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Microbiological and Immunological Investigation of adult patients with Chronic Sinusitis

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Abstract

The present study was conducted in Al-Diwaniya Teaching Hospital ENT department at the period from November 2013 to March 2014 One hundred adult patients clinically presented with chronic sinusitis were selected for this study , swabs were taken from middle meatus by special designed swab under endoscopic guidance from both patients and controls(50 healthy individuals)to identify the causative microorganisms of sinusitis. It was found that from 100 patients, only 10% have no growth while 90% yielded growth of various microorganisms of which the most common pathogens were *Staphylococcus aureus* , *Streptococcus pneumoniae* ,*Haemophilus influenzae and Moraxella catarrhalis.* The immunological investigations (IgG, IgM, IgA, C_3 & C_4) revealed that 99 patients were immune competent while (3) patients showed some immune defect .

Introduction

Sinusitis is one of the common diseases both in developed and undeveloped countries including Iraq and it is one of the major causes for longstanding morbidity and suffering of patient.[(1)]Paranasal sinuses are considered as part of the upper respiratory tract and they are composed of four pairs sinuses which open by special ducts system into the nasal cavity.[(2)]

Chronic sinusitis is one of the most prevalent illnesses in Iraq. it affects as many as one million Iraqi and is the principle diagnosis in almost 2% of all office visits to physicians .[(3)]

Sinusitis is an infection in one or more of our sinus cavities surrounding the nose. It opening to a sinus passage becomes legged a resultant infection can occur causes of this are allergies .nasal polyps ,deviated septum ,and enlarged adenoids.[(4)]Often sinusitis occurs as a complication of a cold. it can cause a lot of facial pressure particularly in the areas of the cheeks and eyebrows. In addition it may also cause nasal discharge ,a bad taste in your mouth ,an upper tooth ache ,fever ,sore throat or cough.[(5)] Several factors ,including hypertension, diabetes mellitus ,a high intake of alcohol, preceding incident of intoxication with alcohol and smoking are associated with an increased risk[(6)]of a relation may also exist between various infections and sinusitis is a well known complication of bacterial infection other infections that are more common in the population may ,however ,also be

implicated.[6.7]Thus several case reports and studies based on a few patients have suggested that preceding respiratory infections is a risk factor for brain infraction especially in children and young adults.[8] Accurate assessment of patient outcome after sinus surgery requires the collection of valid and reliable data symptom-based surveys were administered in a prospective manner patient with chronic sinusitis.[9]

Results of the chronic sinusitis survey also correlated significantly with subscales of a general health assessment in the extent to which chronic sinusitis limits physical activity .[10] interferes with work or other activities. and affects patient perception of bodily pain the chronic sinusitis survey is an efficient and reliable method to follow health status and health-related quality of life outcome in patients with chronic sinusitis.[11]

Aim of the study:

The aims of this study can be summarized as follows:

1- Invention of new technique to obtain representative sample by using special swab under endoscopic guidance.
2- Determination of normal flora of the middle meatus in healthy individuals.
3- Isolation and identification of microorganisms responsible for chronic sinusitis in adult patients.
4- Determination of immunoglo

Statistical Analysis

The results were analyzed statistically by Chi-square (X^2) test at the level of significant when P-value_0.05 [12] .

Material & Methods
Microbiological samples collection
Sample Collection

To perform any successful microbiological study the method for sample collection must be:

1-Easy to perform.
2-Representative of the clinical disease .
3-Collected under sterile condition .

In sinusitis the common methods of sample collection are :
1-Antral lavage (antral wash out)
2-Swab taken under endoscopic guidance.

Antral Lavage (Antral wash – out)

The procedure was done by a specialist under anesthesia (usually local anesthesia, but sometime can be done under general anesthesia). It is performed by making a small hole in the wall of sinus (usually maxillary sinus) then aspiration of sinus content is done to obtain sample for microbiological examination. After that antral wash (antral lavage) is done for therapeutic purposes.(13)

Swabs

It is taken from middle meatus near the sinuses Ostia where secretions from anterior group of sinuses are drained.(14) It is done under local anesthesia by a specialist.

