Buffy Coat smear and culture: An early diagnostic procedure in bacteremic and septicemia Pediatric Patients

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ABSTRACT

Blood culture is the single most important procedure to detect systemic infection due to bacteria, it provides valuable information for management of febrile. In this study trying to use Buffy coat smear methods and culture by using unclotted peripheral blood in haematocrite capillary tube, which may yield important early diagnosis procedure in bacterimic and septicemic baby especially in critically ill and low birth weight infant.

Among sample of Iraqi childhood a study done on 280 babies patients clinically diagnosed to had bacterimia and septicemia. the frequency among male babies was higher than that of female, the ratio 1.5: 1 , the occurrence was more noticeable under one year age group represent 58.9% and decline among other age group in which positive culture showed in 75%(210/280) were male represent 60% of the isolate while female represent 40%. As a conclusion from this study Eshericha coli considered the main organism isolated represent 16.7% (35/210), Pseudomonase spp represent 15.2%(32/210), Streptococci spp 14.8%(18/210), Klebsiella spp 13.3%928/210), Eterobacter spp 11.4%(25/210), Salmonella 8.6%(18/210), finally staph-albus isolated from 6.2% immuncompromised patients.

Associated disease status as arise factor for bacterimia and septicemia including meningitis 18.6%, urinary tract infection 17.6% , chest infection 13.4%, intestinal obstruction 12.4% , jaundice 11.9%, pneumonia 9%. Kal-azar and galactecemia represent 5.2% respectively.

Key Words: Bacterimia, Septicemia, Buffy coat

INTRODUCTION

Bactremia and sepsis caused by gram-negative Bacteria and especially in hospital have increased greatly in incidence and severity.[1,2]

Many reports have recorded the mortality resulting from gram negative bacteria and documented the influence of several factors on this mortality. [3, 4] Most of this study has dealt specially with gram-negative bacteria, few studies have reported the distinction between acquired in the

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community and that acquired within the hospital. Physicians and microbiologist have long recognized that the presence of living microorganism in the blood of the patient carries with it considerable morbidity and mortality. Indeed, because invasion of the blood stream on of the most important and frequently performed test in clinical microbiology laboratory. (4, 5, 6) Many septic episodes are nosocomial and in some hospital represent a majority such episodes may be due to microorganisms with increased antimicrobial resistance and are associated with greater mortality than are community acquired episodes. (6)

Studies of bacteremia and septicemia with gram negative and gram positive bacteria had directed the attention to infection with these agents.

This study will focus on the clinical importance of blood culture diagnostic strategies by using Buffy coat smear and culture as a new methods to be applied in Iraq for early diagnostic procedure in bacteremia and septicemic infant and children.

MATERIAL AND METHODS

All patients need blood culture submitted to the clinical microbiological laboratory of children's Al-Mansoor Hospital in Baghdad .

The unclotted peripheral blood were collected a septicaly in 3 hematocrit capillary tubes.

Gram-stain smear of the E from the first capillary tube removed to a clean glass slide and spread according to the usual blood smear technique, after the smear is air dried and heat fixed, cover it with water for 5 minutes, the red blood cell will layse and float off the smear, thereby leaving a less confusing scene. Method for gram's stain smear were done with crystal violet (10 second), Gram's iodine (10 second), decolorize with 95% ethanol, safranine (10 second). A gram stain smear of the Buffy coat (W.B.C) may yield important early diagnostic information in septicemia patients. (7)

Procedure for Culture:

Each sample from patient showed gram stain of Buffy coat positive for gram positive bacteria or for gram negative bacteria, procedure for culture were done form the second and third capillary tubes, were both inoculated into brain heart infusion (BHI) 10 ml bottle containing sodium polyanethol sulfonate.

Routine subcultures were performed on all macroscopically negative bottles between 1 and 14 hr (early) after inoculation of the culture, and again after 48hr of incubation,

by removing a sample of broth with a sterile needle and syringe and inoculating it onto blood agar plates, chocolate blood agar plates and, which were incubated at 37C in 10% CO2 for 48hr. (8, 9)

MacConkey agar plates were used for gram negative bacteria. No routine anaerobic subculture performed. [9, 10]

Subculture of positive culture was made immediately to the appropriate media to isolate the microorganism seen in the gram stain smear. Microorganism diagnosed by biochemical and serological test

RESULT

All patients in this study were admitted to Al-Mansoor hospital for children in Baghdad area during the year 2001 -2002, started from first Sep 2001 to 30 dace 2002.

