DISCUSSION
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The present study was conducted in Department of Paediatrics in collaboration with Department of Pathology; Department of Microbiology and Department of Obstetrics & Gynaecology at M.L.B. Medical College, Jhansi over a period of one year from July, 1992 to June, 1993.

The prime aim of the present venture was to evaluate the role of Micro ESR, serum C-reactive proteins and leucocyte alkaline phosphatase activity in early diagnosis of neonatal septicemia. At the same time we also evaluated the clinical, bacteriological profile and antimicrobial sensitivity pattern for neonatal sepsis. Further an attempt was also made to observe the role of above mentioned parameters in superficial infections. As shown in table - I, a total of 61 cases were selected for the study. The case material was broadly divided into study group and control group. The study group was further subdivided into three subgroups depending upon the nature of infection. Group A consisted of 14 neonates of clinically suspected septicemia with positive blood culture designated as proved sepsis, Group B consisted of 14 neonates of clinically suspected septicemia and negative blood culture designated as probable sepsis, while 18 cases of superficial infections were included in Group C.
The values of these three laboratory tests studied in proved sepsis, probable sepsis and cases with superficial infections, were also compared with 10 normal healthy neonates. It was our endeavour to observe the effect of gestational maturity on three tests both in diseased as well as healthy neonates. Accordingly 5 healthy neonates without any risk factor for development of neonatal sepsis were also included as control cases. In the light of observation depicted in table - I to XXIII following inference have been drawn and discussed in detail.

It is evident from table - II that out of 28 cases of neonatal sepsis, 9 (32%) were premature babies. The incidence of prematurity in our study is comparable to 29.44% and 21% observed by Saxena et al (1980) and Mondal et al (1991) respectively. However, Khatua et al (1980) observed prematurity in 63% of cases in their study.

A significant finding of our study regarding birth weight was that more than half of cases of neonatal sepsis were low birth weight babies as is shown in table-III viz, 50% cases of proved sepsis and 64.3% cases of probable sepsis were low birth weight babies. However, 61.1% cases of superficial infection had birth weight above 2.5 kg.
Our finding are in agreement with the observation of Philip et al (1980) and Sinha et al (1986), who reported the incidence of low birth babies as 58.24% and 64.9% in proved sepsis respectively. However, Boyle et al (1978) found no significant difference in birth weight of infected as well as control babies. A higher incidence of low birth weight babies presenting with deep infection reported by us as well as by others, suggests that these babies are more prone to infection because of depressed immunological, chemotactic and phagocytic functions as compared to normal weight babies.

The fact that neonatal septicemia is more common among males is a well documented fact which has also been proved by us in the present study. We observed that 78.5% cases in proved sepsis were male. One more interesting fact that is also shown in table 4 IV regarding the sex distribution was a higher percentage of males in the group with proved sepsis (78.5%) as against 64.3% and 50% in the probable sepsis group and group of superficial infections respectively. Among other workers Philip et al (1980), Saxena et al (1980) and Khatua et al (1980) reported that 59%, 59.17% and 70.7% of cases in their series were male. A higher incidence of septicemia in males as observed
by us and other is explained on the basis that factors regulating the synthesis of gammaglobulin are probably located on X chromosome. Presence of one X chromosome in male infant thus confers less immunological protection compared to its counterpart. Apart from this, the socio-cultural outlook of our community wherein the male babies are given better care and attention might also be playing a big role in making the incidence high in favour of male babies.

It is evident from table - V that, 78.5% babies of proved sepsis and 64.3% cases of probable sepsis in our study were below ten days of age. A higher incidence of neonatal sepsis below 10 days of age is easily explainable because of depression of immunological profile in early postnatal period than in the late neonatal period.

