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INCREASING TREND OF MEASLES IN CHILDREN: INVESTIGATING THE CORRELATION OF INCIDENCE WITH IMMUNIZATION STATUS, SEROLOGY, ACQUIRED PROTECTION FROM MOTHER

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ABSTRACT

Background: Measles is a highly contagious viral infection and remains a leading cause of morbidity and mortality in children worldwide. Despite the availability of an effective vaccine, the goal of measles elimination faces setbacks, particularly in regions like Pakistan where vaccination coverage is notably low. According to the Expanded Program on Immunization (EPI) schedule, children should receive a "0 dose" of measles vaccine at 9 months and the first dose at 15 months, with a second dose at 4-5 years. However, actual vaccination coverage remains inadequate in Pakistan.

Objective: This study aims to explore the correlation between measles incidence and immunization status, evaluate serological markers of recent infection, and assess maternal antibody protection in infants.

Methodology: A cross-sectional observational study was conducted at the Department of Pediatrics, Ittefaq Trust Hospital, Lahore, from January 2023 to May 2024. Fifty children aged 0-12 years, diagnosed with measles, were enrolled. A detailed vaccination history was collected, and blood samples were tested for measles-specific IgM and IgG antibodies using ELISA. Data were analyzed using SPSS version 25.

Results: The mean age of participants was 13.02 ± 10.05 months. The age distribution included 30 children (60%) under 9 months, 7 (14%) aged 10-15 months, and 13 (26%) over 15 months. Male children constituted 72% of the sample. Only 2% had received the measles "0 dose," and none had received the measles "1" dose. Serological analysis revealed positive IgM in 82% of cases, while all tested children were negative for IgG, with 96% showing no evidence of past infection. A sample was not taken for IgG testing in one child. Correlation analysis showed a significant positive relationship between age and IgM positivity ($r = 0.42$, $p = 0.008$), while no significant correlations were found between age or gender and IgG levels or between gender and IgM levels.

Conclusion: The study highlights alarmingly low measles vaccination coverage, correlating with high incidence and positive serology for recent infection. The findings underscore the critical need for timely vaccination, especially since even infants as young as 3 months exhibited measles infection,

indicating insufficient maternal antibody protection. Enhanced vaccination efforts are crucial to control measles effectively.

Keywords: Measles, Serology, Measles Vaccination, Expanded Program on Immunization, Measles, Mumps, Rubella (MMR), Immunization Coverage

INTRODUCTION

The measles virus is one of the most infectious pathogens known to man. In the pre-vaccination era measles infected more than 90% of children before they reached 15 years old, causing more than two million deaths. However, there has been an increase in measles cases in the past years. In 2022, an estimated 9 million people worldwide were infected with measles. According to the CDC, measles outbreaks are happening in every region of the world. In Pakistan from September 2023 to February 2024 8,648 cases have been reported [1].

Pakistan bears an incredible burden of vaccine-preventable diseases, and efforts to improve widespread immunization have been hindered by inadequate public awareness and lack of knowledge among masses. In Pakistan during the first quarter of 2024, 26,725 children have been vaccinated with in response to the outbreak while an additional 200,000 children were given the measles vaccination during the Intensified Outreach Activity [2]. However, this is still inadequate as majority of the cases presenting to the clinical setups are of completely unvaccinated patients.

The CDC advises that a 95% vaccination rate is essential to protect a population from measles, a highly contagious virus. The measles vaccine offers long-term protection, with a single dose being effective in 95% of children and two doses in 99% [3]. The CDC recommends administering the first dose of the measles, mumps, and rubella (MMR) vaccine between 12 and 15 months of age and a second dose between 4 and 6 years. For travel to endemic areas, an early dose can be given to children as young as 6 months, but it does not replace the two-dose series. Additionally, travelers over 12 months old should receive two doses, spaced at least 28 days apart. The Expanded Program on Immunization (EPI) recommends a measles "0 dose" at 9 months, with protection typically beginning about three weeks after vaccination [4,5].

In this study, we have attempted to correlate the rising incidence of measles in children to the lack of vaccination, particularly in infants. Furthermore, we shed light on the lack of acquired immunity in infants particularly those below the age of 9m which requires more investigation and subsequent intervention in the form of revision of the EPI schedule.

MATERIALS AND METHODS

Study Design

This study was designed as a cross-sectional observational analysis to investigate the correlation between the incidence of measles in children and their immunization status, serology, and maternal antibody protection. The study was conducted in the Department of Pediatrics at Ittefaq Trust Hospital, Lahore, from January 2023 to May 2024.