The study includes 280 infants age group 1 day -2 years and child from 2-6 years as ill patients with PUO. In table (1): showed the distribution of 280 PUO patients according to the age and sex (infant and children), male female ratio 1.5: 1. In both sex a high mortality noticeable among infants fewer than one year age, male infant represent 56.6% (20/168) while female infant showed 62.5 %. On the other hand this percentage was sharply decreased among patient 1 -2 years which represent 11.9% (20/ 168) among male patient and female patient

represent 8.9% (10/112). Male age group 3-5years represent 25% (43/168); while female represent 22.3% (25/112). Over 5 years old only 5.9 %(l0/168) of the male child, females child represent 6.3(7/112)

suffering of PUO o

examined 75% (210/ 280) were positive the significant isolated were obtained demonstrated on table (2). Escherichia coli represent the high common microorganism 16.7% (35/210) followed by *pseudomonas spp* 15.2 (32/210) of the total isolate Streptococci 14.8 %(13/210), Staph aureus and *klebsiella spp* represent 13.3 %(28/210) respectively *Enterobactor spp* represent 11.9 (25/210) *Salmonella spp* 8.6 %(18/210) and finally Staph albus represent 6.2 %(13/210).

Positive blood culture among male baby represent 60 % of the total isolate, the most common organism isolated *Streptococci spp, Klebsiella spp*. Escherichia coli represent 15.7 %(20/26) followed by *Psedomonase spp* 14.3% (18/126), Staph aureus 12.7% (16/126), Enterobacter 11.9 %(15/126), *Salmonella spp* 7.8(10/126) and Staph albus 5.6 %(7/1226).

Female baby 40% gets positive culture in which Eshericha coli represent the commonest isolated organism 17.9%(15/84), *pseudomomase spp* represent

16.7%(14/84), Staph aureus represent 14.2 %(12/84), Streptococci 13 %(11/84), Salmonella and *Klebsiella spp* represent 9.6 %(8/84) respectively and finally staph albus isolated from 72% (6/84) of immuncompromised patient. Spectrum of clinically significant type of

disease as predisposing bacteremia was demonstrated in table (3). Among male patient the majority of cases had bacterimia due to meningitis 20.6 %(26/126), followed by urinary tract infection 12.6(16/126), pneumonia 11.6 %(15/126) chest infection as well as acute

gastroenteritis represent 10.3 %(13/126), respectively. Intestinal obstruction 9.5 %(12/126), kala-az represent 8.7 %(11/126). Female patient showed that urinary tract infection was the main cause of bacterima represent 25(21/84), while jaundice represent19.1 %(16/84) chest infection 17.8 %(15/84), intestinal obstruction 16.6 %14/48) due to meningitis 15.5(13/84) and pneumonia 4.8(4/84), and finally due to anemia one case female baby represent 1.1

Table (1): the distribution of 280 PUO patients according to the age and sex

TOTAL NO	%	FEMALE NO	%	MALE NO	%	AGE GROUP
165	58.9	70	62.5	95	56.5	Under 1 year
30	10.7	10	8.9	20	11.9	1 – 2 years
68	24.3	25	22.3	43	25.6	3 - 5 years
17	6.1	7	6.3	10	5.9	Over5 years
280	100	112	100	168	100	Total

Table (2): Spectrum of clinically significant organisms isolated from 210 patients

TOTAL NO	%	FEMALE NO	%	MALE NO	%	Microorganism
32	15.2	14	16.7	14.3	18	Pseudomonase spp
25	11.9	10	11.9	15	11.9	Enterobacter spp
28	13.3	8	9.6	20	15.9	Klebsiella spp
35	16.7	15	17.9	20	15.9	Escherichia coli
18	8.6	8	9.6	10	7.8	Salmonella spp
28	13.3	12	14.2	16	12.7	Staph - aureus
31	14.8	11	13.0	20	15.9	Streptococci spp
13	6.2	6	7.2	7	5.6	Staph -albus
210	100	84	100	126	100	TOTAL

Table (3): Spectrum of clinically significant type of diseases as predisposing factors of bacterimia

TOTAL NO	%	FEMALE NO	%	MALE NO	%	DISEASES
25	11.9	16	19.1	9	7.2	Jaundice
11	5.2	0	0	11	8.7	Galactcemia
13	6.2	0	0	13	10.3	Acute gastroenteritis
1	0.5	1	1.1	0	0	Anemia
39	18.6	15.5	13	26	20.6	Meningitis
19	9.0	4	4.8	15	11.6	Pneumonia
11	5.2	0	0	11	8.7	Kala-azar

37	17.6	21	25	16	12.6	Urinary tract infection
28	13.4	15	17.8	13	10.3	Chest infection
26	12.4	14	16.6	12	9.5	Intestinal obstruction
210	100	84	100	126	100	TOTAL

DISCUSSION

Bacterimia frequently potent the life threatening it is early detection is essential. Blood culture is the single most important procedure to detect systemic infection due to bacteria it provide valuable information for the management of febrile acutely ill patients with or without localizing symptom and sign and is essential in any patient in whom infective endocarditis is suspected even if the patient did not appear acutely or severely ill .

