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RESEARCH ARTICLE

"TO STUDY CONJUNCTIVAL BACTERIAL AND FUNGAL FLORA IN DIABETIC AND NON-DIABETIC INDIVIDUALS AND THEIR ANTIBIOGRAM IN TERTIARY CARE HOSPITAL"

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Abstract

Introduction: The conjunctiva is a clear mucous membrane that borders the interior of the eye lids and protects the sclera. It consists of non-keratinized, stratified columnar epithelium with goblet cells. Normal microbial flora constitutes organisms which are present in eyelids and conjunctiva without causing any disease. The flora of the ocular surface consists more of gram positive microorganisms[2].Gram positive organisms, particularly coagulase negative Staphylococci, are widely recognized to be the primary inhabitants of the normal eye. At birth eyes are sterile but they are later infected by various organisms. Diabetes mellitus is a multi factorial disease that can affect all ocular structures.

Aim: To evaluate the Conjunctival bacterial and fungal flora of Non diabetic and diabetic individuals.

Material &Method: This was anCross sectional, Observational Study conducted on a total of 204 patients attending Department of Microbiology of People's Medical College and Hospital, Bhopal, India between February 2022 to February 2023. Conjunctival swab was collected from lower eyelid to expose the Conjunctival membrane of eyes by sterile swab sticks. to assess the conjunctival flora and antibiotic sensitivity pattern in diabetic and non-diabetic individuals without any pre-existing ocular diseases.

Result: Conjunctival flora of a total of 204 patients, 102 diabetic and 102 non diabetic, were screened. Among the 102 diabetic patients, there were 51(49.5%)male and 51(49.5%)female. The most common isolated bacteria in diabetic and non diabetic groups were staphylococcus aureus (diabetic 26.92%, Non-Diabetic 61.11%). There was a statistically significant difference in microbial flora

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pattern between the diabetic and non diabetic group. There was higher proportion Gram negative bacilli grown in Diabetics.

Conclusion: Present study found statistically significant difference in the microbial flora between diabetics and non diabetics. The observation of the present study and various other studies, infer that staphylococcal infections are common among both Diabetic and Non-diabetic individuals. Gram-negative bacteria Escherichia coli and Klebsiella pneumoniae were detected in higher ratios in the conjunctival flora of diabetic patients. Candida albicans were more common among the diabetics as compared to non- diabetics.

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Introduction:-

The conjunctiva is a clear mucous membrane that borders the interior of the eye lids and protects the sclera. It consists of non-keratinized, stratified columnar epithelium with goblet cells. Normal microbial flora constitutes organisms which arepresentineyelidsandconjunctivawithoutcausinganydisease.Atbirtheyesaresterilebutafterbirtheyeisinfectedbyvariousorganisms.Grampositiveorganismsaremostly inhibiting inocularflora.

Themicroorganismshaveanimportantroleinthemaintenanceofnormalconjunctival functionsandthepreventionofocularinfections[1].Thefloraoftheocularsurfaceconsists moreofgrampositivemicroorganisms[2].Grampositiveorganisms,particularlycoagulasenegativeStaphylococci,arewidely recognizedtobe the primary inhabitants of the normal eye. These are the major source of post-operative infection[3].

The conjunctival flora is altered under special circumstances as in newborn, acquired immune deficient patients, contact lence wearers and patient using immune suppressive drugs.[4,]

Neisseria gonorrhoeae and Chlamydia trachomatis also infection a conjunctiva. Staphylococcusepidermidisandcertain corny forms,includingsuchPropionibacterium acnes,arethemostcommon.[5]Type2diabetesisacomplicateddiseasethataffectsmany aspectsoftheeye.[6]

Diabeticpeopleareatahigherriskofdevelopingpostoperativeendophthalmitisthan non-diabetic ones[7].Themostofpathogenicbacteriaimplicatedinpostoperative endophthalmitisarefoundintheconjunctivaflora,lids,andeventhenasalmucosa.[8]