In this study only 10 patients were subjected to antral lavage and in the rest ,the specimens taken for microbiological study were obtained by swab method.

Micropur Swab Design

The microbiological sample was obtained by a special swab designed as follow : a (10) length of 28 gauge nichirome wire was bend completely back on itself at a length of 1 mm from the tip end to avoid accidental injury to the middle meatus by the sharp wire end .then a small wisp of cotton wool is applied at the tip to make the swab .This was immersed in a plastic tube of 12.5 cm length sterilized by autoclave . The autoclaving removes the entrapped oxygen in the swab and keep it in reduced state to increase the chance of collecting.

Isolation, Diagnosis and Identification of Microorganisms:

The result of cultivation of the collected samples appear after (24-48hours) for aerobic culture and (1-7 days) for anaerobic ,fungul cultures .After visual examination to the colonies of the bacteria and fungi ,they were identified as follow:(3)۞

1-Morphology including :
-Cultural characteristics
-Gram stain for bacteria
-Lactophenol cotton blue for fungi
2-Biochemical tests including:
Catalase ,Coagulase ,Oxidase ,Bile solubility and sugars fermentation tests)
Other test: Growth on manitol salt agar .
This test used for primary identification then confirmed by using :
The kit of Api Staph for all the *Staphylococcus* and *Micrococcus* .
The kit of Api 20E for Enterobacteriaceae and other non – fastidious Gram-negative bacteria.
Maintenance of The isolates:
1Forrapidgrowingorganisms*(Staphylococcus,Pseudomonas,Enterobacteraceae)***.**
-Brain heart infusion with 15% glycerol in screw capped tubes.

-Store at 20^0C.

Immunoglobulins and Complement Components Determination

Immunoglobulins and complement components were determined by Endoplate Single Radial Immunodiffusion Test , this procedure is done as follow[4]®

1- The Endoplate and Reference (Standard) sera were deft to equilibrate to room temperature.
2-Reference sera and and patient sample mixed thoroughly by inversion .
3- From Reference sera and each patient sample were dispensed into the wells of Endoplate.
4-Firmly replaced lid, incubated at room temperature (23 ± 2^oC)on level surface for 72 hours.
5-The immune precipitin ring diameter were measured to the nearest (0.1) mm by specific ruler.
6- For each Immunoglobulin and complement component a standard curve was prepared by plotting the results of human Reference sera of high medium and low concentrations.
7-The patient sample was determined by plotting the result on appropriate standard curve.

Results &Discussion

The favorable material for microbiological examination was pus aspirated from infected sinus by antral lavage .[15] , but this procedure is very painful ,it require cooperative patients, and it carries the risk of complications like bleeding , the puncture point of entry to the sinus must be sterile to avoid contamination ,also patient should be on antibiotic therapy (prophylactic therapy)to avoid septicemia , and must be performed under anesthesia (local or general).[16]

Therefore antral wash –out can be done only in small group of patients ,and it more practical to obtain are preventative sample by nasal swab taken from middle meatus under endoscopic guidance.[17] Therefore in this study most of samples were collected by nasal swab done under endoscopic guidance.

Endoscopy is particularly helpful in investigation ,not only to obtain accurate sample for microbiological examination ,but in the diagnosis of sinusitis and assessment of any mechanical obstruction.[18]

Isolation of microorganisms

Control Group

The presence of normal flora in the upper respiratory tract in appropriate number is important for normal physiology because it represent a host barrier against pathogenic bacteria by competition against pathogenic bacteria for nutrient requirement, also

normal flora are important to stimulate the immune system to produce proper immune response against pathogenic bacteria.[19] However, overgrowth of normal flora ,or its presence outside the normal sites may lead to infection ,Also normal flora may behave as opportunistic pathogens when the host immunity decline[20]

In the present study (50) healthy individuals were chosen as a control group to detect the normal flora of the middle meatus (Table 1). Each one was examined carefully to exclude any upper respiratory tract infection.

