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Evaluation of Hematological Parameter, Glycemic Status and Risk Factors Responsible for Neonatal Septicemia and Bacterial agents Causing Neonatal Sepsis in Chittagong, Bangladesh

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FN, RHC and SA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MKJ and MMUQ managed the analyses of the study. Author CMMH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was undertaken to assess haematological parameter, glucose status, the possible maternal and neonatal risk factors responsible for neonatal septicemia and the pattern of bacterial agents causing neonatal sepsis. Blood cultures were performed for 147 newborn babies (0-28

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days) admitted into the hospital with a clinical diagnosis of neonatal sepsis where 66% were males, and 34% were females resulting in an overall male to female ratio of approximately 2:1. Among them, 57.1% neonates were presented with EONS, 42.9% with LONS and 21.8% were preterm. Approximately, 37.4% neonates with sepsis had low birth weight (< 2500 g), 6.8% had very low birth weight (< 1500 g). The most prevalent clinical features were reluctance to feed 56.5%, respiratory distress 36.7%, jaundice 19.7% and convulsion 15.0%. Among the 147 neonates, 8.2% had high WBC count (>18,000/cm) and 2.0% had low WBC count (<5000/cm). Immature/total neutrophils ratio ≥0.16 and <0.16 was observed in 8.8% and 91.2% peripheral blood smear examined respectively. While 36.7% had neutropenia (<54%), 48.3% had higher (>62%) neutrophils. 23.8% neonates had low, and 76.2% had higher haemoglobin where 35.4% had higher C-reactive protein level. Out of 147 neonates 27.9% were hypoglycaemic (< 45 gm/dl) and 19.0% were hyperglycaemic (>140 gm/dl). Among the neonates with EONS, 40.5% were hypoglycaemic, and 17.9% were hyperglycaemic while with LONS, 11.1% were hypoglycaemic and 20.6% were hyperglycemic. As for the neonatal risk factors, A significant difference was found for C-reactive protein Levels ($p<0.05$) and neutrophils ($p<0.05$) between the culture positive and negative culture sepsis. However, 29.3% had positive blood culture for bacteria. *Klebsiella* spp. accounted for 13 (30.2%) of the total isolates followed by *S. aureus* 8 (18.6%). The Gram-positive and Gram negative bacteria accounted for 34.9% and 65.1% respectively.

Keywords: Neonatal risk factors; glucose status; C-reactive protein; haematological parameters.

1. INTRODUCTION

Despite advances in maternal and neonatal care, infection remains a frequent and important cause of neonatal and infant mortality and morbidity [1]. Neonatal sepsis may have subtle, diverse and nonspecific symptoms and signs; moreover, a delay in the diagnosis and commencement of treatment results in high morbidity and mortality rates [2]. Neonatal sepsis is a clinical syndrome characterised by signs and symptoms of infection with or without accompanying bacteremia in the first month of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections. Superficial infections like conjunctivitis and oral thrush are not usually included in neonatal sepsis. Neonatal sepsis remains as an important cause of morbidity and mortality among infants in developing countries accounting for 30-50% of total deaths each year [3]. Neonatal sepsis is divided into two categories: early-onset sepsis (EOS) and late-onset sepsis (LOS). EOS refers to sepsis presenting in the first 7 days of life (although some refer to EOS as within the first 72 hours of life), with LOS referring to presentation of sepsis after 7 days (or 72 hours, depending on the system used) [4,5]. The reported incidence of neonatal sepsis varies from 7 to 38 per 1000 live births in Asia [6,7], from 6.5 [should be 7] to 23 per 1000 live births in Africa [8,9] and from 3.5 [should be 4] to 8.9 [should be 9] per 1000 live births in South America and the Caribbean [10, 11]. By comparison, rates reported in the United States and Australasia range from 1.5 [should be 2] to 3.5 [should be 4] per 1000 for EOS sepsis and up to 6 per 1000 live births for LOS sepsis, a total of 6–9 per 1000 for neonatal sepsis [12,13] and in Europe 0.3-3 [approx. 3] per 1000 live births [14]. EOS usually progresses rapidly and has multiorgan involvement. Infants with EOS had a significantly higher risk of respiratory distress syndrome, severe intraventricular haemorrhage or periventricular leukomalacia [15, 16]. It is an important cause of neonatal death. Mortality ranges from 4 to 50% [17-19]. Particularly, mortality of the early-onset GBS sepsis is 2–30%. Mortality of LOS is usually much lower than in EOS [18,20]. Neonates with sepsis may present with one or more of the following symptoms and signs like hypothermia or fever, lethargy, poor cry, refusal to suck, poor perfusion, prolonged capillary refill time, hypotonia, absent neonatal reflexes, bradycardia/ tachycardia, respiratory distress, apnea and gasping respiration, hypo/hyperglycemia, metabolic acidosis. A definitive diagnosis based on the culture of blood, cerebrospinal fluid (CSF), or urine is usually reached only after a delay of a day or two. Initiation of antibiotic therapy before diagnostic results are available is recommended for neonates with clinical signs or epidemiologic factors associated with neonatal sepsis [2]. C-reactive protein (CRP) is a non-specific, acute-phase protein that rises in response to inflammatory processes. Sufficient evidence exists to support the use of CRP measurements in conjunction with other established diagnostic tests, such as a total and differential leukocyte

