DISCUSSION
DISCUSSION

Malaria continues to be an important health problem in most of the countries of South East Asia Region and requires a flexible epidemiological approach with available resources. However, the success in integrated malaria control could be seriously impeded without a sound knowledge of local epidemiology of malaria. Since during past three decades, socio-economic status as well as the habitats and eco-systems of malaria vectors and parasites, deforestation, flooding, irrigation, the green revolution, vectorial efficiency of anophelines and their sibling species and uncontrolled population movements have also had a significant impact on malaria epidemiology (T.R.S. 347).

The serology of malaria has a long and varied history. Only in the last few decades, new serologic techniques such as the indirect fluorescent antibody test (IF) and Enzyme linked immunosorbent assay (ELISA) have been explored for use in solving problems associated with epidemiology, specialise and diagnosis.

1. **Population under study**

The study under discussion was conducted in rural area of Chirguna, Jhansi (U.P.). A population survey for
slide and seropositivity for malaria was carried out in the area. The studied population consisted of 1520 individuals from 269 families out of 290 families. An attempt was made to include all ages and both sexes in the study to know the serological profile of the population. The study area was known hyper-endemic (API 2.6) for malaria. Hence it was decided to have 20 percent sampling of the population to look for correlation between serological and slide positivity.

Lobel et al (1976) in sero-epidemiological investigation of malaria in Guyana, where malaria control was in maintenance phase included 20 percent of the estimated population of survey sectors. Mantani et al (1979) took a small sample. They examined only 60 and 50 households in first and second surveys. The total population of surveyed area was 30,023.

The survey was done during September-October 1987, since it was peak of transmission season. Srivastava et al (1978) studied in the same district and showed, August, September and October were the favourable months for the transmission of both the species viz. Plasmodium vivax and Plasmodium falciparum, with September and October the peak months. It was due to increased vector mosquito density.
1.1 Male Female ratio :

The male and female ratio of sample in our study was 1000 : 818 as compared to 1000 : 845 for the population of area (Census, 1981). The male female ratio was less as compared to Census 1981. The young adults male and females contributed 30.92% and 31.37% respectively. The paediatric population sampled in the study was 31.72 percent. Male accounted for 16.03 percent and female 15.69 percent. However, only 1 (0.07%) individual was in age group ≤ 1 year sampled during survey. This disparity was due to non co-operation of parents of children and adults were not available during day time and due to unavoidable circumstances, could not be contacted in the evening in that particular village.

There was poor co-operation from adult females due to practice of purdah. They refused to come out from the house for interrogation and investigations. But this was not expected to affect the results as sex differences do not produce variation in serological studies for malaria (Lobel et al., 1976).

2. Bio-social Characteristics of Population :

2.1 Age :

The present study revealed that the slide positivity rate (SPR) for malaria varied with age. The higher (1.68%) slide positivity rate was observed amongst
individuals of 25 years and above. It was found lower (0.50%) amongst individuals belonging to age group 5-9 years. Slide positivity was 3.65 percent in the 1-4 years age group which indicates that fresh transmission is occurring in this area. No comment on age group ≤ 1 year could be made due to non-availability of samples from this age group. There was no significant (p ≤ 0.25) statistical difference in slide positivity rates between age group 1-4 years and 15-64 years.

Choudhary et al (1988) carried out a study and classified the population on the basis of the clinical history of malaria observed that all age groups were affected by the disease but that there was a progressive increase of malaria attacks to 16-25 years of age, where the rates reached the maximum level.

Pattanayak et al (1978), while attempting to analyse the dynamics of re-establishment of malaria endemicity, found that the malaria incidence in the age group 9-14 years was quite high even at initial stages of malaria epidemics in comparison to other age groups. The prevalence of malaria among different age groups is subject to wide variations. According to MacDonald (1957) in the zone of epidemic malaria, there had been a trend for malaria prevalence to remain more or less evenly distributed among different age groups during malaria epidemics. The value of malaria indices among children and adults were
used extensively for identification and specification of endemicity in malarious area (Fumana, 1969). The dynamics of these indices in areas subject to anti-malarial measures served as a basis for assessment of these measures (Bruce-Chwatt, 1985).

Verna et al (1981) observed overall slide positivity rate of 3.62%, without any significant difference for various age groups. This corroborates well with our study.

