DISCUSSION
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The present study has been carried out to study the immunological profile in 80 newborn babies, delivered in M.L.B. Medical College, Jhansi, over a period of one year. Our study group comprised of 20 cases each of low birth weight newborns, neonates with hyperbilirubinemia and those suffering from neonatal infection. An equal number of full term healthy normal weight healthy newborns delivered vaginally acted as control for the present study. The primary aim of our study was to evaluate the humoral immunity in all the study groups and compare these values with those observed in control group of cases.

An attempt was also made to assess the complement activity by measuring the $C_3$ levels in all the groups of cases which hitherto has not been attempted by many workers in the recent past. Besides evaluating the humoral immunity and complement activity, a thorough physical examination was done in each and every case to categorise the newborns in our study groups. Based on the observations depicted in table 1 to 13, various inferences has been drawn and discussed in details herewith.
Of the total 80 newborn babies selected for the present study, there were 52 male, while the rest 28 were female. As is evident from table 2, males predominated over the females in the low birth weight, as well as the group of cases of neonatal hyperbilirubinemia. Since the weight of the baby has a direct impact on the immunological status, care was taken to select only full term normal weight babies in all the groups except the group of low birth weight babies. The gestational age in our study was assessed by the morphological and neurological characteristics and tallied with the history of last menstrual period as given by mother. Immunoglobulin estimation was done by the method of single radial immunodiffusion technique of Mancini et al (1965).

**Immunological profile in Control group of cases**:

Serum IgG levels in the control group of cases had mean value of $1365 \pm 453.41 \text{ mg}\%$ with range of 1050 - 2500 mg\% while the serum IgM values had a mean of $22.70 \pm 4.76 \text{ mg}\%$ with a range of 17 - 28 mg\%.

Amongst the 20 control group of cases there were 8 cases having gestational age of 38 weeks, 2 cases of 39 weeks whereas 10 cases were of 40 weeks. On evaluating the IgG levels in these sub-groups of control group of cases a significant finding observed was that cases having gestational age of 40 weeks had mean values of $1610.00 \pm$
549.31 mg% which was found to be highly significant from the mean values \((1137.5 \pm 110.86 \text{ mg\%})\) observed in the 8 cases having gestational age of 38 weeks \((P \leq 0.05)\). Our observation of the IgG levels therefore amply demonstrate that there is a linear correlation between increasing gestational age and the increasing levels of Immunoglobulin IgG. However, no such linear correlation of the increasing gestational age to the IgM values was observed in our study.

The immunoglobulin IgG values have been determined by various workers in the past two or three decades. Our values of \(1365 \pm 453 \text{ mg\%}\) in control group of cases are in conformity and more or less similar to the values observed by many other workers in the field (Hobbs and Davis, 1967, 1220 mg\%, Evans et al, 1967, 1088 mg\%, Sethi et al, 1980 1162.58 \pm 137.43 mg\%, Hariharan et al, 1984, 1259 \pm 51 mg\% and Kolhatkar et al, 1987 – 1377 mg\%). However, few workers have reported much lower values in control group of cases viz. Yeung et al, 1968 (879 mg\%), Prasad et al, 1971 (446.6 \pm 20.4 mg\%), Malik et al, 1977 (253.6 \pm 137.5 mg\%).

Kaur et al (1979) and Sharma et al (1986) are some of the workers to have reported higher values (1478.5 \pm 305.71 mg\% and 1402 \pm 132.3 mg\% respectively).

The exceptionally low levels of IgG in full term healthy control babies observed by Prasad et al (1971) and Kaur et al (1979) has been attributed by these workers to a
concomitently lower levels of IgG in the mothers of these babies. The view, by many of these workers (Hobbs & Davis, 1967; Yeung et al, 1968; Evans et al, 1971; Sethi et al, 1980; Tandon et al, 1984 and Bhatia et al, 1987), that there is a direct correlation of increasing IgG to the gestational age and birth weight has been substantiated in our study as has been discussed earlier.