count (TLC and DLC) and blood culture to establish or exclude the diagnosis of sepsis in full-term or near-term infants [2]. Hypoglycemia and hyperglycemia are a common metabolic change in neonatal sepsis. A high or low blood glucose level may have a significant effect on the outcomes in patients of neonatal sepsis. A recent study in Bangladesh found that those patients with neonatal sepsis with altered glucose level had higher mortality [1]. The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries. Overall, Gram-negative organisms are more common and are mainly represented by *Klebsiella, E. coli, Pseudomonas*, and *Salmonella spp*. [7,21]. Of the Gram-positive organisms, *Staphylococcus aureus (S. aureus),* coagulase-negative staphylococci (CoNS), *Streptococcus pneumoniae (S. pneumoniae)* and *Streptococcus pyogenes (S. pyogenes)* are most commonly isolated [21-23]. *E. coli, Enterobacter, Enterococcus,* and *Listeria spp.* are often associated with EOS disease. *Klebsiella, Acinetobacter*, and *S. aureus* are associated with both EOS and LOS while *Pseudomonas, Salmonella,* and *Serratia spp*. are typically associated with LOS disease [21,24,25].

2. METHODS AND MATERIALS

2.1 Place of the Study

The study was conducted in the biochemistry, haematology, clinical, Pathology & Microbiology lab, Chattagram Maa-O-Shishu General Hospital, Agrabad, Chittagong.

2.2 Study Design

It was a case-control study. The subjects were admitted to the hospital ward.

2.3 Study Period

This study was done from December 2010 to October 2011.

2.4 Types of Study

This study was prospective which was based on current data. It is very reliable data. These data are stronger.

2.5 Study Subjects

Total 147 subjects were included in this study. There was no specific predilection for race, religion and socioeconomic status. The study subjects comprised of the following two groups.

1. Culture positive sepsis: 43 neonates with Culture proven sepsis.
2. Culture negative sepsis: 104 neonates with Culture unproven sepsis.

2.6 Collection of the Subject

Subjects were collected on every day of the week from 9-00 am to 2-00 pm from the pediatric unit who admitted neonates into the hospital mainly with the complaints of one or more clinical signs and symptoms.

2.7 Study Procedure

During the period of December 2010 to October 2011, a total of 147 neonates (0 to 28 days of age) admitted at Chattagram Maa-O-Shishu General Hospital with suspected cases of early onset sepsis (n=84) and late onset sepsis (n=63) were investigated. Written informed consent was obtained from their parents/guardians. Following a detailed clinical examination, neonates with suspected sepsis having any one of the clinical symptoms and signs as shown in Fig. 1 were investigated for bacterial etiologic agents. Admitted neonates who did not fulfil the above clinical criteria were excluded from the study.

2.8 Inclusion Criteria

- In all cases of suspected sepsis belonging the age group of 0-28 days.
- Voluntarily agreed to be included in this study.

2.9 Exclusion Criteria

- Above 28 days old babies were not included in this study.
- Incomplete medical records and self-discharge against medical advice.
- Local infection at the puncture site.
- Neonates who don't fulfill the clinical criteria described in literature were excluded from the study.

2.10 Ethical Consideration

Informed parental consent was taken before enrolling the children in the study. The procedure was fully explained to the parents and they were

informed that if they desired they could withdraw from the study without affecting the quality of treatment received. Permission was also taken from the hospital authority, Departmental heads of the Paediatric, Biochemistry, Microbiology and Pathology units in order to undertake the study.

2.11 Development of Questionnaire

A questionnaire was developed to obtain relevant information of demographic and socio-economic data. The questionnaire also included anthropometric data, birth history, immunization history, past medical history and other relevant clinical information. The questionnaire had both open- and close-ended questions. It was coded and pre-tested before finalisation.

2.12 Sample Collection, Handling and Transport

Using aseptic technique by applying Povidone iodine and 70% alcohol at the site of vein puncture, 2 ml venous blood was drawn from the antecubital or femoral vein by the attending nurse. One ml blood was inoculated into Tryptone Soy Broth (TSB) for culture and the remaining 1 ml blood was used for white blood cell count and differential count. The specimens were transported within one hour to the Laboratory of Chattagram Maa-O-Shishu General Hospital, Agrabad, Chittagong.