The higher (7.8%) slide positivity was observed by Mantani et al (1982). Upaty et al (1982) observed 6.8% slide positivity rate in afebrile healthy children of 2–9 years of age, although the positivity was higher (45.5%) in febrile individuals.

In our study, the sera-positivity rate of 19.23 percent was seen in 1–4 years age group and higher (75.34%) in 55–64 years & above age groups. Age has been an convenient variable in interpreting the results of serological technique. This can tell whether transmission occurring is fresh or it is previous experience of malaria. All age groups are expected to be involved where fresh transmission of malaria is occurring. The immunity to malaria rises as the age of individual increases with consequent reduction in parasita rate (Dessruits et al., 1965). The N.I.C.D. has conducted serological studies in erstwhile hypo-endemic malarious areas of the country.
during 1970s, and it was observed that the population below 5 years of age had hardly any malarial experience. It was only higher age group who showed high titres.

This correlated very well with our observations that the sero-positivity was significantly low in the age group 1-14 years when compared with 15-44 years and 45-64 years age group (P < 0.001).

The overall sero-positivity was 45.7 percent. Out of the total ELISA positive individuals, 60.96% individuals were positive at 1:32 dilution and point by indirect immunofluorescence (IIF) test and 50.14 percent at 1:64 dilution were also positive by IIF test.

In the literature, the data on comparative studies of ELISA and IIF are extremely scarce, our findings are in accordance with Collins et al (1972) who observed percentage positive to *Plasmodium falciparum* antigen ranging from 47.2% to 96.6% and for *Plasmodium falciparum* or *P. malariae* antigen percentage positive ranging from 60.0 to 90.6% and for *P. malariae* from 52.0 to 80.4%.

Collins et al (1972) also reported 78.4 percent sero-positivity for younger groups and 92.8 percent for older groups. It is apparent that in younger age group the IIF test failed to detect a number of those with patent parasitemia.
In our study, 100 percent of malaria cases could be detected by a *P. falciparum* ELISA test and 90.00 percent at 1:32 dilution and 77.50% at 1:64 dilution by IIF test. This corroborates well with Srivastava *et al.* (1983) who observed 100 percent sero-positivity of malaria patients (Group I), 28.78 percent of patients varied origin (Group II), 39.75 percent of random hospital patients (Group III) and 19.36 percent of the normal healthy subjects (Group IV). Ray *et al.* (1983) obtained 85.1 percent sero-positivity in malaria cases by a *P. falciparum* ELISA test. Agarwal *et al.* (1981) obtained 98.7 percent sero-positivity by using *P. vivax* antigen and 79.9 percent sero-positivity rate by IIF test, using *P. knowlesi* antigen.

2.2 **Sex:**

Female dominated the scene in present study as slide positivity was higher (2.77%) than males (2.51%). The difference was not significant ($P > 0.75$). On the contrary, Srivastava *et al.* (1973) observed higher slide positivity rate for males (4.60%) as compared to females (3.29%). Males sleep outdoor more than females, thus resulting in a frequent non-mosquitoes contact.

Beljaev *et al.* (1986) in Nayurbhanj District (Orissa), did not observed significant difference in slide positivity rates in males (12.5%) and females (10.6%).
It was not clear whether it was due to higher exposure of adults males to malaria or due to influence of local socio-economic status, ethnic groups, or the attitude of parents especially mothers towards male and female children regarding treatment and ignorance about availability of free services in the village. There was no relationship of sex to species of malaria parasites and no significant difference between infection rates of males and females with any species of parasite (Sweet, 1933).

Sero-positivity rate was higher in males than females and the difference was statistically significant ($p \leq 0.05$). Similar observations were made by Collins et al (1971) in a study carried out in Ethiopia, found sero-positivity 36.7 percent and 4.3 percent at low and high altitude respectively. The sero-positivity was higher among males than females.

2.3 Religion & Caste

In our study, majority (49.60%) belonged to backward caste, followed by scheduled caste (35.72%), upper caste (14.8%) and Muslims (0.86%). However, the slide positivity rate was highest (7.69%) for Muslims, whereas for scheduled and backwards it was found to be 3.69 and 1.89 percent respectively. This difference was not significant ($p \geq 0.10$).
Srivastava et al (1975), in the same district found more cases amongst Hindus which largely reflect the population composition during recent years.