Just like a majority of other studies on neonatal septicemia, the clinical features, remained vague, non specific and subtle in the present study also. As shown in table-VI refusal of feeds seen in 82% of cases was the most common and consistant presenting symptom, whereas, lethargy observed in 64.2% cases was commonest clinical sign in our study. Saxena et al (1980) reported refusal of feeds in 70.41% cases and lethargy in 53.66% cases,
while Kalra et al (1985) found these features in 86.6% and 71% cases respectively; Chandna et al (1988) in their study documented low incidence of refusal of feeds (46%) but observed lethargy in 70% of cases. Temperature changes were found in 50% of suspected cases of neonatal sepsis in our study. Fever reported in 32.2% cases of sepsis by us, is comparable to 30% observed by Guha et al (1978) and 24.8% respected by Khatua et al (1986). In contrast to us few studies like Saxena et al (1980) and Chandna et al (1980) observed hyperthermia only in 6% and 14% cases respectively. Four out of five babies who had hypothermia were premature and incidence reported by us was 17.8%. This incidence was nearly equal to 18.18% and 12% reported by Ronney et al (1971) and Chandna et al (1988) respectively. However, studies by Khatua et al (1986) and Mondal et al (1991) reported incidence of hypothermia as high as 46.8% and 50% respectively. Irregular fluctuations in temperature have also been described as an important feature of neonatal sepsis by Krugman and Ward (1973). One premature baby of 30 week gestational age, developed sclerema and died. This supported the observation of other workers like McCracken and Schine field (1966) that sclerema always carried a bad prognosis.
Positivity of blood culture reported by various Indian workers varies from 30-75%. In the present study (Table-VII) microrganism were isolated in 50% of clinically suspected cases of neonatal sepsis. Namdeo et al (1985) and Chandra et al (1988) like us also reported positivity in 50% and 48% cases respectively. Relatively high positivity were reported by Mittal et al (1980) and Mehrotra et al (1985) as 60% each, however, Chaudhary et al (1975) and Bhakoo et al could isolate microrganism only in 30.6% and 32% cases respectively. The relatively low incidence of bacterial isolation in their study might be due to clinical over diagnosis to err on the right side giving the patient the benefit of doubt, prior administration of antibiotics and other technical snags.

We could isolate only two microrganism viz, staph. aureus in 57.2% cases and E coli in 42.8% cases from blood culture, in the ratio of 4 : 3 (Table - VII). Like us, Somu et al (1978), Saxena et al (1980), Mishra et al (1985) and recently Mondal et al (1991) are the workers to report that staph. aureus is the commonest bacterial isolate in neonatal sepsis. E coli which was the second isolate and detected in 42.8% cases by us was reported as commonest organism by certain others Indian workers viz, Bhakoo et al

We also observed profile of superficial infections and found that umbilical sepsis was the commonest manifestation which was present in 50% cases alone and in 16.5% cases alongwith other superficial infections, viz, pyoderma and conjunctivitis (Table VIII). Our report in this aspect is similar to that of Taneja and Ghoshroy (1961), who also observed umbilical sepsis as commonest superficial infection. Contrary to these reports Chaudhary et al (1975) reported that pustules and conjunctivitis were more frequent in their study. The workers also opined that low incidence of umbilical sepsis in their study might be due to application of gention voilet in majority of cases.

As shown in table-IX, staph. aureus was isolated in 31 out of 32 (97%) cases of superficial infection and 16 out of 17 (94%) cases of umbilical sepsis by us.
Chaudhary et al (1975) were the only workers in the recent past to report the incidence of different bacteria from different site and isolated staph. aureus in 96% cases of superficial infection and 100% cases of umbilical sepsis, which was nearly equal to present study.

William (1969), Chaudhary et al (1975), Aiyenger et al (1991) were of the opinion that poor handling of baby by uncleaned hand, poor sterilisation, nursery colonisation and delivery and followup in unclean environment are some of the factors which increase the incidence of staphylococcus aureus. The greater incidence of this bacteria in present study might be due to association of above mentioned factors.

In table-X, we made an attempt to observe the sensitivity pattern of commonly used antibiotics and also tried to evaluate the efficacy of newer third generation cephalosporins. High resistance to ampicilin reported by many Indian workers, also confirmed by us, is a serious matter because it has been used for first line of treatment in majority of centers in our country. Sensitivity for this drug in 53% cases observed by us is comparable to 64% reported by Gluck et al (1964) and 36% each by Nicolo- poulous et al (1970) and Shakoo et al (1974).
Efficacy of Gentamicin, the second commonly used antibiotic was observed to be definitely better than Ampicillin, as it was sensitive in 75% cases of staph. aureus, which was in agreement to the observation of Mishra et al (1975) and Monga et al (1986) who found it to be sensitive in 70% and 75% cases respectively. Similarly for E. coli the sensitivity observed by us was 83.3%, which was also found to be in close approximation to 80% by Mishra et al (1985) and 84.4% by Monga et al (1986). Another significant finding amongst the sensitivity pattern of antibiotics particularly aminoglycoside was that the newer antibiotic amikacin is in no way better than the conventional and time trusted Gentamicin for treatment of neonatal sepsis.