From this table ,it was found that out (50) normal individuals only (30%)yielded no growth and (70 %)yielded (41) different isolates ,the most frequent microorganisms encountered were :*Staphylococcus epidermidis*(30%), *Diphtheroids* (18%) and S*taphylococcus aureus* (12%).Other members of normal flora were *bacillus spp.,Neisseria spp., micrococcus spp.,* and *Streptococcus spp.,* while neither anaerobic bacteria nor fungi were detected as normal flora.

Table (1)

Types and Numbers of Microorganisms Isolated from Control Group

Types of the microorganisms	Individual Number	*%*
Staphylococcus epidermidis	*15*	*30*
Diphtheroid	*9*	*18*
Staphylococcus aureus	*6*	*12*
Bacillus spp.	*4*	*8*
Neisseria spp.	*3*	*6*
Micrococcus spp.	*2*	*4*
Streptococcus viridians	*1*	*2*
Streptococcus pneumonia	*1*	*2*
Anaerobic bacteria	-	-
Fungi	-	-
No growth	*15*	*30*

The presence of these bacteria as normal floras in the middle meatus in acceptance with the results of previous studies done by[17] who found that the normal flora of middle meatus comprise *Staphylococcus epidermidis* (42%) ,*Diphtheroids* (23.7%), *Staphylococcus aureus* (14%),*Neisseria* spp., and non-hemolytic streptococci in low percentages ,while [22]found that the most common flora of middle meatus were coagulase-negative *staphylococcus*(53%),*Corynebacteria* (24%) and *Staphylococcus aureus* (14%) .*Neisseria spp* .,and non –hemolytic *streptococci* in low percentage ,while [15] found that the most common flora of middle meatus were coagulase –negative *staphylococcus* (53%), *Corynebacteria* (24%)and *Staphylococcus aureus* (14%).Intraoperative culture of antral mucosa seems to provide the most reliable finding of bacterial flora in chronic sinusitis. [21][22]

In the present study the isolated microorganisms were in accordance with other previous studies but differ in the reduced percentages of isolation which may be

explained by the fact that most of people consumed antibiotics randomly in large amount without consulting physicians which result in reduction of the normal flora found in the upper respiratory tract , and as shown from the table(1) ,(30%) of control group yield no microbiological growth.

Patients

The results of microbiological growth in chronic sinusis in patients were demonstrated in table (2) and (3).

Table (2) showed that out of (100) patients (90%) developed microbial growth and (10%)yield no growth.this findings was in agreement with the results of[23] who concluded that (20%)of the patients had no growth ,while[24] ,found that (8%)of sinusitis patients yielded no growth. The high incidence of negative microbial growth (20%) may be related to other microbial agents like parasites ,chlamydia ,leptospira or mycobacterium ,which need special media and techniques for isolation ,which is not the aim of this study,in addition the difficulties in obtaining samples ,transportation ,and culturing techniques may increase the rate of negative growth.[25]

The nature of microbial growth can be divided into two groups. Pure growth (63%) and mixed growth (27%),this agrees with[14] who stated that in chronic sinusitis often more than one bacterial species can be found ,in contrast to what is seen in acute sinusitis.[26]

Table(2) The result of the microbial growth in chronic sinusitis

Culture	No. Of patients	%
Microbial growth No growth	90 10	90 10
Total	100	100
Pure microbial growth Mixed microbial growth	63 27	63 27
Total	90	100
Culture	Number of isolate	%
Growth of aerobic & facultative anaerobic bacteria Growth of anaerobic bacteria Growth of fungi	116 10 5	88.5 7.7 3.8
Total	131	100
Classical pathogens Non-classical pathogens	90 16	95.4 16.9
Total	106	100

The total isolates (131) which were isolated from (90) patients can be graduated as follows : aerobic and facultative anaerobes (88.5%) , anaerobic bacteria (7.7%) and fungi (3.8%) . The bacterial isolates can be classified as classical pathogens(95.4%) and non classical pathogens(16.9%) . Classical pathogens were defined as organism that were highly pathogenic with marked potential to cause disease , while non classical pathogens were defined as organisms with lesser degree of pathogenicity , and found as part of normal flora of the skin and respirator mucous membrane.[27]