2.4 Marital Status:

The present study revealed high (3.19%) slide positivity rate in married and lower (1.93%) in unmarried. The difference was significant ($p \leq 0.01$). The sero-positivity rate was highest (65.80%) in widow/widower/divorcee, followed by (62.22%) in married and lowest (22.77%) in unmarried individuals. The difference was significant ($p \leq 0.001$). It is due to the fact that married individuals belong to higher age group.

2.5 Literacy Status:

Our study revealed that malaria is more common amongst illiterates. Slide positivity rates amongst illiterates and literates were 4.29 percent and 2.35 percent respectively, which was statistically significant ($p \leq 0.025$). On the contrary, Verma et al (1984) did not observed any significant association of slide positivity rate with literacy status.

The sero-positivity was 50.70 percent in illiterate and 34.00 percent in literate. It was found that sero-positivity rate declined with increasing literacy status.
2.6 **Occupation**

Our study revealed a significant ($P \leq 0.025$) association between slide positivity of malaria and various occupations. Association between sero-positivity and various occupations was also significant ($P \leq 0.001$). The slide positivity rate was higher (11.11%) in individuals engaged in service in thermal power project, railways etc. and declined amongst farmers (2.94%), housewives (3.19%), followed by labour (2.04%). It was lower amongst student (1.85%) and children (1.66%).

In India, the majority of total annual malaria cases occur among various categories of agricultural labour (Pattanayak, 1981). The rest of cases occur in urban and other areas of the country (Sharma, 1986; Kondrashin & Dizit, 1985). The risk to acquire malaria is higher among mobile workers and among those exposed to mosquito bites in open air on account of their occupational requirements (Kondrashin, 1986). SFR and slide falciparum rate (SFR) in particular, was higher in labour force engaged in tea plantations, in forest economy bamboo cutting in jungles, as compared with same index among local inhabitants of neighbouring area (National Malaria Eradication Programme, 1984). Construction workers at development projects, fishermen, coal miners and labour employed in number of thermal power projects
as well as railways in the peripheral part of the country show a relatively new pattern of labour movement and had shown higher SPR and SFR. There was explosive malaria situation with evidence of chloroquine resistant P. falciparum (Ray, 1984; Raj Copalan, 1984; Panicker et al., 1984; Panicker & Raj Copalan, 1986).

Comparative sero-epidemiological studies among migrant workers and the sedentary population residing around Sathanam Reservoir in Tamil Nadu revealed that the former had a higher positivity rate as compared with the latter (Nynja & Ramesh, 1989).

3.7 Social Class

Our study revealed a higher (4.35%) slide positivity rate amongst individuals from Social Class V to those from Social Class IV (2.62%) and Social Class III (2.41%). No individual was found positive amongst Social Class I and Social Class II. The difference was significant ($P < 0.05$). It was due to low socio-economic status, individuals were living in ill-ventilated, ill-lit and unhygienic houses surrounded by various types of water collections. Poor people usually live in huts/kutcha houses and keep cattle inside their residences, thus resulting in mosquito nesting places with them. Verma et al. (1983) has reported higher SFR (3.61%) for those belonging to social class V as against about 2% for Social Class IV.
The sero-positivity rate was higher (34.48%) in Social Class V, followed by Social Class IV (46.10%) and Social Class II (40.74%). Difference was significant ($P < 0.010$). Malaria, though common in all groups of society, has been significantly increasing among economically backward classes, inhabiting areas with difficult accessibility on the periphery, and where malaria eradication was never achieved (Jay, 1979; Kondrashin, 1963).

2.6 Over-crowding:

Sero-positivity rates observed by us were 2.93 percent and 2.22 percent in individuals residing in over-crowded and uncrowded conditions respectively. The difference was not significant ($P > 0.05$). The sero-positivity rate for individuals residing in crowded and uncrowded conditions were 49.99% and 45.41% respectively. This difference was insignificant ($P > 0.01$).