Given the rising prevalence of diabetes, every surgeon in India is dealing with the patient of diabetes for cataract. Pathogenicity of these microorganisms forming the flora affects the development of conjunctivitis as a harmful bacterium, S.aureus may be found in the conjunctival flora. The most common agent identified from neonatal bacterial conjunctivitis is Staphylococcus aureus. Patients with diabetes mellitus are prone to infection because glucose in the skin, urine, mucous membranes, and tears promotes growth of microorganisms. Conjunctival flora Neisseria gonorrhoeae and Chlamydia trachomatis play a pathogenic role when immune function is compromised, which can lead to serious infection.[9, 10, 11, 12, 13]

People with diabetes, due to potential ocular complications, represent a group at risk of ocular infections. Furthermore, Staphylococcus a saprophyticus remained the most common infection in this group, with an increase in Staphylococcus identified in cultures from individuals with proliferative diabetic retinopathy.

Material And Methods:-

It is a cross sectional, observational study the study will be carried out in the Department of Microbiology, People's Collage of Medical Science & Research Center Bhopal of Microbiology, which includes 204 consecutive conjunctival swab samples during period of FEB 2022 TO FEB 2023 after consideration of inclusion and exclusion criteria and informed consent from the subjects. Total 204 patients (102 diabetic and 102 non diabetic) who came to OPD with complaints other than eye infections were included.

Inclusioncriteria:
Allgenderwillbeincluded, DiabeticPatientsareincluded and Non-DiabeticPatientsareincluded.

Exclusioncriteria:
Patientswithdiseaseotherthandiabetes**,** To exclude infective condition of the eye and Patientsnotwillingtogivesample

Procedure:
The samples were collected from lower eyelid to expose the Conjunctival membrane of eyes by sterile swab sticks. The samples were inoculated to sterile blood agar, macConkey agar, SDA medium and incubated at 37°C for 24 hours. Gram staining and Potassium Hydroxide (KOH) mount were then done for the Inoculum of each plate.

Gram positive organisms found were staphylococcus sp, streptococcus spp. For gram positive cases catalase test, coagulase test and mannitol salt agar test, Bile-esculin, Salt tolerance test, Optochin disk and PYR test were done.

Gram negative organisms found were E. coli and Klebsiella spp. For Gram negative organisms identified by Indole, Urease, Citrate, Triple sugar iron agar test, Methyl red (MR), Voges Proskauer (VP), Nitrate reduction and Sugar fermentation test were done.

Mucoid purple colony gives confirmatory evidence of Klebsiella pneumonia.In the diabetic patients' eye swab, staphylococcus aureus and Klebsiella pneumonia were not found in same plate.

Antibiotic sensitivity tests were done on Muller Hinton agar plate by Kirby Bauer disk diffusion method. The zone of diameter was measured in centimeter (cm). The antibiotics used for Bacterial isolates were Cefoxitin, Ciprofloxacin, Co-trimoxazol, Doxycycline, Clindamycin, Erythromycin, Gentamycin, Imipenem, Piperacillin/Tazobactum, Amikacin, Levofloxacin, Penicillin, Vancomycin, High level Gentamycin and for fungal isolates Nystatin, Itracanazole, Fluconazole, Amphotericin 'B', Ketoconazole, Miconazole.

Result:-

Conjunctival flora of a total of 204 patients, 102 diabetic and 102 non diabetic were screened. the Diabetic Group includes 49.5% male and 49.5% Female; the control group includes 42.2% male and 57.8% female.Bacterial growth occurred in the conjunctival cultures of 12.74% of diabetic patients and 8.82% of non-diabetic individuals.The following bacteria were identified in cultures from the non-diabetic group: Staphylococcus aureus
(Gram positive cocci) in 11 cultures (61.11%), coagulase negative staphylococci (Gram positive cocci) in 4 cultures (22.22%), candida non-albicans in 1 culture (5.55%), mixed colonies S. aureus with candida non-albicans in 1 cultures (5.55%) and S.aureus with candida albicans in 1 culture (5.55%).
In the diabetic group, the following bacteria were identified: Staphylococcus aureus in 7 cultures (26.92%), staphylococcus epidermidis in 2 cultures (7.69%), streptococcus spp in 2 cultures (7.69%), enterococcus in 1 culture (3.84%), CoNs in 5 cultures (19.23%), Escherichia coli (gram-negative bacilli) in 2 cultures (7.69%), Klebsiella pneumoniae in 1 culture (3.84%), candida albicans in 1 cultures (3.84%) candida non-albicans in 1 cultures (3.84%) mixed growth S. aureus with candida albicans in 1 culture (3.84%), S. aureus with candida Non-albicans in 2 cultures (7.69%) and CoNs with candida non-albicans in 1 culture (3.84%). Gram negative bacteria were more common in diabetics than in non-diabetics.
The microbial isolates detailed in table:

Discussion:-

In this study, the conjunctival flora of diabetic patients and healthy individuals were compared. Specimens obtained for this purpose were seeded on different media. The diabetic group showed no significant difference in frequency of bacterial growth compared to the control group, but yielded less gram-negative bacteria cultures.

Although the conjunctival flora forms a defensive barrier against infection, it also includes major pathogens of ocular infections. In healthy individuals, the conjunctival flora is frequently comprised of same microorganisms as the skin flora.[22] Gram-positive bacteria constitute the main elements of bacterial floral, though the positive culture rate and microorganisms grown show diversity.[23,24]

Higher rates of bacterial colonization are expected in situations that weaken the immune system such as diabetes advanced age and corticosteroid use.[25] However, it has been reported in the literature that infections that substantially suppress the immune system, such as HIV, don't cause significant changes in

Positive culture or the variety of bacteria found in conjunctival cultures.[26, 27] Similar changes in diabetic patients have also been reported in this study.

A total of 204 patients were screened to study the conjunctival flora in the diabetic and non-diabetic group in ophthalmology (OPD).

Type of infection	Diabetic Individuals (n=26)	Non- Diabetic individuals (n=18)
Staphylococcus aureus	26.92%	61.11%
S. epidermidis	7.69%	
S. pneumoniae	7.69%	
CoNs	19.23%	22.22%
Enterococcus	3.84%	
Escherichia Coli	7.69%	
Klebsiella	3.84%	
Candida Non-albicans	3.84%	
candida albicans	3.84%	5.55%
S. aureus, c. albicans	3.84%	5.55%
S. aureus, c. non-albicans	7.69%	5.55%
CoNs, c. non-albicans	3.84%	

Organism isolated in diabetic and non- diabetic samples:
In the diabetic group were growing following organisms:
Monomicrobial organisms 22(84.61%) were Staphylococcus aureus (26.92%), Staphylococcus aureus (7.69%), Streptococcus pneumoniae (7.69%), CoNs (19.23%), Enterococcus (3.84%), E.coli (7.69%), Klebsiella spp. (3.84%), candida non-albicans (3.84%), Candida albicans (3.84%) and Polymicrobial organisms 4(15.38%)were staphylococcus aureus with candida non-albicans (7.69%) and coagulase negative staphylococcus aureus with candida non-albicans (3.84%).

In the non-diabetic group were growing following organisms:
Monomicrobial organisms 16(88.88%) were Staphylococcus aureus (61.11%), coagulase negative staphylococcus aureus (22.22%), Candida non-albicans (5.55%) and Polymicrobial organisms 2(11.11%) were Staphylococcus aureus with candida non-albicans (5.55%) and Staphylococcus aureus with candida albicans (5.55%)were grow.

In our study, of the 204 (100%) samples Showing positivity of 43 (21.7%), Diabetic (12.74%) and Non-diabetic (8.82%)]. This is in accordance with the study done by **Kalpana Suresha et al.** showing positivity of The total positive cultures were 148 from 100 patients (diabetic-72 and non diabetics-74). Study by **Rajeshkannan R et al.,** showed that diabetics had a positive culture of 68% as compared to non diabetics[7]. Another study showed that the microbial growth in diabetic patients was 62.27% compared to 46.67% in non diabetics.[28] similar result was also found in study by **Martins EN et al.**[29]

In our study positivity rate was 26 (12.74%) in diabetic group and 17 (8.33%) in non-diabetic group. This is in accordance showing in the study done by**AdamM et al.**[30]in (2015) examined 96 samples (53 diabetic and 43 non-diabetics) out of which 38.5% sample were positive of diabetic patients and 34.9% of non-diabetic individuals.

