

Isolation and Identification of MRSA from Leukemia Patient Under Chemotherapy

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The risk of bacterial infections is related to the severity and duration of neutropenia. Recurrent infection typically occurs with neutropenia but the neutropenia Per se is not necessarily a disease[5].

Patients with neutropenia are not increased risk of parasitic and viral infection, bacterial infection are the most common, although, fungal, infection can occur early in the course of immunocompromised as well in most cases the pathogen is endogenous. the modified sites of infection among them are mouth, pharynx, nose, sinuses, soft tissue and gastrointestinal infection[6].

Aim of Study :-

The current study was conducted aiming to :-

- 1- Isolate the most common microorganisms, if present in blood, throat, nose and urine from different types of leukemia.
- 2- Determine the correlation between neutrophil count and finding of isolated microorganisms.

Materials and Method's:-

Sample collection:-

Leukemic patients according to the type of leukemia, were further divided into two groups; chronic and acute. in addition to other information were observed such as sex, duration of the diseases, complications, treatment with cytotoxic or antibiotic drugs, age and neutrophil count.

Also 50 healthy people were taken as a control group; throat and nasal swabs were taken with all other information required.

Introduction :-

Leukemia is a cancer of the blood, it develops in bone marrow, the soft spongy center of the long bones that produces the three major blood cells, white blood cells to fight infection, red blood cells that carry oxygen, and platelets that help blood clot and stop bleeding. for unknown reason. the bone marrow produces abnormal cells that reproduce very quickly and do not function as healthy cells especially white blood cells to help fighting infections[1].

Neutropenia is a severe drop in infection - fighting white blood cells (neutrophils), a common side effect of some kind of chemotherapy.

chemotherapy, the use of drugs to treat cancer, works by seeking out and destroying fast growing cells. unfortunately, because chemotherapy can not differentiate between cancer cells and normal cells, healthy cells also killed including the white blood cells that protect against infection.

Neutropenia and infection are major dose - limiting side effect of chemotherapy [2].

Infection in the neutropenia patients have remained a major clinical challenge for over three decades, while diagnostic and therapeutic intervention have improved greatly during this period, increases in the number of patients with neutropenia, changes in the etiologic agents involved, and growing antibiotic resistance have continued to be problematic [3].

Surveillance cultures from throat, skin, and feces in febrile neutropenic patients have to be done. blood culture has been shown to be useful surveillance culture to be performed during period of persistence fever in leukemic patients [4].

All swabs need to be transported to the laboratory immediately into blood agar plates (or in appropriate medium as quickly as possible; with in 30 minutes).

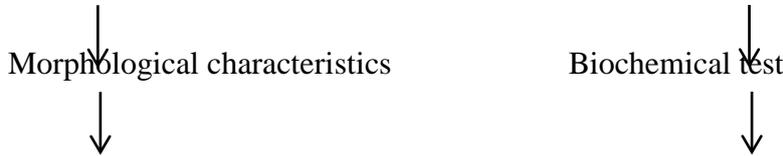
The collected samples were treated immediately by using the following procedures :-

- 1- Direct microscopic examination with Gram's stain.
- 2- Cultivation on blood agar for 24-48 hours at 37C.

Specimens:-

Throat and Nasal swabs:- Samples were taken by a sterilized swab for each site, throat (near the pharynx and tonsils) and nose , this was do ne by rolling the cotton to rub thoroughly over the tonsil and the posterior wall of pharynx avoiding heavy contamination of the swab with saliva.

Identification of isolated microorganisms



<p>A- Colonial morphology and culture Characteristics (were determined by Examining growth on differential and Selective media).</p> <p>B- Gram's stain (can di vided all bacterial Species into two groups , Gram stain Film was made initially from both Media irrespective of the signs of Bacterial growth ,also it was done For bacterial isolates from colonies that were obtained from swab)][7].</p>	<p>A-Coagulase test (slide method) (Coagulate is an enzyme produced by Pathogenic ,which causes clotting of Plasma .it has been shown that factor Known as activator must be present in the Plasma of certain animals by which the test becomes demonstrabie)][8].</p> <p>B- mannitol fermentation test (in gram staining, all isolates which ferment Mannitol appeared to be Gram's positive Cocci more detailed biochemical tests Were perfonned on each isolates to Determine wh ether or not they could be Identified as stain of staphylococci or Other genus .these involved the determination of hemolysis activity coagulase)][9].</p>
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when the antibiotics reach inhibitory concentration or more in the age, there will be no growth of the microorganism and zones of inhibition will be found around the antibiotics disc [11].

Three to five colonies of microorganism were touched with sterile loop and seeded into 5b ml of BHIB , which was then incubated at 37 C for few hours . A sterile disposable wooden stick cotton swab soaked in the broth culture, the surplus suspension was removed from the

Methicilline susceptibility test staph aureus:

Antimicrobial susceptibility tests of the isolated microorganisms were perfonned by using Bauer- Kirby disc diffusion method [10] .

those method employs an plate inoculated with the test microorganism . di sc of filter paper containing known amount of antibiotic (methicilline) is placed on the plate. as the microorganism commences to grow , the antibiotic diffuses from the discs.

The current study was conducted on 50 patients, with leukemia their distribution regarding the type of leukemia were shown chronic lymphocyte leukemia (CLL), 10 (20%). The role of the duration of disease in leukemia patients. Although the multiple infection was found in both two groups (less than 6 months and more than 6 months). It seems that multiple infection is little more in the first group (less than 6 months) and that with significant value, this applied in both nasal and throat swabs. The longer duration of disease leads to severe multiple infection, because the severe drop of neutrophil count in this group of patients makes them susceptible to all of the common Gram-positive and fungal infection. In addition, prolonged periods of therapy-induced neutropenia have been associated with an increased frequency of invasive mycoses mostly caused by mRSA. [14]. mRSA was the most common microorganism isolated in both swabs (33;66%) and 28 (56%), [15].

coagulase-negative, mRSA are the predominant, the changing from Gram-negative to Gram-positive microorganisms that occurred is not absolutely clear, and is probably multi-factorial. All these factors play important role in this finding.