Malaria is an exclusively local phenomenon governed by the presence of parasite vector and suitable environmental conditions in the community. Its distribution varies from village to village, and town to town and even from ward to ward in the same community depending on malarious conditions. Kondrashin & Orlov (1985) observed positive correlation between *P. vivax* incidence and population density as such the most intensive foci of *P. vivax* are situated usually in over-populated plain areas.
3. Slide positivity and seropositivity in relation with Clinical Manifestations:

3.1 Hepatomegaly:

In our study, slide positivity rate was higher (17.31%) in hepatomegalic individuals and lower (2.11%) in individuals without hepatomegaly. Hepatomegaly was significant in slide positive individuals ($P \leq 0.001$). The sero-positivity rate was higher (94.33%) in individuals with hepatomegaly and lower (0.30%) in individuals without hepatomegaly. The difference observed was significant ($P \leq 0.001$). Decovitz & J.J. Saave (1963) in a study of immunity to malaria in protected and unprotected groups showed the liver enlargement rates, for all age group were lower than spleen rate but with the advance of age there was a decrease in liver enlargement rates. Liver rates were strikingly decreased of the protected population in contrast to unprotected population.

In congenital malaria, hepatomegaly and jaundice with haemolytic anaemia is common in an infant. The diagnosis is confirmed only by detection of the malarial parasite in the peripheral blood of the infants (Thompson et al. 1976).

3.2 Splenomegaly:

Out of 1520 individuals, 144 (9.47%) had splenomegaly and showed 12.9 percent slide positivity.
Further analysis of these 144 individuals with splenomegaly 92.36 percent showed sero-positivity and rest were sero-negative. Slide positivity was 1.60 percent in individuals without splenomegaly. The difference was statistically significant (P < 0.001).

Thomas et al. (1991) conducted a sero-epidemiological study on aboriginal children in Orang Asli Malaysia, revealed that the falciparum antibody prevalence rate was 64.6% as against to spleen rate (61.6%) and parasite rate (43.3%). There was positive correlation between sero-epidemiological study and spleen rate, particularly in the age group up to 9 years old. Splenomegaly is a good clinical manifestation for diagnosis of malaria during epidemics and in hyper-endemic areas as it gives on the spot results but it is of no value in low endemic areas where it does not depict the true prevalence, nor it is useful in monitoring the progress of malaria control programme. All patients with splenomegaly do not have malaria and all patients of malaria do not have splenomegaly. In view of the fact that the population in this rural community do take prompt presumptive treatment which prevents spleen from becoming enlarged, and that there is negligible difference in individuals with and without parasitemia. However, higher sero-positivity rate was found in individuals with splenomegaly, consequent upon a sustained malaria challenge.
It was unlikely that malaria was the aetiological factor in the splenomegaly of these individuals. These results therefore confirm that spleen enlargement is an unreliable method for epidemiological assessment of malaria when, as at present, widespread use of anti-malarials is prevalent. Vander Kaaq also obtained similar results in an epidemiological study carried out in Surinam in 1972-74. Kantani et al (1979) also obtained similar results in a serological survey for malaria in a rural community of Delhi.

3.3 Analysis:

In the study, slide positivity rate was zero in anaemic individuals, but all the individuals were serologically positive. This is due to the fact that they have suffered from malaria in the past and there may be other causes of anaemia in the population studied. All of the 40 individuals with slide proven parasitaemia did not show anaemia at the time of survey. It was clear from the results of slide positivity that anaemia is not a constant manifestation in recent infections, whereas, 2.65 percent slide positivity in non-anaemic individuals indicate that anaemia is common in chronic malarial infections as a remote manifestation.
4. **Past History :**

4.1 **Past history of fever :**

There were 47.50% individuals with past history of fever and 52.50% individuals without past history of fever. The slide positivity rate was higher (3.60%) in individuals with past history of fever and lower (1.75%) in individuals without past history of fever. The difference was statistically significant ($P \leq 0.01$). The sera-positivity rate was higher (92.94%) in individuals with past history of fever and it was lower (6.16%) in individuals without such history. The difference was statistically significant ($P \leq 0.001$).

Fever was classified as typical when it was intermittent and associated with chills and rigors, and atypical when it was either continuous or remittent without chills and rigors. Sharma et al. (1985) observed body temperature in malaria patients ranging from $37.5^\circ C$ to $41^\circ C$. Fever was more acute in P. falciparum than P. vivax malaria.