In our study, gram-positive bacteria were the major bacterial flora element and among the gram-positive cultures, Staphylococcus aureus was most common in diabetic and non diabetic group. CoNs were the second most common microorganism in the diabetic and non-diabetic group. The present study showed that staphylococcus aureus is the most common isolate in both the diabetic and non diabetic group, as is found by **Adam M et al**.[30] Similar to our study, **Birinci et al.**[11] identified Staphylococcus aureus as the most common bacterial flora element in diabetic patients. **J.A Capriotti, et al** (2009) and **Martins, et al** (2004) found CONS to be more common among the

diabetics.[31, 32, 33, 34, 35]Similarly **DavoodAghadoost, et al** (2007) also found CONS to be the most common organism constituting the normal flora of the conjunctiva.[32, 33, 34, 35, 36]

In the present study isolation rate of staphylococcus epidermidis were 7.69% in diabetic group. **Ashtamkar S et al.,** found Staphylococcus epidermidis to be the most common organism (13.2%) isolated from diabetics.[37] Study by **Venkataraman M et al**., showed coagulase negative Staphylococci as the most common organism in diabetics. similar results were found in other studies.[38, 39, 40, 41]

We did not find a significant difference in bacterial isolation rate between the diabetic and non-diabetic groups. In our study found more positivity in cultures rate in diabetic group as compared to non diabetic group (12.74% and 8.33% respectively). Similar study done by **Karimsab and Razak et al.** found a higher positive culture rate in their diabetic group compared to their non-diabetic group (34% versus 24%, respectively).

In our study the rate of gram-negative bacteria was 11.53% in the diabetic group and no growth in non-diabetic individuals. The most common gram-negative bacteria were Escherichia coli.**Rajeshkannan R et al.,** found that gram negative organisms were more common among diabetics. Study done by **J.A. Capriotti, et al (2009)** showed 9.8% of gram negative bacteria among the healthy individuals in the rural population.[44,45] whereas our study showed 11% among the non diabetics.

Antimicrobial susceptibility patterns:
The sensitivity pattern of Staphylococci aureus showed maximum sensitivity to Co-Trimoxazol (90%) and Doxycycline (90%) in Diabetic group, whereas in the non-diabetic group it was (69.23%). Staphylococcus aureus showed maximum resistance to Erythromycin (60%) in the no diabetics group, whereas in the non-diabetic group it was (46.15%). Similarly the study conducted by **Kalpana Suresha et al.**Staphylococcus aureus showed maximum resistance to Erythromycin (27%) in the non diabetics group, whereas in the diabetic group it was 38%.

In the present study was found that the gram positivestaphylococci sp. has maximum resistance against erythromycin (60%) and cefoxitin (60%) among the diabetic group and in the non diabetic group maximum resistance was seen with ciprofloxacin and Doxycycline (53.84% and 52.80% respectively). In other study found that the gram positivestaphylococci sp. has maximum resistance against erythromycin (38%) among the diabetic group and in the non diabetic group maximum resistance was seen with Ampicillin (30%)

Resistance of (40%) to ciprofloxacin by Staphylococcus aureus in the diabetic group and (53.84%) in the non diabetic group was seen in our study.

Conclusion:-

A higher positive culture rate was seen in diabetic individuals as compared to non-diabetic individuals. The diabetic patients were more prone to ocular infection.Diabetic individuals are at a higher risk of developing other ocular infections as they are more prone to have a positive culture rate of microorganisms. The observation of the present study and various other studies, infer that staphylococcal infections are common among both Diabetic and Non-diabetic individuals. Gram-negative bacteria Escherichia coli and Klebsiella pneumoniae were detected in higher ratios in the conjunctival flora of diabetic patients. Candida albicans were more common among the diabetics as compared to non- diabetics. Conjunctival flora can become a pathogen in diabetic patients following any injury to the eye. Over usage of antibiotics should be restricted to prevent resistance among the bacteria.

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