Guha et al (1978) reported few important and interesting fact about Gentamicin. In their study some of the septicemic babies treated with this drug initially showed improvement but after a course of 7-8 days, signs of deterioration in form of not accepting feeds, weight loss, ashen gray colour was observed and these babies died ultimately. The workers failed to explain the cause of death as these babies showed initial improvement with negative blood culture subsequently. Another fact, that
had been reported by them was that neonates with septicemia due to staphylococcus aureus and treated by Gentamicin had multiple abscess in various organs on autopsy. The workers, therefore, concluded that although Gentamicin possesses good activity against staph. aureus in vitro, its role in the treatment of neonatal septicemia is doubtful. One important finding of our study regarding antimicrobial sensitivity pattern is the high efficacy of newer cephalosporins. We found ceftriaxone to be sensitive for 87.9% cases of staph. aureus and 100% cases of E coli while sensitivity of cefataxime for these two bacteria was 87.5% and 66.6% respectively.

Superiority of third generation, cephalosporins viz, ceftriaxone and ceforanxime in the treatment of neonatal sepsis as reported by us is in agreement to findings of Chugh et al (1989) and Bhave et al (1989). Anand et al (1990) emphasized the need for judicious use of third generation cephalosporins to prevent emergence of resistant organisms, while Gurmeet et al (1990) suggested frequent change in routinely used antibiotics from time to time to check the development of resistance.

The values of micro ESR which is a simple, inexpensive test requiring only few drops of capillary blood have been shown in table - XI. As evident from table XI,
we observed elevated values of micro ESR in 76.5% cases of proved neonatal sepsis. This observation was similar to 71.4% reported by Parida et al (1980), 80% reported by Namdeo et al (1985), 74% reported by Singh et al (1986) and 73% by Mishra et al (1989) in proved cases of neonatal sepsis. we observed raised value of micro ESR (710) in 64.3% cases of probable sepsis which is comparable to 62% that has been reported by Singh et al (1987) in this group. However, contrary to the our study Parida et al (1980) and Mishra et al (1989) reported elevated values in significantly lower percentage of cases viz, 24% and 22% respectively in cases of probable cases of neonatal sepsis. On the other hand micro ESR was raised only in 38.8% cases of superficial infection in present study.

No other worker like us evaluated the role of this parameter in superficial infections and hence a comparison could not be done.

In table XII, micro ESR values were statistically analysed in different groups of infection. We observed mean values of micro ESR as 15 ± 7.36 in proved sepsis and 12.85 ± 8.44 in probable group of infection. Statistically the values were found to be highly significant in proved sepsis (P<.001) and significant in probable sepsis (P<.05)
when compared with control group. Sharma et al (1973) were the only workers to have elaborated their observations statistically like us and reported micro ESR values as $18.2 \pm 8.8$ and $19.3 \pm 13.3$ mm respectively in culture positive and negative cases of neonatal sepsis. Our statistical analysis is in agreement with that of Sharma et al (1993), who also observed elevation in the values of micro ESR in cases of neonatal sepsis which were found to be statistically significant ($P \leq 0.001$) when compared with values observed in normal healthy control babies. Like the present study, these workers too, reported no difference between culture positive and negative cases, values being statistically insignificant ($P > 0.05$).