2.13 White Blood Cell and Immature Neutrophil Count

Automated complete blood counts were performed using samples collected on the day of diagnosis and 48 hours post-diagnosis. Horiba ABX Diagnostics automated machine was used to test haematological parameters. Peripheral blood smears were prepared and stained by Wright's stain as described by Dacie and Lewis (1994) [26]. White Blood Cell count, differential count and immature neutrophil count were performed after which the Immature/Total neutrophil count (I/T) ratio was calculated.

2.14 Measurement of C-reactive Protein

The neonatal blood was taken into the test tubes containing a fixed amount of anti-CRP antibody. A beam of infrared light was then passed through the tube. The amount of scattering of this light was measured using a nephelometer, turbidimeter or automated analyser. This is proportional to the amount of CRP–ligand complexes. Nephelometry is the most widely used technique, taking only 10 min using a fully automated analyser and has an analytical sensitivity of about 0.04 mg/l [27].

2.15 Estimation of Blood Glucose by Glucose Oxidase Method

2.15.1 Principle

In this method, the aldehyde group of glucose is oxidised by glucose oxidase to give gluconic acid and hydrogen peroxide. Then, the formed hydrogen peroxide is broken down to water and oxygen by a peroxidase in presence of an oxygen acceptor (phenol and 4-aminophenazone) which itself is converted to a coluored compound (red quinoeimine), whose intensity of coluor is proportional to the amount of glucose, which can be read colorimetrically. An enzymatic colorimetric test was done on the basis of Trider - Reaction. Glucose

$$\text{Glucose} + H_2O + O_2 \xrightarrow[\text{oxidase}]{} \text{Gluconic acid} + H_2O$$

$$H_2O_2 + \text{Phenol} + \text{4-aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Quinoeimine} + H_2O \text{ [23]}$$

2.16 Data Analysis

Collected data was checked for its completeness, correctness. Editing was done by employing Statistical Package for Social Science (SPSS version 11.5) software package. Comparisons were made using Chi-square test with Fisher exact tests. A p-value of <0.05 was considered indicative of a statistically significant difference.

3. RESULTS

A total of 147 admitted neonates (0 to 28 days of age) with suspected case of sepsis were investigated for bacterial infection between December 2010 and October 2011 at Chattagram Maa-O-Shishu General Hospital, Agrabad, Chittagong. Among them, 97 (66.0%) were male and 50 (34.0%) were female encompassing about an overall male to female ratio of approximately 2:1. A total of 84 (57.1%) neonates were found with EONS and 63 (42.9%) found with LONS. Among the neonates with

EONS, 55 (65.5%) were male and 29 (34.5%) were female. Among neonates with LONS, 42 (66.7%) were male and 21 (33.3%) were female. In terms of socio-economic status, number of upper class was 11 (7.5%), middle class 49 (33.3%) and lower class 87 (59.2%), indicating that the lower class population are more susceptible to sepsis.

Of the 147 neonates, 32 (21.8%) were preterm (gestational age less than 37 weeks) and 115 (78.2%) were term. 53 (36.1%) neonates were born at home, 69 (46.9%) in the hospital and 25 (17.0%) in the clinic. 41 (27.9%) of them were delivered by Caesarean section/instrumentation and 106 (72.1%) by vaginal delivery. Approximately, 55 (37.4%) neonates with sepsis had low birth weight (<2500 g), Out of these 10 (6.8%) had very low birth weight (< 1500 g).

The clinical features of EONS and LONS are summarized in Fig.1. The most prevalent clinical features were reluctant to feed 83 (56.5%), respiratory distress 54 (36.7%), jaundice 29 (19.7%) and convulsion 22 (15.0%). Common clinical features like hyperthermia, hypothermia, respiratory distress and reluctance to feed were observed in both EONS and LONS.

132 (89.8%) of them had normal white blood cell count (5000-18,000/cm), 12 (8.2%) high WBC count (>18,000/cm) and 3 (2.0%) low WBC count (<5000/cm) (Table 1). Immature to total neutrophils ratio ≥.16 and <.16 were observed in 13 (8.8%) and 134 (91.2%) peripheral blood smear examined respectively. However, 22 (15%) of them had normal neutrophils, 54 (36.7%) had neutropenia (<54%), 71 (48.3) had higher (>62%) neutrophils (Table 2). A total of 112 (76.2%) neonates had higher haemoglobin where low haemoglobin was found in 35 (23.8%) of them (Table 3). However, 52 (35.4%) had higher and 91 (61.9%) had normal C-reactive protein level (Table 4).