In addition to its diagnostic significances, recovery of infectious agents from the blood provides valuable aids in determining anti-microbial therapy.

Every efforts should there fore be made to isolate the causative organism in bacterimia, in clinically ill very low birth weight infant's volume of one ml or more may be difficult to obtain. In this study trying to use a Buffy coat smear methods as indicator for bacterimia and using unclotted peripheral blood in hematocrite capillary tube for culture.

This may yield important early diagnostic procedure in bacterimic and septicemia patient. Data from this study afforded a good opportunity to document such change in occurrence and character of bacterimia and septicemia infection.

Among sample of Iraqi childhood study done on 280 baby patients clinically diagnosed as bacterimic and septicemia, that male/ female ratio 1.5: 1, that mean the frequency rate among male is higher than that of female. On the other hand occurrence was more noticeable among the age under one year, which represent 58.9 %(165/280) then sharp decline among age group 1 -2 years to reach 10.7 % (30/280) and slightly increased among age group 3-5 years which represent 24.3 (68/280) and over 5 years old represent 6.1 % (17/280). Many studies have shown that bacterimia and septicemia is not uncommon with the

greatest risk 5 – 10 % accruing in infant between the age 5 months and 2 years who have temperature above 38.9 C and leucocytosis. [6, 10]

The spectrum of organisms causing childhood bacterimia is different than that causing the disease in adults. [11] The spectrum of organism causing bacterimia in this study , out of 280 babies patients 210 had had positive culture results which represent 76.6 (210/280) in which 60% of the isolate from male babies and 40 % of the isolate from female babies.

The most common micro-organism isolated from male babies , Streptococci spp, Klebsiella spp , Escherichia coli represent

15.9 % respectively Pseudomonase spp 14.3 %(18/126) , Staph-aureus 12.7(16/126) , Enterobacter 11.9% 15/126) , Salmonella isolated 7.8 % (10/126) of the patients , finally Staph-albus isolated from very critical patients5.6%(7/126) .

On the other hands microorganism isolated from female baby the commonest organism were Escherichia coli 17.9% (15/84),Pseudomonase 16.7% (14/84), Staph- aureus 14.2% (12/84) , Streptococci spp represent 13%(11/84) , Enterobacter 11.9%(10/84) , Kebsiella spp 9.6% (8/84) and Staph- albus 7.2 % (6/84).

As conclusion from this study Esherichia coli considered the main organism 16.7% (35/210) of the total isolate followed by Pseudomonase spp l5.2% (32/210), Streptococci spp 14.8% (31/210)

Klebsiella spp 13.3% (28/210) ,Enterobacter 11.9%(25/210), Salmonella 8.6%(18/210) and Staph-albus 6.2%(13/210) . The important organism found in other studies [10,11] they found that certain bacteria particularly Staph- aureus and Strept pyogens may be associated with unusually sever systemic inflammatory response syndrome such as Staphylococcal toxic syndrome. Septicemia may follow untreated bacterimia and may develop rapidly in the immunocompromised child such as the child with leukemia, nephritic syndrome or sick collapse for septic shock. [11, 12, 13] In the preterm infant infection due to coaggulase-negative streptococci is common. [14]

Associated disease status as risk factor for bactrimia and septicemia including meningitis 18.6 % (39/210), urinary tract infection 17.6 %(37/210), Chest infection 13.4%(28/210), intestinal obstruction 12.4%(26/210), Jaundice 11.9 % (25/210) Pneumonia 9%(19/210), Kal-azar and Galatcemia represent 5.2%(11/21)) respectively , and due to anemia one case only .

The relation in this study between the disease and bacterimia originating from were shown before in many studies.[15, 16, 17]

Finally the conclusion and suggestion obtained from this study to certainty that early diagnosis is essential with minimum volume of blood by using Buffy coat smear and culture of two or more if it is easily to obtained by capillary tubes to be assessed as small sample submitted for critically ill newborn and very low birth Wight infants .

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