In table XIII and XIV we also tried to see the effect of gestational maturity on micro ESR. We thus, analysed these values separately among preterm and full term babies of all the three study group as well as control cases, but observed that the difference was statistically insignificant. So our study concluded that gestational maturity per se has no effect on micro ESR values. Evans et al (1978), like us also reported that no correlation exists between gestational age and micro ESR value.
C-reactive protein is an acute phase reactant, which is elevated in any acute inflammatory conditions. Its elevation and return to normal level once the infection is controlled, is a matter of few hours. This response is quicker than other acute phase reactants like fibrinogen, heptoglobulin and alpha one glycoproteins etc. As shown in table - 2V positive CRP test was seen in 64.2% cases of proved sepsis and 71.5% cases of probable sepsis. Vyas et al (1985) reported positivity for proved and probable cases of sepsis as 82.5% and 46% respectively. Singh et al (1987) reported these values to be 80% and 94%, while Chandna et al (1988) observed these values as 83.3% and 57.7% for above mentioned two groups. In our institution a study by Sethi et al (1990) reported positive CRP test in 100% cases of confirmed deep bacterial infections. Vyas et al (1985) in addition, also observed significant difference in CRP values of proved sepsis and probable sepsis, which could not be distinguished by this test in present study.

In superficial infection, however, raised serum CRP was observed only in 27.8% of cases which is markedly less than the value observed for the proved and probable cases of sepsis. Vyas et al (1985) and Sethi et al (1990),
were the workers, who evaluated this test in superficial infections and found it to be positive in 42% and 50% cases respectively.

In our study false positive cases i.e. percentage of detection of CRP in normal healthy control was 10%, which is comparable to that which have been reported as 8% by Vyas et al (1988) and 9% by Singh et al (1987).

We also made an attempt to observe CRP positivity separately in full term and preterm babies (table XVI and XVII). It was seen that the percentage of detection was similar in different study groups of full term and preterm babies, proving that serum CRP is probably independent of gestational maturity.

Leucocyte alkaline phosphatase activity determined by cytochemical method is increased in bacterial infections of adult but little work has been done concerning LAP activity in neonatal infections. Donato et al (1979) were first to describe its importance in neonatal infections. The leucocyte alkaline phosphatase activity may differ from laboratory to laboratory in addition to an objective criterion of assessment, however, normal score observed by us among healthy neonates was 91–154 with a mean 128.5 ± 19.67, which was significantly higher than that observed
in cases of proved sepsis (102.86 ± 16.53) and probable sepsis (109.07 ± 14.98), as is evident from table—VIII. It was found, that the decrease in the LAF value was also statistically highly significant (p≤.01) for proved sepsis and significant for probable sepsis (p≤.05) when compared with normal healthy control.

Our study on this aspect was in complete agreement with various other studies. Donato et al (1975) demonstrated, that newborn infants with severe bacterial infection had low LAF score (114 ± 16.6) as compared to healthy control babies (216± 26.9). In their study this difference was also found to be statistically highly significant (P<.001). Sharma (1980) and Sharma et al (1983) using the same method also observed significantly low LAF activity in severely infected neonates of the same age group (F value ≤.001 and ≤.05 respectively).

Sharma et al (1985) studied LAF activity separately in preterm and full term babies and compared the values in normal babies to the values observed in confirmed bacterial infections and probable infection in two different gestational age group. Their findings revealed that decreased LAF activity in both preterm and term babies in proved as well as probable sepsis was found to be statistically significant when compared with control cases (P<.000). However, contrary to above report we could find these values statistically significant in only proves sepsis in the full
term gestational age group and proved as well as probable sepsis in premature babies (table AIX and XX).

Trubowitz et al (1957) observed that bone marrow neutrophils had 50% less LAP activity than do circulating older neutrophil. Physiologically and chronologically older neutrophil contain more leucocyte alkaline phosphatase enzyme than young polymorphs. Within the morphological boundaries of segmented polymorphonuclear neutrophil, the cells are still in stage of cytoplasmic maturation and LAP activity inside these cells increases with time. The LAP activity might therefore, be a cell maturation index. Based on such observations the finding of decreased leucocyte alkaline phosphatase scores in septicemia of newborns could be explained by rapid release of functionally immature neutrophil by bone marrow. Bandue et al (1980) in their study opined that raised level of steroid present in various diseased states, probably play some role in increased LAP activity in these conditions. In infected neonates, however, the level of steroids is usually normal or even increased due to increased endogenous secretion, because of acute stress, thus decreased LAP activity found in infected neonates is not due to steroid effect and a cause effect relationship can be ruled out.
Contrary to our study and above mentioned reports, Paul and Kumar (1964) reported high LAL activity in neonatal infections. They explained that low scores, obtained by other workers might be due to inclusion of more premature babies in the study group in whom the LAL score is distinctly lower. However, the results of our study contradicted their explanation. We observe, that there was no significant difference in values of LAL activity of preterm and full term babies in the study group of cases as well as in the control group (P 0.05). In this regard our finding was also in contrast to the fact that have been reported by Halbrecht and Chabtay (1972) who observed low LAL activity in prematures. They considered LAL activity as an important criterion for prematurity.