Table 1. Distribution of neonates according to white blood cell counts

White blood cells (/cm)	Frequency	Percent
<5000 /cm (Low)	3	2.0
5000-18000 /cm (Normal)	132	89.8
>18000 /cm (High)	12	8.2

Glucose status of EONS and LONS is summarized in Fig. 2. Out of 147 neonates, 41(27.9%) were hypoglycaemic (<45 gm/dl), 46 (31.3%) were normal and 28(19.0%) were hyperglycaemic (>140 gm/dl). Among the neonates with EONS, 34 (40.5%) were hypoglycaemic and 15 (17.9%) were hyperglycemic. However, neonates with LONS, 7 (11.1%) were hypoglycaemic and 13 (20.6%) were hyperglycemic.

Neonatal risk factors, associated/not-associated with blood culture proven sepsis are outlined in (Table 5). As for the neonatal risk factors, a significant difference was found for C-reactive protein levels ($p<0.05$) and neutrophils ($p<0.05$) between the culture positive and culture negative sepsis. Frequency of low and high neutrophils was found higher in culture negative sepsis than culture positive sepsis ($p=0.041$). Elevated C-reactive protein level was higher in culture negative sepsis than culture positive sepsis ($p=0.017$) [16,28]. Other clinical features, laboratory findings and risk factors showed no significant differences between the culture positive and culture negative sepsis.

Table 2. Distribution of neonates according to neutrophils counts

Neutrophils	Frequency	Percent (%)
<54% (Low)	54	36.7
54-62% (Normal)	22	15.0
>62% (High)	71	48.3

Table 3. Distribution of neonates according to haemoglobin status

Haemoglobin (gm/dl)	Frequency	Percent (%)
<14.5 gm/dl (Low)	35	23.8
14.5-22.5 gm/dl (Normal)	112	76.2

Table 4. Distribution of neonates according to C-reactive protein

C-reactive protein (mg/l)	Frequency	Percent (%)
<6 mg/l	91	61.9
6-10 mg/l	4	2.7
>10 mg/l	52	35.4

Out of 147 neonates, 43 (29.3%) had positive blood culture for bacteria. The Gram-positive and negative bacteria accounted for 15 out of 43 (34.9%) and 28 out of 43 (65.1%). *Klebsiella spp. (K. pneumoniae and K.terrigena)* accounted for 13 (30.2%) of the total isolates followed by *S.*

aureus 8 (18.6%), *E. coli* with prevalence of 7 (16.3%), Gram negative bacilli 5 (11.6%), Viridans streptococcus 5 (11.6%), *Acinetobacter spp* 2 (4.7%) *Enterococcus* spp with an incidence of 2 (4.7%) and *Pseudomonas spp* 1 (2.3%) (Fig. 3). Among the 147, neonates admitted with suspected cases of EONS, 34 (40.5%) had proven sepsis confirmed by positive blood culture while 9 (14.3%) neonates admitted with suspected cases of LONS, were positive for

Table 5. The values of risk factors with culture-proven or unproven sepsis

	Culture positive No.	Culture-negative No.	Total No.	P value
Gestational age (weeks)				
<37 Weeks(Pre term)	11	21	32	
37-42 Weeks(Term)	32	83	115	0.471
Birth weight (gm)				
<1500 gram(VLBW)	4	6	10	
<2500 gram(LBW)	20	35	55	
2500-4000 gram (Normal weight)	19	61	80	0.273
>4000 gram(Overweight)	0	2	2	
Place of delivery				
Home	19	34	53	
Hospital	15	54	69	0.170
Clinic	9	16	25	
Mode of delivery				
Vaginal	30	76	106	
Caesarean	13	28	41	0.684
Glucose status				
Hypoglycaemia	13	28	41	
Mild decrease	2	12	14	
Normal	14	32	46	0.769
Mild increase	6	12	18	
Hyperglycaemia	8	20	28	
White blood cells				
<5000 /cm(Low)	1	2	3	
5000-18000 /cm(Normal)	36	96	132	0.250
>18000 /cm(High)	6	6	12	
Neutrophils				
<54%(Low)	12	42	54	
54-62%(Normal)	7	15	22	0.041
>62%(High)	24	47	71	
Lymphocytes				
<25%(Low)	20	43	63	
25-33%(Normal)	9	17	26	0.526
>33%(High)	14	44	58	
Monocytes				
<3%(Low)	40	97	137	0.957
3-7%(Normal)	3	7	10	
C-reactive protein				
<6 mg/l	19	72	91	
6-10 mg/l	2	2	4	0.017
>10 mg/l	22	30	52	
Haemoglobin				
<14.5 gm/dl(Low)	9	26	35	0.598
14.5-22.5 gm/dl(Normal)	34	78	112	
IT ratio				
≥.16	5	8	13	0.445
<.16	38	96	134	

bacterial culture. Among EONS, *K. pneumoniae* was the commonest isolates 10 (29.4%) followed by *Staphylococcus aureus* 6 (17.6%) then *E. coli* 6 (17.6 %). The Gram-positive and Gram negative bacteria accounted for 10 out of 34 (29.4%) and 24 out of 34 (70.6%) respectively. However, *Klebsiella pneumoniae* accounted for 3 (33.3%), Viridans streptococcus 3 (33.3%), *E. coli* accounted for 1 (11.1)% and *Staphylococcus aureus* 2 (22.2%) in neonates with LONS. The total bacteria count both for EONS and LONS is shown in Fig. 4.