It would therefore be safe to conclude that fever remains an important and reliable symptom of malaria. Sehgal et al. observed the same findings in Thailand. Nantani et al. (1979) also observed the same phenomenon in the rural community of Delhi and suggested that malaria can occur in a typical form more often than the physicians might believe.
4.2 *Past history of treatment (Presumptive/Radical)*:

The slide positivity rate was higher (2.95%) in individuals with past history of presumptive treatment and lower (1.43%) in individuals without past history of treatment. The difference was not significant \((P > 0.75)\).

The sero-positivity rate was higher (97.07%) in individuals with past history of treatment taken and lower (1.43%) in individuals without past history of treatment taken. The difference was statistically significant \((P \leq 0.001)\).

Seroepidemiology in general, confirmed and extended results of slide examination and it was successful where slide examination failed, in detecting persons with malaria contact and possibly with sub-patent parasitemia. Some of these reactions may have represented residual antibody from cured infections. Following anti-malarial treatment, antibody titre declined. Sufficiently long drugs or natural immune responses of individuals may decrease antibody titres from significant levels. Such infections in individuals may exist for almost 1 year but this is only a minimal time (Horstein et al., 1983).

5. *Parasite Rate:*

In the study, the overall parasite rate was 1.03 percent. It was higher (2.10%) in females and lower (1.00%) in males. In the age group 2-10 years, the higher sero-positivity (18.33%) was observed in males as compared to females (12.33%). Similar finding was reported by
Mantani et al. (1979) in a rural community of Delhi, who observed parasite rate 3.22 percent. Verma et al. (1983) revealed an overall parasite rate of 3.42 percent which was higher (4.68%) for males and lower (2.29%) for females. The difference was not significant. On account of quite scanty work in recent years on the type of subject considered, the results of present study are difficult to be interpreted widely. However, in a study in unadventurous area, Upadhyay et al. (1982) obtained 6.8% slide positivity rate in afebrile healthy children of 2–9 years, although the positivity was higher (48.3%) in febrile cases. Slide examination can only indicate the presence and absence of patent parasitemia at the time of examination. It does not indicate individual malaria experience (Kagan, 1972). Absence of patent parasitemia can be misleading since patency is influenced by immune status of individuals and use of anti-malarials.

A survey carried out in the Gambia (Narverson, Wilson & Hall, 1966) showed good concordance between the parasite rate and F.A. tests in the rural area where the transmission was at high level. Teitel et al. (1980) conducted a study in West Africa Savanna, using ELISA technique, found that many young infants were ELISA negative even though they had previous proven parasitemia. This may be due to these young infants are not sufficiently mature to mount an effective humoral response and immune-depression might play a part.
6. **ELISA Test**

6.1 **Validity of ELISA test**

Enzyme linked immunosorbent assay has been applied in the diagnosis of many infectious diseases (Voller et al., 1976). The present study reports evaluation of micro-ELISA in malaria and confirms the finding of Ray et al. (1983). That in vitro cultured *P. falciparum* is an excellent source of antigen for this test to detect antibodies. Between the three antigen batches the *P. falciparum* strain had undergone over 100 more sub-cultures. Parasite did not show any changes so far as antigenicity is concerned with regard to micro-ELISA test. It was also obvious that with higher parasitaemia of the culture, the yield of antigen was more. The data on replicate testing of the test and reference sera within the same batch as well as with three different batches of antigens showed consistent results indicating the usefulness of the test. In this report, at a serum dilution of 1:400, 93.4 percent of normal individuals showed reaction up to 0.4 whereas the rest showed reading between 0.4 - 0.6 0.2. In contrast, Spencer et al. labelled a reading of 0.3 at dilution of 1:40 as negative and no positive reaction was noted amongst normal individuals at 1:80. In a study on a normal healthy Indian population (Nahajan et al., 1981), using *Atues P. falciparum* antigen, non-specificity was found to be 6.06 percent which is comparable to our results. However,
in this study the results were read visually 1 : 100 was considered as the cut off point.

In our study, at a serum dilution of 1 : 400, 52.98 percent of individuals showed reaction up to 0 - 0.4 O.D. \( (E_{492}) \). 24.81 percent showed 0.4 - 0.6 O.D. at \( (E_{492}) \) reaction. 14.81 percent individual showed 0.6 - 0.8 O.D. \( (E_{492}) \). 6.05 percent showed 0.8 - 1.0 O.D. \( (E_{492}) \) and rest other showed up to 1.0 O.D. \( (E_{492}) \). All the slide positive individual showed more than 1.0, O.D. \( (E_{492}) \) reaction.