The pattern of immunoglobulin IgM in full term neonates as observed by various workers has varied markedly in different series. As with IgG, only few workers viz. Hardy et al (1969) and Prasad et al (1971) have reported more or less similar IgM values of 20 and 17.88 mg% respectively. Most of the workers (Steihm & Fundenberg, 1966; Malik et al, 1977; Kaur et al, 1979; Khatua et al, 1984 and Sharma et al, 1986) have reported much lower values than that observed by us. The difference in the IgM values as observed by various workers may be attributed to the difference in the type of sample studied as well as the method of detection. No linear correlation of IgM values to the increasing gestational age and birth weight was observed by us or by any other worker in the field.

Immunological profile in low birth weight babies -

Twenty low birth weight babies which included 10 premature and 10 full term small for date babies were taken up for immunological assessment. Irrespective of the
sub-groups, the mean IgG and IgM values were 1050.25 ± 515.00 and 30.05 ± 15.02 mg% in our study, respectively. It was seen, that two cases weighing less than 1000 gms and having a gestational age of 30 weeks had the minimum values of immunoglobulin IgG (450 mg%) and IgM (11 mg%). On comparison of the IgG and IgM values observed in low birth weight babies to that of our control group of cases, it was seen that the IgG values were lower while the IgM values were higher, values in both being statistically significant ($P_G \leq 0.05$ and $P_M \leq 0.05$).

The decrease in immunoglobulin IgG in low birth weight babies in our study is mainly accounted by the premature babies in the low birth weight group, while the increase in the immunoglobulin IgM in low birth weight group babies when compared to the control is mainly accounted by rise of immunoglobulin IgM in IUGR group of babies. Amongst the low birth weight babies, it was observed (Table 3) that premature babies had mean values of IgG and IgM 800.50 ± 232.38 and 18.1 ± 7.30 mg% while IUGR babies had mean values of IgG and IgM 1300.00 ± 501.24 mg% and 42.00 ± 9.88 mg% respectively. As has already been mentioned, the two most premature babies of 30 weeks gestational age having birth weight of 800 gm each, manifested the least values of serum IgG. It is evident from our observation that the premature babies manifested a highly significant decrease (800 mg%) as compared to the control values (1365.00 ±
453.41 mg%), values were found to be highly significant \( P \leq 0.01 \). However, no statistically significant difference was observed in the IgG values in the IUGR babies and the control group \( P_g > 0.05 \). Since the major portion of the IgG of the newborn is derived transplacentely from the mother continuously during the third trimester of pregnancy, the decrease in level of this class of immunoglobulins in premature babies can be accounted for the shorter period of gestation available in these neonates for the transfer of this immunoglobulin.

The IUGR babies did not show any significant difference in the IgG levels from the control group of cases, possibly due to the fact that all our IUGR babies were having mild intra-uterine growth retardation. Similarly on analysing the IgM values it was observed that premature babies demonstrated lower levels of immunoglobulin IgM while IUGR babies manifested with highest level of IgM, both these values were found to be statistically significant from that of control group of cases. The rise of IgM in IUGR babies is easily explainable since all these babies were an outcome of deliveries in which the mother had some systemic disease or infection.

Umpteen workers in the past have evaluated the humoral immunity in the low birth weight babies and practically all of them are of the opinion that premature babies manifest with decreased level of IgG, while the IUGR
Various workers have studied the levels of immunoglobulin IgG and IgM in premature and small for date infants separately. In our study, value of immunoglobulin IgG in premature babies (800.5 mg%) was found to be more or less similar to the values obtained by other workers in the field (Evans et al, 1971 - 850 mg%; Singh et al, 1978 - 691.92 ± 242.4; Kaur et al, 1979 - 829.66 ± 174.09 and Tandon et al, 1984 - 708 ± 195.3 mg%). Few workers have reported much lower values in premature babies viz. Yeung & Hobbs, 1968 - 512 mg%; Prasad et al, 1971 - 467 ± 85.16 mg% and Sethi et al, 1978 - 478.2 ± 70.71 mg%; Raghvan et al, 1976 - 1366.6 ± 792.8 mg%; Shapiro et al, 1981 - 1363 ± 357 mg% and Sharma et al, 1986 - 1267.3 ± 84.0 mg% are the few workers to have reported higher values.