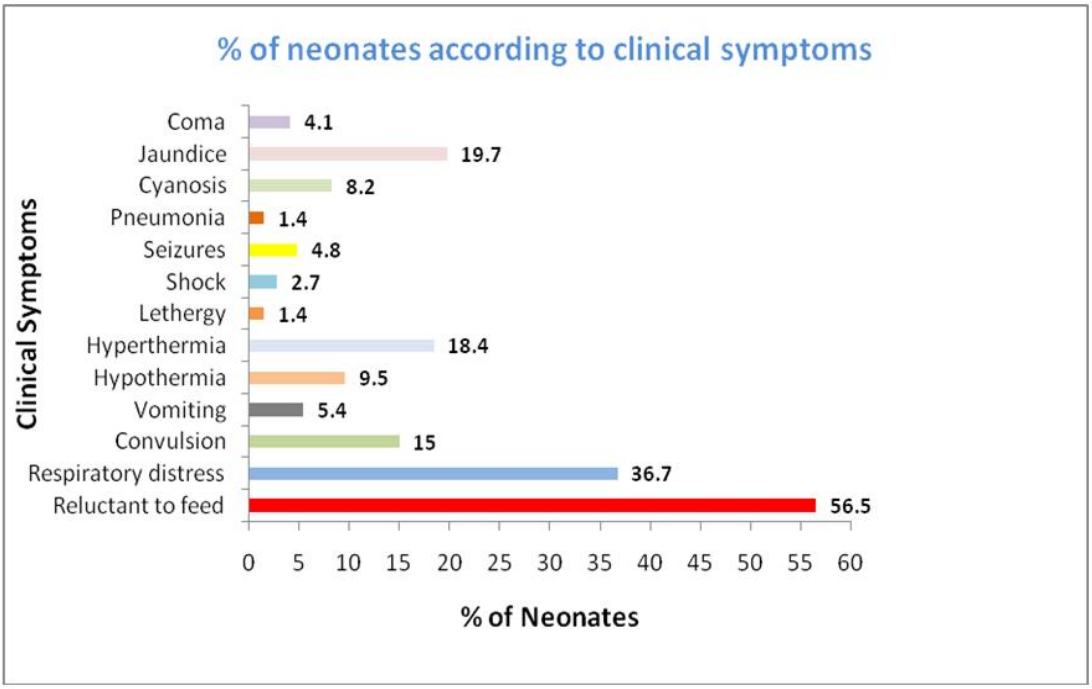

Fig. 1. Bar diagram of percentage frequency of clinical symptoms in neonatal sepsis