The sensitivity and specificity were calculated at cut off point \( (\overline{7} .40) \). The 100 percent sensitivity of the test in individuals with patent \textit{P. vivax} parasitemia reported here confirmed earlier studies. There was no difference in sera-positivity in case of first attack and more than one attack showing that this test can detect very early antibodies (Nahajen et al. 1981). Roy et al. (1983) also observed 100 percent sera-positivity in all the 11 cases of \textit{P. falciparum} infection from Maryana State. Similar observation were made by Voller and colleagues who found that this test was positive in 19 out of 20 Tanzanian sera and in all the 41 Iranian patients who were parasitologically positive for malaria. Dutta et al. (1983) reported, using \textit{P. falciparum} antigen, sera at a single dilution (1 : 200) from 143 malaria patients (Group I), 70 patients of varied origin (Group II), 75 random hospital patients (Group III)
and 75 normal healthy subjects (Group IV) were tested at a cut off point equivalent to 95% confidence limit of normal subjects (Group IV). 100.0, 6.6 and 12% cases respectively of Group I to IV gave positive ELISA reaction. Srivastava et al found that using P. falciparum antigen, the ELISA test at 99 percent confidence limit gave 99.3 percent positive results among 143 malaria patients while none of the 75 patients of pyrexia and 75 random hospital patients gave positive reaction. These findings confirm previous reports on other serological tests, (Mathews et al, 1978; Ray et al, 1983; Agarwal et al, 1968 and Wilson et al, 1975). Chandramani et al (1981), Kagan et al (1969), Mahajan et al (1981, 1982) observed lower sensitivity as compared to present study.

The specificity was observed 93.92 percent at cut off titre (7N.40) at which sensitivity and specificity were more acceptable. Similar observation was made by Ray et al (1983) who compared IFA, IIF and ELISA, and did not reveal significant differences as shown by Wilson et al and differences observed in the three tests suggest that antibodies detected may comprise similar and dissimilar classes. Mahajan et al found ELISA to be far superior to IFA and IIF in acute malaria infection using P. knowlesi antigen.
6.3 **ELISA Test by age and sex**;

In our study, ELISA value was increased with the increase in age up to the age of about 54 years and the mean ELISA value showed a decreasing trend in the elderly age group i.e., over 55 years at cut off point $\leq 40$ O.D. ($E_{492}$) with serum dilution 1 : 400. Our finding corroborates with Voller et al. (1980), who observed in a longitudinal study of malaria in West Africa savanna, in unprotected and protected population after one year of protection that showed ELISA values increased with age and reached a plateau by age 19 – 28 years in unprotected population. In the protected population, the ELISA values were significantly lower in age groups 1-4 to 9-18 years, but there was less difference in the older age groups at cut off point ( $\geq 0.2$) with serum dilution 1 : 100. High ELISA value correlates well with degree of exposure. Malaria control activities result in low ELISA value. ELISA may give negative results in infants with proven parasitaemia (Voller et al., 1980).

In our study, mean ELISA value was higher in males than females. It is due to the fact that males are involved in outdoor activity more frequently than females thus resulted to high degree of malaria exposure. This test has been used in large number of studies of malaria serology (Ambrose Thomas et al., 1978; Mcleod et al., 1979; Srivastava et al., 1981, 1983; Mahajan et al., 1981;

Spencer et al. (1979 a) used in vitro culture of P. falciparum antigen for micro-ELISA. Positive (±700) ELISA antibody responses were found in persons with parasitemia. In all the semi-immune individuals titre were ±700, and reciprocal titre rose rapidly to levels ±7350 by 2nd to 9th day of patent parasitemia and gradually decayed after curative therapy. In non-immune individuals titres were lower than in semi-immune individuals. However, positive titres do appear rapidly with patent parasitemia. In another study (1979 b) they observed discordance between IIF & ELISA in 23% samples from Vietnam and 29.6% from Honduras. ELISA was negative in considerable number of parasitologically positive cases. Edrisen et al. (1979) observed higher ELISA values in unprotected population in comparison to protected population.