The decrease in the IgG levels in premature babies, in our study as well as the values reported by others, (as has already been suggested) is due to the decreased transplacental passage of immunoglobulin IgG in the last trimester of pregnancy. The workers who have reported higher values of IgG in premature babies than us too, had significantly low levels of IgG as compared to their control, which had nearly double the values (2028.8 ± 1100.0 mg%).

A significant finding in our study was that each and every premature baby had measurable immunoglobulin IgM, which is in contrast to some other workers who have
not reported measurable levels of IgM in all the premature babies (Kaur et al, 1979; Tandon et al, 1984 and Sharma et al, 1986).

Our observations of low IgM values in premature babies as compared to our controls has been substantiated by other workers too, viz. Prasad et al, 1976 - 6.6 ± 3.6 mg%, Raghvan et al, 1976 - 10.04 ± 7.51 mg%, Singh et al 1978 - 12.5 ± 12.32 mg%, Sharma et al, 1986 - 7.4 ± 3.9 mg%. This observation goes to prove that immunoglobulin IgM has got no correlation to the gestational age as has also been mentioned by these workers.

Amongst the IUGR babies as has been mentioned earlier, no significant difference was observed in the IgG values as compared to our control group of cases. Our values of 1300.00 ± 501.24 mg% in IUGR babies are in conformity to those of some workers (Singh et al, 1978; Kaur et al, 1979; Shapiro et al, 1981; Tandon et al, 1984 and Sharma et al, 1986), while few workers have reported low values than us viz. Sethi et al, 1980 and Khatua et al, 1984.

Although in our study we did not categorize cases of IUGR into mild (≤10th percentile), moderate (≤ 3 to 10th percentile) and severe (≤ 3rd percentile) IUGR groups, Kaur et al (1979) and Tandon et al (1984) having done this, reported significantly low values of IgG from the controls only in the severe IUGR group of cases. The mean
level of IgM obtained in our study in IUGR babies was 42.00 ± 9.88 mg% which is significantly higher than the value obtained in control (22.70 ± 4.76 mg%) group of cases (P < 0.01). Value observed by us, in our series is much higher as compared to the values given by other workers, viz. Singh et al (1978) - 13.6 ± 6.55 mg%, Kaur et al 1.76 ± 4.7 mg% and Khatua et al (1984) - 12.78 ± 24.36 mg%.

The difference in the IgM values as observed by various workers can be due to the type of sample studied as well as the method used for the assessment of the immunoglobulin. Higher mean values of IgM in IUGR babies in our study was possibly due to intra-uterine infection in these cases which triggered and heightened the levels of IgM in these cases.

**Immunological profile in cases of Neonatal Hyperbilirubinemia**

Twenty neonates having bilirubin level between 10.8 mg% and 30 mg% were selected for the present study. The mean IgG and IgM values were 1339.25 ± 319.85 mg% and 50.60 ± 14.30 mg% respectively in our study.

It is evident from our observations (Table 4) that infants with hyperbilirubinemia did not reveal any significant alteration in the mean serum IgG values, as compared to control group of cases (P < 0.05).

Contrary to the mean IgG values, the mean value of IgM was higher (50.60 ± 14.30) in the neonatal hyper-
bilirubinemia group as compared to the control group of cases (22.70 ± 4.76 mg%) values being statistically significant ($P_M \leq 0.01$).

Very few workers have assessed the humoral immunity in cases of neonatal hyperbilirubinemia. However, Ansaldi et al (1968) found a slight decrease in the levels of IgM levels in neonates with serum bilirubin levels above 16 mg%. However, Mantalenaki et al (1975) did not find any significant difference in the levels of immunoglobulins IgG and IgM between hyperbilirubinemic infants and controls. Sethi et al (1989) have been the only worker in the recent past to have recorded values of immunoglobulin IgG in cases of neonatal hyperbilirubinemia. Their values of 995.87 ± 163 mg% were more or less similar to the IgG values in our study. The rise of IgM in cases of neonatal hyperbilirubinemia could possibly be because of associated infections in many of our cases, which is substantiated by the fact that highest values of IgM were found in severe neonatal hyperbilirubinemia which are more prone to infection. Since there is paucity of data in the humoral immunity in cases of neonatal hyperbilirubinemia, no comparison could be done in this regard.