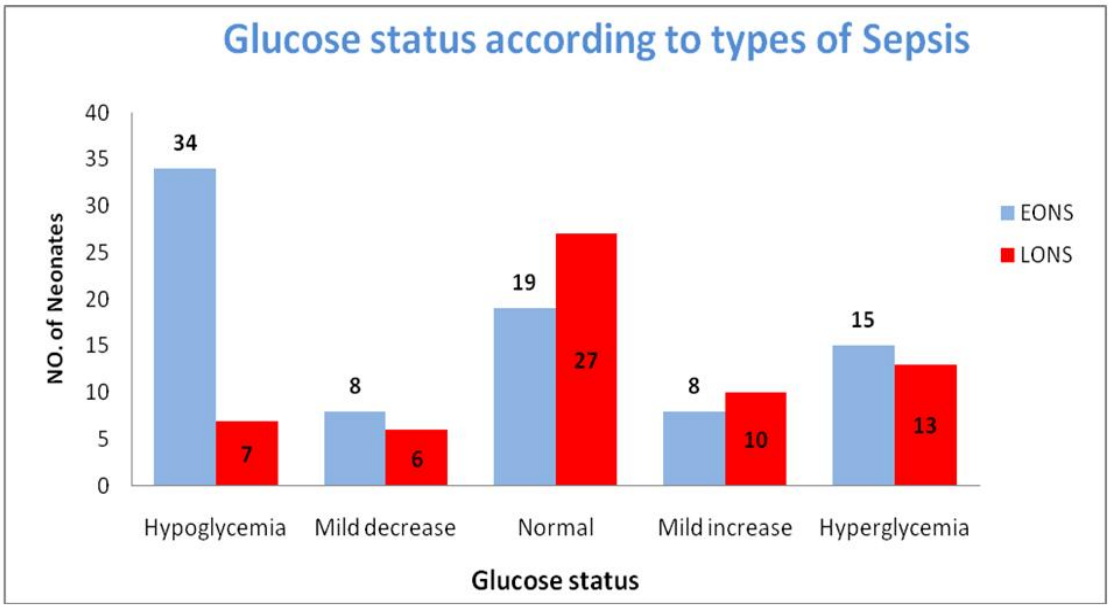

Fig. 2. Bar diagram showing the distribution of neonates according to glucose status

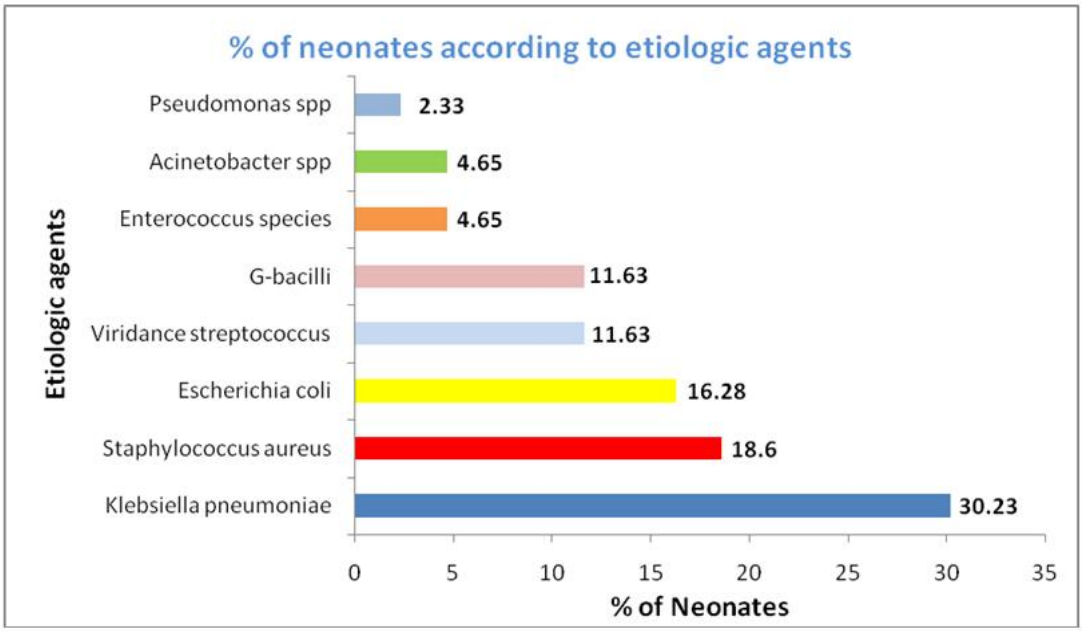

Fig. 3. Bar diagram of percentage frequency of aetiologic agents in neonatal sepsis