Classical pathogens include : *Staphylococcus aureus*; *Streptococcus peneumoniae*; *Haemophilus influenzae* ; *Moraxella catarrhalis*; *Streptococcus pyogenes; proteus mirabilis; Kelbsiella spp*. ; *Escherichia coli* ; *pseudomonas aeruginosa* and anaerobic bacteria . while non-classical pathogens include *Staphylococcus epidermis; Streptococcus .viridans;* Diphtheriods *and* bacillus spp. These results were disagree with the results obtained by[28] who found that (31.5%) of chronic sinusitis were caused by classical pathogens and (68.5%) were caused by non classical pathogens .

Diphtheroids and bacillus spp. These results were disagree the results obtained by[28] who found that (31.5%) of chronic sinusitis were caused by classical pathogens and (68.5%) were caused by non classical pathogens.

While table (3) illustrated that among (100) patients the most frequent aerobic and facultative anaerobic bacteria isolated from chronic sinus were *Staphylococcusaureus*(17.5%);*Staphylococcus.epidermids*(14.1%);*Streptococcusneu moniae*(12.5%) ; H.*influenzae*(10%) and *Moraxella.catarrhalis* (10%) . Other less frequent bacteria were:*Streptococcus.pyogenes*(7.5%); *Streptococus.viridans*(6%) ; *Diphtheriods*(4.2%) ; *Proteus .mirabilis*(3.3%) ; *Klebsilla spp* (7.5%) ; *E.coli* (1.7%) *; pseudomonas aeruginosa* (1.7%) and *Bacilus spp* . (1.7%) . while anaerobic bacteria recovered only from (8.3%) , and include *streptococcus spp.* (2.5%) ; *Bacteriods spp.* (2.5%) *fusbacterium spp*. .(1.7%); *peptococcus spp* (0.8%) and *Veillonella spp* .(0.3%) . on the other hand fungi were found in (4.2%) and it includes *Aspergillus fumigatus*(2.5%) and *Aspergillus niger* (1.7%) .

The causative agents of chronic sinusitis include variety of microorganisms and often more than one bacterial species can be found , so it is difficult to compare the results of various studies with each other because : [29]

1- Different clinical samples were used to detect these microorganisms, e.g. swabs from middle meatus , aspirate by antualwas out and biopsy .
2- Differences in patients selection (age , duration , extent of disease pre-surgical treatment with antibiotics , …. etc) .
3- Differences in site of clinical specimens , which used to obtain culture (maxillary , ethmoidal sinuses) .
4- Differences in transport method , type of media , and culturing techniques.[30]

Table (3)Types of Microorganisms Isolated from Chronic Sinusitis patients

Types of microorganisms	No.of patients	%
Aerobic and facultative an aerobic		

Bacteria		
Staphylococcus aureus	21	17.5
Staphylococcus epidermidis	17	14.1
Streptococcus peneumoaniae	15	12.5
Haemophilus influenzae	12	10
Moraxella catarrhalis	12	10
Streptococcus pyogens	9	7.5
Streptococcus viridnas	6	5
Diptheriods	5	4.2
Protues mirabilis	4	3.3
Kebsiella planticola	4	3.3
*Kebsiella pne*umonia	3	2.5
Kebsiella oxytoca	2	1.7
Escherichia coli	2	1.7
Pseudomonas aeruginosa	2	1.7
Bacillus spp .	2	1.7
Anaerobic bacteria :		
Peptosreptococcu spp.	3	2.5
Bacteriods spp.	3	2.5
Fusobacteriumsp.	2	1.7
Peptococcus spp.	1	0.8
Veillonella spp.	1	0.8
Fungi		
Aspergillus fumigatus	3	2.5
Aspergillus niger	2	1.7