Thus in nutshell we see that hyperbilirubinemia per se has no effect on the immunoglobulin IgG, though the values of IgM may be increased due to associated infection. However, further work has to be done to substantiate our
Immunological profile in Neonatal Infections group -

Twenty newborn infants suffering from various infections were selected for the present study. Care was taken to select only full term normal infants to exclude the detrimental effects of prematurity or fetal malnutrition on the immunological apparatus of the studied cases. All the cases were subjected to various tests for the assessment of serum IgG and IgM in neonates having infections. The mean IgG and IgM values were 865.00 ± 97.32 mg% and 66.05 ± 18.36 mg% respectively in study conducted by us. It was seen, that, whereas the cases of neonatal infection had lower values of IgG as compared to the control (P ≤ 0.01), values of immunoglobulin IgM were found to be much higher as compared to the control group of cases (P ≤ 0.01). This significant finding of decrease in IgG and increase in serum IgM in cases of neonatal infection is easily explainable on the basis, that depression of IgG is the cause of neonatal infection whereas the rise of IgM is effect of infection.

The mean IgG value in our study (865.00 ± 97.32 mg%) was much higher as compared to the value obtained by Prasad et al (1971) - 479.8 ± 32.4 mg% and Malik et al 243.6 ± 107.3 mg%, though unlike us, these workers did not observe a decrease in IgG values as compared to the control group of cases.
The findings of raised levels of immunoglobulin IgM in response to infections have been uniformly observed by several other workers viz. Alford et al (1967) - 13 - 130 mg%, Hardy et al (1969) - 30 mg%, Khan et al (1969) - 55 mg%, Prasad et al (1971) - 39.2 ± 8.36 mg% and Malik et al (1977) - 98.7 ± 58.7 mg%. The elevated levels of immunoglobulin IgM as a response to infection is possibly an exaggeration of the normal response to a myriad of antigens in the extra-uterine environment.

**Complement C₃ activity in various study groups**

Complement C₃ was assessed in all 80 newborn babies selected for the present study. As is evident from Table - 6, the mean C₃ value in control group of cases was 51.40 ± 18.76 mg%. Tandon et al (1984) have reported nearly similar values of complement C₃ (51.50 ± 14.94 mg%) as reported by us. However, Kaur et al (1979) and Shapiro et al (1981) have reported a higher mean value of complement C₃ in their control group of cases (124.72 ± 44.62, and 90 ± 18 mg%) respectively. In the low birth weight babies, the mean serum complement C₃ levels was 42.55 ± 6.59 mg% which was found to be statistically insignificant from control group values (P > 0.05). A significant finding in the complement activity of the low birth weight babies was, that premature babies had statistically significant lower values (P < 0.05) as compared to the controls, whereas the values of C₃ in IUGR
group had no statistical significance (p > 0.05) from the control group of cases. The depression of complement activity in premature babies is well documented fact in literature, which accounts for one of the factors enhancing infection in the premature babies because of depressed complement activity.

Very few workers have assessed complement C₃ in low birth weight babies. Tandon et al (1984) have reported nearly similar values in premature and IUGR babies as observed in our study (33.8 ± 11.18 mg% and 47.5 ± 19.75 mg%). However, Kaur et al (1979) have reported much higher values of complement C₃ in premature babies than our values, though like us their values in premature babies too were much low than the values in their control group of cases. Kaur et al (1979) like us, too have opined, that pre-term babies have lower levels of complement in proportion to their immaturity and since complement plays a role in the heat labile opsonic system and enhances phagocytosis of organisms, a depression of this factor of immune response predisposes the premature baby to greater infection. Another fact highlighted by our observation was, that IUGR babies had more or less normal complement activity, akin to that observed in full term babies.

