (1 year -80 years old). we agree with this study in the affect of the environmental factors on the results, in our study the major potential allergen was the hot air , also the age range may affect the adequacy of the samples obtained in which the elder population showed high frequency of sample adequacy [2].

In this study the population have many factors suggested to make an effect on the conjunctival cytology smear picture, the occupation or job determined their range of exposure hours to direct sun rays and involvement of particular potential allergen. In Somnath M *et al* study they sub grouped the job into outer doors and indoors, while in our study the population was grouped according to their occupation

and the most frequent jobs were (house wives, students, workers and drivers. Each group studied for their common eye diagnosis, the particular potential allergens and counting numbers of the allergens in their environments, infection as a type of diagnosis shown the highest percentage among house wives and students groups , while in groups of workers and drivers.

the most frequent diagnosed in typing of (others) that included normal cases and intra ocular diseases such as glaucoma, cataract and refractive errors.

Subsequently the particular potential allergens in each job group was determined by asking the participants and the results that no wide variation between groups as the category named (other) that included (hot weather and cold air of condition devices) as source of allergic reaction, had the highest percentage of answers, and (dust) get secondly. The house wives group shown the highest number of involved allergens factors and according to their data results we agree with them.

Koss *et al* 1992 used the scrapping cytology for diagnosis of the malignant lesions on conjunctiva ocular surface, in our study this not expectable by ophthalmologists in the clinic because they are not familiar with the procedures [24].

Kobayashi TK, *et al* (1997) studied the conjunctiva ocular cytological changes in dry eye status in patients with Kerato conjunctivitis sicca (KCS), in our study the frequent of conduction of patient with this disease was 1.08% from total population, and the dry eye status appear as a secondary involvement in conjunctiva ocular disease so not include in typing of eye diagnosis done by the ophthalmologist [9].

Thiel M *et al* (1997) in their study impression cytology techniques were evaluated to analyze their diagnostic potential in viral infections of the ocular surface. A biopore membrane device instead of the original impression cytology technique was used to allow better quality and handling of the specimens. The impressions were processed, using monoclonal antibodies and immunoperoxidase or immunofluorescence techniques. Immunocytological staining of these samples provided diagnostic results for all three viruses in patients with viral surface disease [10].

However, and based on this study we found that conjunctival cytology as a diagnostic and screening tool for patient of different ocular diseases is simple, safe, cost-effective a virtually quick for the vast majority of infectious agents and non infectious ones such as Candida ,bacteria, degenerative changes due to eye drops prolonged uses as in glaucoma patients, crystals appear due precipitation of environmental matter, and complementary to the clinical ophthalmology diagnosis as the swabbing and scrapping collection method used in our study exhibited high percentage of matching of cytological finding with the clinical ocular diagnosis (89.40%), with scrapping method had impressive percentage of yield adequate samples (90%).

Charles Gerald Connor (2003) described that dry eye disease includes Keratoconjunctivitis (KCS), age-related dry eye, Stevens-Johnson syndrome, Sjogren's syndrome, ocular cicatricial pemphigoid, blepharitis, Riley-Day syndrome, and congenital alacrima. Dry eye disease can also be caused by nutritional disorders or deficiencies (including vitamins), pharmacologic side effects, eye stress and

glandular and tissue destruction, environmental exposure to smog, smoke, excessively dry air, airborne particulates, autoimmune and other immunodeficient disorders, and comatose patients who are unable to blink (25) And also Satici, A *et al* (2003) concluded that chronic smoking has a negative effect on the ocular surface and affects some tear characteristics. The chronic ocular irritative effects of cigarette smoking may lead to defects in ocular surface defence [26]. In our study there was no facilitates of time and resources to detect the specific effects of factors include smoking, cosmetics creams or soaps used on face and the wearing glasses, on cytology of the conjunctiva ocular surface, although the information about events of these factors were collected from population under study by quesnnaire and plotted in frequency figures as results. In this study the relation between the general medical status of the patient , the presence of family history of ocular diseases or non ocular ones and conjunctiva cytology finding in certain eye diagnosis type is limited . As the majority of the population as filled in healthy, with no family history of eye disease or other diseases [10, 11].

Application of conjunctival cytology in the diagnosis of ocular diseases, can help us to get information about effect of eye drops or systemic administrated drugs on conjunctiva cells, observation of the type of tissue reaction against different environmental factors and to reveal evidence of infestation allow subsequently submitted for microbiological examination. So we can carry out it as complementary examination.

conjunctival scraping is preferred to swabbing because it yields more epithelial cells and causes less contamination due to inflammatory debris from the ocular surface.

Also I recommend by :

- The involve of the malignant eye patients in the future studies.
- The use of high applicable and more specific tools in collection of cytological samples.
- Increase the knowledge and practice of the ophthalmologists and cytologists about this type of cytological procedures.

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