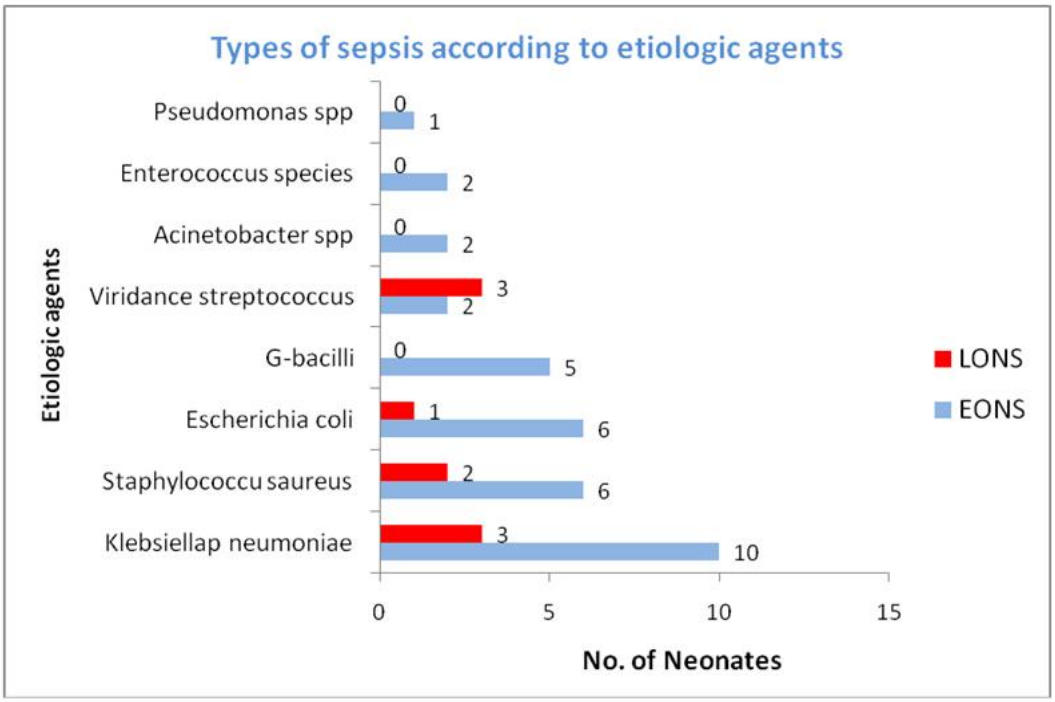

Fig. 4. Types of sepsis according to etiologic agents

Where LONS and EONS represent late-onset neonatal sepsis and early-onset neonatal sepsis respectively.

4. DISCUSSION

Although extensive research is available worldwide [29,30], very few reports are available on neonatal sepsis in Bangladesh. The present study was undertaken to evaluate glucose status, hematological parameter and etiologic agents in neonates with clinical diagnosis of septicemia

admitted at Chattagram Maa-O-Shishu General Hospital from December 2010 to October 2011. An attempt has been also made to identify the possible risk factors responsible for neonatal septicemia.

In the present investigation, it was found that early onset sepsis (EONS) was more common than late onset sepsis (LONS), which is in agreement with the reports from other developing countries e.g. in Iran (77.5% vs.22.5%) and Bangladesh (70.7 vs. 29.3%), but in contrast with reports from Saudi Arabia (39% vs. 61%) and Pakistan (42% vs.58%) where LONS is more common [31-34]. The possible explanation for a lower frequency of LONS in this study might be the early discharge tendency in the hospital.

In terms of socio economic status, it was found that neonatal sepsis occurred regardless of the economic status, however, lower class were found to be more vulnerable. Preterm neonates were found less in number considering the number found in term neonates. Around 72% neonates were delivered by vaginal delivery whereas 28% of them were delivered by Caesarean section. However, 55 among total 147 neonates had low birth weight (<2500 g) and out of these 10 were found with very low birth weight (<1500 g). Often the early signs of neonatal sepsis are non-specific, such as respiratory distress, temperature instability, difficulty in breathing, lethargy, reluctance to feed, and unexplained jaundice [35]. Clinical assessment using a combination of symptoms and signs are useful guides to provisional diagnosis of neonatal sepsis [36]. In recent years, various investigators have evaluated some markers (e.g. serum interleukin-6 (IL-6, IL-8, C-reactive protein and procalcitonin) to diagnose neonatal sepsis. Although these markers are highly sensitive and specific, they require sophisticated and expensive kits and therefore, are impractical for routine clinical work-up in community health delivery systems, particularly in developing countries. Various hematological parameters, a complete blood count, WBC count and differential, immature neutrophil count, band form count and platelet count, immature /total neutrophil count and immature/mature neutrophil count ratios individually and in combination have been evaluated for their ability to predict neonatal sepsis [37]. The white blood cell count (WBC) and differential count are useful for assessing a neonate who may have sepsis and for evaluating a neonate being treated for proven sepsis. Among white blood cell count changes, neutropenia is the most reliable predictor of neonatal sepsis; it reflects the severity of sepsis and represents depletion of neutrophil reserves, and requires specific therapeutic measures for management [38]. In the present investigation, immature/total neutrophil count ratio ≥0.16 and <0.16 was observed in 8.8% and 91.2% peripheral blood smear examined respectively. An increased concentration of immature neutrophil series cells and an immature/total neutrophil count ratio of >0.16 has been reported to have moderately increased specificity for sepsis. The immature/total neutrophil count ratio takes into consideration the normative values over the first days of life [39]. Diagnosing neonatal infection, however, is a challenge, since clinical signs and symptoms are often nonspecific for a particular infection. As a consequence, deciding whether to treat or not, balancing optimal patients care with aspects such as possible adverse events or antibiotic resistance, may be difficult. In line with this idea, the recognition of the risk factors for neonatal infections is extremely relevant in the clinical setting, since it contributes to the diagnostic reasoning and supports clinical decisions. Also, this knowledge offers the target to control strategies that may minimize the morbidity, mortality and, consequently, the high costs associated with hospital acquired infections. Our result showed that elevated CRP level is predictably associated with sepsis. Previous studies have already shown that CRP is a sensitive marker of inflammation, but it responds with a lag time of 24-48 hours. Repeated measurement of CRP level has therefore been suggested as a method to rule out sepsis, rather than a method of detection. In our study a significant difference was found for C-reactive protein Levels ($p<.05$) between the culture positive and culture negative sepsis. Most neonates in our sample had blood glucose changes, with hypoglycemia being more prevalent than hyperglycemia .Waeschle et al. [40] made a major contribution to the association between poor prognosis in septic patients and blood glucose changes, as well as the role of fluctuations between hypoglycemia and hyperglycemia during sepsis as predisposing factors for death. Blood culture to isolate the offending pathogen remains the mainstay of diagnosis for neonatal sepsis. The results of blood culture may take about a week, necessitating initial empirical treatment of suspected septicemia. In this study out of 147 neonates admitted with suspected cases of