Complement activity which was assessed in all the 20 cases of neonatal hyperbilirubinemia had mean value of 70.45 ± 23.65 mg% which was found to be higher and
statistically significant from the values observed in the control group of cases ($P_C \leq 0.01$). No study till date has been found with regards to the complement activity in cases of neonatal hyperbilirubinemia. The rise of complement activity in these cases is not easily explainable since some of the cases of neonatal hyperbilirubinemia were also having infection but Cooper et al (1967) have reported that complement activity may be normal or elevated earlier in the disease and declines in the late terminal stages of the infection. Why there was a rise of complement activity in hyperbilirubinemia remains still to be answered and further work has to be done to elucidate the effect of hyperbilirubinemia on the complement activity.

It is evident from Table - 6 that $C_3$ complement levels in the neonates having infections ($34.60 \pm 6.87$ mg%) were significantly lower as compared to the non-infected control group of cases ($P_C \leq 0.01$). Few workers in the past have also reported low complement $C_3$ activity in the infected newborns (R.P. Singh, 1986).

A decrease of complement activity in infected newborns has been explained on the basis of consumption of various components of the complement system in various infections.
Immunological profile according to severity of neonatal hyperbilirubinemia -

In our study we also tried to find out a correlation between different serum bilirubin levels to the changes in the immunoglobulin and the complement C₃ activity in cases of neonatal hyperbilirubinemia. Accordingly, cases of neonatal hyperbilirubinemia were sub-grouped into three categories depending on the serum bilirubin levels (Table - 7).

A significant finding of our study was that there was an inverse correlation between the increasing serum bilirubin levels to the decrease in the serum IgG as well as complement C₃ activity in cases of neonatal hyperbilirubinemia, values being maximally decreased (991.66 ± 218.42) in cases with serum bilirubin above 20 mg%. However, on statistical analysis no significant difference was observed in the various sub-groups, as described in table - 7, for both immunoglobulin IgG and complement C₃ values (P₀ & P₀ 7 0.05). On the other hand a direct correlation was observed in serum IgM values with increasing severity of jaundice, values being highest (53.34 ± 15.68 mg%) in those cases having serum bilirubin more than 20 mg%.

Besides our best efforts we could not compare our findings due to paucity of the data in this field.
Immunological profile of neonates having infection according to the severity and causative organism -

An attempt was also made in our study to observe a correlation of the severity of neonatal infection, to the immunological profile of the newborn babies. It is evident from our observations (Table - 10) that whereas serum IgG values decreased with increasing severity of neonatal infection, the values of IgM on the contrary increased with severe infection. Neonatal septicemia with multiple pyemic abscesses, the most severe form of neonatal infection in our study, recorded the lowest values of IgG and the highest values of immunoglobulin IgM. However, cases of umbilical sepsis, the milder form of neonatal infection recorded higher values of immunoglobulin IgG and low levels of IgM. As has been explained earlier, a fall and rise of IgG and IgM respectively are the cause and effect of neonatal infection having a direct bearing on the severity of the neonatal infection.

Since none of the worker in the past have evaluated the IgG level in cases of neonatal infection, a comparison to our values could not be ascertained. Khan et al in 1969 have however reported high values of immunoglobulin IgM in 88% cases of meningitis and pneumonia.

Highlight of the present study in neonatal infection was, that staphylococcus aureus was found to have a greater
impact on the humoral status of the newborn baby as compared to the E. coli infection. It is evident from table - 12 that cases of staphylococcal infection had a more profound alteration of the humoral immune system as manifested by a significant decrease of immunoglobulin IgG when compared to an equal number of cases of E. coli infection ($P < 0.01$) and a greater rise of immunoglobulin IgM than that observed with E. coli infected cases. However, further work has to be done in greater number of cases to substantiate these findings.

Complement activity however was found to have no significant difference as regards the nature or the causative organism of the neonatal infection.