Table (4) demonstrated the mean values of immunoglobulins and complement with each types of microbial infection in (110) patients regarded as immune competent ,because they have normal values of immunoglobulins and complement. from this table ,we can see that there is no relationship between the type of microbial infection and values of immunoglobulins and complement ,therefore we take the mean values of immunoglobulins and complement ,

It is important to remember that local and systemic immune defense mechanisms are separate and a normal serum IgA doesn't exclude deficiency of secretory IgA in nasal mucosa ,The secretory IgA is estimated by special technique not available so some of these patients might have local immune deficiency as predisposing factor for sinusitis, and this agrees with[31] who stated that a small group of patients lack IgA in the saliva and nasal mucosa , despite the normal serum levels .

Therefore ,the results of two studies cannot be identical and the results of different studies may be conflicting, but in general *Staphylococcus aureus* was found to be the most frequent pathogens of chronic sinusitis and this agrees with the results of[32,33,34] .This is mainly because *Staphylococcus aureus* has many virulence factors and present in the upper respiratory tract specially nasal cavity as normal flora and has the potential to cause infection :therefore we suggest that if empiric antimicrobial therapy is to be used for chronic sinusitis ,it should be primarily be effective against *Staphylococcus.aureus*.[35]

Another interesting points is the presence and importance of anaerobes. The frequency of anaerobes reported in chronic sinusitis ranges from (0%) [32] to (100%) [20] .The involvement of anaerobes in chronic sinusitis probably is related to the persistent oedma and swelling within the sinus cavity which reduce blood supply, ostial obstruction ,and poor drainsge ,these lead to mucociliary dysfunction ,increase PCO_2,decrease PO_2 and reduced pH ,Other factor is the emergence of anaerobic bacterial strain that possess essential virulence factors such as capsule.[14]

Table (4)Mean values of Immunoglobulins and complement with each Type of Microbial Infectious in Immune complements patients.

Types of Microorganisms	Means of Immunoglobulins and complement (mg/dl)				
	IgG	IgM	IgA	C3	C4
Streptococcus aureus	1504	142	212	148	24.9
Streptococcus peneumoaniae	1389	160	197	140	30.7
Haemophilus influenza	1234	107	150	143	33.3
Branhamellacatarrhalis	1550	190	255	156	32.9
Streptococcus pyogens	1300	65	207	131	41.8
Protues mirabilis	1715	106	150	140	33.9
Kebsiella planticola	1603	172	237	166	27.3
Kebsiella pneumonia	1500	188	170	157	30.1
Kebsiellaoxytoca	1715	155	100	124	25.5
Escherichia coli	1332	130	115	124	33.2
Pseudomonas aeruginosa	1349	152	180	157	30.6
Anaerobic bacteria	1287	70	92	110	34.2
Normal bacteria flora	1338	130	226	145	30.9
Mixed of bacteria	1500	180	215	130	34.9
Fungi	1237	85	100	132	30.3
Normal values	844-1912	50-196	68-423	101-186	16-47

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الخلاصة

هذه الدراسة الحالية في مستشفى الديوانية التعليمي (قسم الأنف والإذن والحنجرة)للمدة من تشرين الثاني 2013لغاية آذار 2014 تم اختيار 100 مريض بالغ مصابين سريريا بالتهاب جيوب الأنفية المزمن والذين أخذت المسحات من القناة الوسطى بواسطة مسحات مصممة خصيصا تحت مرشد تنظير باطني من كلا المرضى ومجموعة السيطرة (50)فرد صحي لتوصيف الجراثيم المسببة لالتهاب الجيوب الانفية المزمن ،إذ وجد ان من 100مريض ،كانت فقط 10% مسحاتهم ليس فيها نمو بينما 90% أعطت نمو لجراثيم متنوعة وكانت معظمها ممرضات شائعة تمثلت بأجناس *Staphylococcus aureus* , *Streptococcus pneumoniae* ,*Haemophilus influenzae and Moraxella catarrhalis.* التحقيقات المناعية أظهرت (99) مريض كانوا سليمين مناعيا بينما 3مرضى اظهروا خللا مناعيا.