Important considerations include aggressive chemotherapeutic regimens that causes more severe mucositis, with longer duration of diseases and use of prophylactic antibacterial agents with relatively weak coverage of Gram-positive microorganism.

For all the above we must know that the patients of leukemia under the chemotherapy with neutropenia were of find with highly no. of pathogenic or nonpathogenic.

Conclusions:-

1. mRSA was among the most common and frequent isolated microorganisms. In addition, most isolated bacteria in throat may be isolated from the nose.
2. Infection is not the only cause of death in the study group.

swab by being rotated against the side of the tube, then the swab was streaked evenly in 3 planes on the surface of freshly prepared (within four days). After the inoculum's was dried for 3-5 minutes, antimicrobial disc with methicillin was then applied on the inoculated plate with flamed forceps gently pressed down to ensure contact. The plates were inverted and placed in the 37°C incubated within 30 minutes after applying the discs.

After overnight incubation, zone diameters were measured with measuring device at the back of the plate. The end point was taken as the complete inhibition of growth as it appears to the unaided eye. Zone diameter was correlated with known standard values for susceptible and resistant response to methicillin disc.

The standard value of inhibition zone of methicillin disc was to disc contains 5 µg of methicillin antibiotic, 9 mm or more susceptible [12].

Result and Discussion:-

Infection continues to be a significant problem in patients with blood cancer. There are many factors that which increases the susceptibility of immunocompromised cancer patients to infection. The results depends on the type of leukemia, the neutrophil counts and the duration of illness.

One hundred patients were divided into two groups 50 (50%) microbiologically documented while the other group gave negative culture because many factors may contribute to this; patients might be pre-equipped by antibiotics, another microorganisms than the isolated which may need special media for growing viral, fungal, protozoa, etc..... or might need longer duration than usual or other manual procedure.

At the same time, those with negative groups explained the fever of unknown etiology which pyrogenic medications, allergic reactions and administration of blood products and malignancy per se should be not [13].

microorganism (33 ; 66%) and (28 ; 56%) respectively followed by (28 ; 56 %) in throat and (20 ; 40%) in nose .

Kappa test for measuring the degree of agreement was used to test the association between throat and nasal swabs for every individual microorganism isolated .it was (96.7%).

References:-

- 1- Richard C & David G (1987) . leukemia . In : Harrison's principles of internal Medicine, 11th ed. ,Chapter 292. Churchill Livingstone Oxford. PP.
- 2- Hozer et. al . ,1991.
- 3- Donowitz RG (2001) . Hematology . the American Society of Hematology , Infections in the neutropenia patients .
- 4- Haslett et . al . , 1999 ; Kalastrsky , 1999 ; Dennis & Barry, 1988.
- 5- Shin 2002 .
- 6- Mendelson m. (1998). Fever in the immunocompromised host Emergency Medicine. Clinics of North America; 16(4) .
- 7- Finegold and Bron, 1986 .
- 8- Facklam, 1980 .
- 9- Johnson et. al . , 1996 .
- 10- Bauer et. al . , 1996.
- 11- Raphael , 1976 .
- 12- National Committee for Clinical Laboratory Standards (1997) :Methods of dilution antimicrobial susceptibility test. National Committee for Clinical Laboratory Standard, Wayne Pa.
- 13- Pizzo PA (1989) . Evaluation of fever in cancer patients .Eur . J Cancer Clin. Oncol . ; 25 Suppl 2 : 59-116 .
- 14- Sudhoff T, Arning M & Schneider W(1997) . Prophylactic strategies to meet infectious complications in treatment of leukemia . Leukemia ; 11 : 358-41 .
- 15- Donowitz GR , Maki DG , Crnich CJ, Pappas PG & Rolston KVI (2001) . Infection in neutropenic patients . Hematol , 23 :45-49 .

3. the prevalence of bacterial infection is significantly high in chronic type of leukemia compared with acute type of leukemia.

Recommendation:-

More studies in this aspect are needed to detect the pattern of infection and management of leukemic patients.

Abstract:-

A study was conducted at National center of Hematology (NCH) for leukemic patients during the period of February 2003 to February 2004 for which patients were the source of throat , nasal swabs to investigate the most common microorganism through isolation and identification of isolates using usual microbiological methods . it is also to determine the most important factors of infection associated with leukemia as well as the causes of fever in the study group.

In our study the total of the patients were 100 patients , which were divided into two groups , the first group 50 (50%) were microbiologically documented and the second were not.

The total number of isolates were 356 , which were classified into 214 isolates in throat swabs and 142 isolates in nasal swabs .

The neutrophil count and the duration of disease play as important factors in infection of this group .the neutropenia causes mostly seen highly in chronic type of especially CLL patient (8 ; 80%) based on neutrophil count , infection were isolate were found in throat (16; 32%) , while four isolates were found in nasal infection (8 ; 61.5%) . in throat swabs , mRSA mostly seen in neutropenic patients (22; 66.7%) . while in nasal swabs , mRSA is also the commonest microorganism (16; 80%) . according to the duration of leukemia, the infection is mostly seen in early phase of disease (less than 6 months) . in these groups and in both throat and nasal swabs , mRSA is strongly predominant