7. Indirect Immunofluorescent:

7.1 IIF by Age & Sex:

Out of 1930 individuals examined for slide positivity and ELISA positivity, 713 individuals were also further analysed by IIF test. All individuals could not be tested due to paucity of time and available resources in this particular study. Comparative conclusion could not be
drawn between IIF & ELISA. However, higher (30.37\%) reaction grading was observed in age group 55-64 years, followed by 25-34 age group. The lower (8.11\%) reaction grading was observed in age group 10-14 years individuals at 1:32 dilution end point. Similarly, higher (46.22\%) positive reaction was observed in age group 45-54 years, followed by 55-64 age groups and lower (24.32\%) was observed in 10-14 years age group individuals at 1:64 dilution end point. The reaction grading (3+ and above) for males, was 57.92\% and 40.51\% at 1:32 dilution and 1:64 dilution respectively. For females, it was 46.78 percent and 32.39 percent at 1:32 dilution and 1:64 dilution end point respectively. In relation to age zero-positivity rate was increasingly higher (9.14\%) in age group 25-34 years and lower (1.53\%) in age group 1-6 years and thereafter zero-positivity rate showed a decreasing trend upto age group 65+ (1.96\%).

Studies carried out in endemic malarious area of Africa, have shown parallelism between the age dependent rise of immunity to malaria and the level of antibodies measured by immunofluorescent techniques (Bray, 1963; McGregor et al., 1965, 1966; Schindler, 1967; Collins, Skinner & Coidman, 1967). With advancing age and increased exposure to degree of transmission, the inhabitants of endemic and holo-endemic areas show a proportion rise of the fluorescent antibody (F.A.) titre. McGregor et al. (1965)
indicated that the rate of increase in antibody is rapid in young children but slows down in adolescence and adult life.

Collins et al (1971) in a study at Ethiopia, observed 36.7 percent and 4.3 percent IFA positivity at low and high altitude. This corresponded with other malarialometric indices. The positivity was higher among males than females. While studying antibody response in persons previously exposed to malaria, Bruce Chuwatt et al (1972) concluded that about 50 percent showed a positive response at low titre against P. falciparum and P. vivax. There was little evidence of persistence of malaria infection in this group.

Warren et al (1973) in a study in Costa Rica observed 0.8 percent and 31.7 percent positivity in population under 15 years and over 15 years respectively. They suggested that positive responses were more likely to be associated with old or imported cases than with current local transmission. Srivastava et al (1982) observed its high diagnostic value since 98 percent of slide positive malaria patients carrying P. falciparum or P. vivax could be diagnosed. Furthermore, positivity observed in patients of pyrexia as well as random hospital patients reflected a low degree of false positivity due to past experience of malaria infection among these cases.
7.2 Validity of IIF Test:

Out of 722 total ELISA positive individuals examined by IIF, antibodies were detectable in 47.5 percent individuals from study area during transmission season. Some of them were not having malaria at the time of survey. In many of them detectable antibodies might have been due to previous experience to malaria. It was hence considered desirable to find out a cut off titre at which the diagnosis of malaria could be made with reasonable sensitivity and specificity.

The sensitivity and specificity were calculated at cut off titres of 32 and 64. The test was very sensitive at these titres and specificity range from 40.76 percent to 50.6 percent. Whereas, at cut off titre 1:32, though the sensitivity was good (90.00%), the specificity was lower (40.76%). At cut off titre 1:64 though the specificity was slightly better but the sensitivity was poor (77.50%). Taking this into consideration, cut off titre of 32 was taken for study at which sensitivity and specificity both were most acceptable and this was 90.00 percent and 40.76 percent respectively. In previous studies Agarwal et al (1981, 1983), Ray et al (1982, 1983) also found cut off titre of 32 to be most acceptable. Similar specificity was also observed by Ray et al (1982).
Higher specificity 67.9 percent was observed as compared to present study using same antigen (Ray et al., 1982). Whereas sensitivity was much lower in evaluation study conducted by Colline et al. (1981) and Warren et al. (1975).

Bruce Chewett et al. (1972) concluded that about 50 percent showed a positive response at low titre against *P. falciparum* and *P. vivax*. 