We also observed LAL activity in cases of superficial infection and obtained a score of 122.94 ± 21.26, which was found to be statistically insignificant (P 0.05), when compared to the value observed in control group. Sharma et al (1985), were the only workers, who like us, studied this test in superficial infections and observed similar results as ours.
In table - XXI we analysed the efficacy of our diagnostic parameters in proven cases of neonatal sepsis. Sensitivity of a test refers to diagnose a disease when it is present, whereas, specificity means ability of a test not to diagnose a case when disease is absent. We observed micro ESR as 78.60% sensitive and 80% specific for proved sepsis, which is in agreement with the observation of Mishra et al, who observed 79% sensitivity and 83% specificity for micro ESR. However, Namdeo et al (1985), observed sensitivity and specificity as low as 68% and 69% respectively, while Singh et al (1987) reported 81% specificity which is also comparable to that which has been observed by us, however, sensitivity reported by them was quite low as 55%. We also analysed our finding in terms of positive predictive accuracy (that is the probability of presence of disease, whenever, the test is positive) and negative predictive accuracy (probability to rule out disease when test is negative). Positive predictive accuracy observed by us was 84.6%, while Namdeo et al (1985) and Mishra et al (1989) reported it, value as 63% and 73% respectively. The negative predictive accuracy for micro ESR as observed by us was 72.7% in cases of proved sepsis. However, Mishra et al (1989), who were the only worker to analyse this statistical test reported its value as 94%.
For C-reactive protein the specificity in proved sepsis as reported by us was 90%, which is in complete agreement with the reports of Singh et al (1987), who reported 91% specificity in their study. Sensitivity observed by us for C-reactive protein was 64.26% which was found to be lower than 80% and 53% reported by Singh et al (1987) and Chandra et al (1988) respectively. No other worker analysed negative and positive predictive accuracy of CRP in recent past so the values of these two statistical test for CRP reported by us as 90.0% and 64.3% respectively could not be compared with others.

Singh et al (1987) like us, were the only worker in the recent past who compared these two tests viz, micro-ESR and C-reactive protein and reported sensitivity and specificity of these parameters. Their report, that out of two tests, CRP is more specific and sensitive is contrary to the findings observed by us. We observed that CRP is more specific to diagnose cases of neonatal sepsis, but its sensitivity is found to be lower than that of micro ESR.

We also analysed our data in all clinically suspected cases of septicemia (proved + probable) and thus tested the efficacy of our laboratory test in the cases where sepsis is proved by blood culture or where blood culture is
negative even in presence of strong clinical suspicion (might be due to prior administration of antibiotics or technical snags). We observed that micro ESR was more sensitive but less specific than CRP test in this group. It also had lower positive predictive accuracy, however, negative predictive accuracy for micro ESR and C-reactive protein was the same (table-XXII). No other workers in past have analysed their data in all clinically suspected cases of septicemia like us, hence a comparison could not be made.

The role of these diagnostic tests were also evaluated in cases of superficial infections. We observed low sensitivity of 38.9% for micro ESR and 27.8% for CRP test in superficial infections. So our findings revealed that both micro ESR and CRP had low efficacy to detect superficial infections. Similarly low negative predictive accuracy for micro ESR (42.1%) and CRP (40.9%) observed by us suggests that both these tests have poor chance to rule out superficial infection in absence of positive micro ESR or CRP test. However, specificity observed by us for Micro ESR and CRP were 80% and 90% respectively and positive predictive accuracy were 77.7% and 83.3% respectively.
No workers in the past have evaluated the role of these test in superficial infection in terms of above mentioned statistical analysis and hence a comparison could not be done.