sepsis, 43 were positive for bacterial culture. The isolation rate of bacteria in this study is comparable to rates reported in Nigeria (45.9%), India (52.6%), Pakistan (54.0%) and Uganda (37.5%) [34,36,41,42]. Lower isolation rates were reported in Ethiopia (23.1%- 27.9%) and studies conducted in other developing countries e.g. in Iran (6.6%), Bahrain (4.2%) [17,31,43]. Similar lower rates were also reported in India (14.0%-25.0%) [37].

The frequency of isolation of gram positive and negative bacteria from blood culture in this study was 34.9% and 65.1% respectively. This finding is similar to that of other studies which showed that gram negative bacteria were responsible in most cases of neonatal sepsis [31,34,37,44]. This was in contrast to other studies where gram positive bacteria were the commonest cause of neonatal sepsis [17,28,42], while another study showed that the frequency of isolation of both gram positive and negatives was equal [33].

In early onset sepsis, in the present investigation, gram positive and gram negative bacteria accounted for 29.4% and 70.6% respectively. Among gram negatives, most cases were due to *Klebsiella spp*. and *S. aureus* was the commonest gram positive organisms. In late onset sepsis, all (n=5) were gram positive bacteria; Viridans streptococcus accounted for 33.3 %(n=3), followed by *Staphylococcus aureus* 22.2% (n=2). Comparable findings have been reported in another study [45].

In general, *Klebsiella spp*. were the most common isolates (30.2%) causing neonatal sepsis in this study. Similar findings have been reported in previous studies done in Bangladesh [46] and elsewhere e.g. in Pakistan [22] Jamaica [45] India [37] and Ethiopia [43]. Study report from Dhaka Shishu Hospital revealed that principal organisms are *Klebsiella, Acinetobacter, E coli, coagulase negative staphylococci* and *Staph aureus* [47]. *Klebsiella species* have often been isolated in hospital setting and are often implicated in nursery outbreaks [48]. *S. aureus* was the second most common organism isolated in this study. Similar finding has been reported in Pakistan [44]. However, in this study, coagulase negative Staphylococci (CoNS) were not found. But recovery of CoNS from blood of septicaemic neonates needs to be reviewed with caution since most of them are regarded as contaminants. CoNS, especially *Staphylococcus epidermidis* are the major normal flora of the skin and they can contaminate blood at the venipuncture site during collection of blood [49]. Generally the spectrum of organisms causing neonatal sepsis in this study is similar to that reported from developing countries, with gram negative bacteria being responsible in most cases. But the pattern of isolated organisms in our study slightly differs from the findings in Iran and India [31,50], where *Pseudomonas aeruginosa* was the most common cause of neonatal sepsis followed by *Klebsiella spp.* and *E. coli*. In a similar study from Bangladesh, Nepal and Pakistan, *E. coli* was the leading cause of neonatal sepsis followed by *Klebsiella spp*. [34]. In other studies gram positive bacteria such as *S. aureus* and group B streptococcus (GBS) were found to be the most common isolates in neonatal septicemia [10,42].

Metabolic changes, haematological parameter and identifying risk factors are very important to identify neonatal sepsis. Identification of etiologic agents by blood culture is important for starting treatment of neonatal sepsis. The classic initial (empiric) treatment of neonatal sepsis and meningitis consists of a combination of a penicillin (benzylpenicillin, ampicillin, or cloxacillin) and an aminoglycoside (most commonly gentamicin) [30,51]. Evaluation of various parameters under this study will help to gather knowledge about neonatal sepsis and will mitigate the higher morbidity, mortality and costs.

5. CONCLUSION

C-reactive protein levels, abnormal neutrophils (high and low) showed a significant change in culture-proven neonatal sepsis. Clinical assessment using a combination of symptoms and signs are useful guides to making a provisional diagnosis of neonatal sepsis. *Klebsiella spp.* and *S. aureus* were the most common organisms causing neonatal sepsis. Metabolic changes, haematological parameter and identifying risk factors are helpful to recognise neonatal sepsis.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee

has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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