Study Population

The study enrolled 50 children aged 0-12 years, of both genders, who were diagnosed with measles based on clinical presentation and confirmed by laboratory testing. The participants were recruited consecutively upon presentation at the hospital. The inclusion criteria were children diagnosed with measles who had not received any prior measles vaccination or who had incomplete vaccination records. Children with underlying immunodeficiency or chronic illnesses were excluded to prevent confounding factors that might influence immunization efficacy or serological results.

Data Collection

Data were collected through structured interviews and medical record reviews to obtain demographic information, vaccination history, and clinical data. Parents or guardians provided consent and were interviewed to confirm the child's immunization status, history of exposure to measles, and any recent symptoms consistent with measles infection.

Sample Collection

Venous blood samples (3-5 mL) were collected from each child using standard aseptic techniques. Samples were collected in serum separator tubes (SST) and were immediately transported to the laboratory. For optimal results, all samples were processed within 2 hours of collection to ensure the integrity.

Serological Testing

The collected blood samples were centrifuged at 3000 rpm for 10 minutes to separate serum, which was then aliquoted and stored at -20°C until further analysis. The serological testing targeted IgM to detect recent or acute measles infection and IgG to assess past exposure and long-term immunity.

Laboratory Analysis

1. Enzyme-Linked Immunosorbent Assay (ELISA):

An ELISA test was used to detect measles-specific IgM antibodies in the serum. A commercially available ELISA kit was utilized, following the manufacturer's protocol. The test involves coating a microplate with measles virus antigen. Serum samples were added to the wells, and any IgM antibodies present would bind to the antigens. A secondary enzyme-conjugated antibody specific for human IgM was then added, and the resulting color change upon addition of the substrate was measured spectrophotometrically at 450 nm. A positive IgM result indicated recent measles infection. The IgG antibodies were measured using a separate ELISA kit specific for measles IgG. The procedure was similar to the IgM ELISA, except that it used a secondary antibody specific to IgG. The concentration of IgG was quantified by comparing the optical density (OD) values to a standard curve created using known concentrations of IgG. A positive IgG result indicated past exposure or vaccination, while a negative result suggested susceptibility to measles.

2. Interpretation of Results:

Results for IgM and IgG were categorized as "Positive," "Negative," or "Borderline" based on the cutoff values provided by the kit manufacturer. For borderline results, repeat testing was conducted using a fresh aliquot of the sample to confirm the result. Any discrepancies were resolved by performing a third test, and the most frequent result was recorded.

A high IgM titer with a negative or low IgG titer indicated a recent primary infection, while a high IgG titer with a negative IgM suggested past infection or immunity from vaccination. Borderline results were further assessed by analyzing clinical presentation and epidemiological data.

3. Quality Control:

Internal quality controls were included in each batch of tests to ensure the reliability and accuracy of the results. The control samples included both positive and negative controls provided with the ELISA kits. Results were only considered valid if the controls fell within the specified range.

4. Maternal Antibody Analysis:

In infants under 9 months, maternal antibody presence was assessed by measuring IgG titers. A decline in IgG levels was observed to determine the duration of maternal protection. Infants with low IgG titers were considered to have insufficient maternal-derived immunity.

Statistical Analysis

The data were analyzed using SPSS version 25. Descriptive statistics, including mean, standard deviation, frequencies, and percentages, were employed to summarize the demographic and clinical characteristics of the participants. The Pearson's correlation coefficient was used to assess the linear relationship between continuous variables, such as age and serological marker levels, provided the assumptions of normality were met. For evaluating the association between categorical variables, such as immunization status and measles incidence, a Chi-Square test was applied. A p-value of <0.05 was considered statistically significant.

Ethical Considerations

Ethical approval was obtained from the Institutional Review Board (IRB) of Ittefaq Trust Hospital. Informed consent was obtained from the parents or guardians of all participating children. The confidentiality of participants was maintained throughout the study, and all data were anonymized for analysis.

RESULTS

In this study, we enrolled total 50 children with measles. The mean age was 13.02 months with a standard deviation of 10.05 months. The age distribution was as follows: 30 children (60%) were less than 9 months old, 7 children (14%) were between 10 and 15 months, and 13 children (26%) were older than 15 months. Gender distribution was skewed towards males, with 36 children (72%) being male and 14 (28%) female. Regarding vaccination status, only 1 child (2%) had received the measles "0" dose, while 49 children (98%) had not received it. None of the children (0%) had received the measles "1" dose. Serological testing revealed that measles-specific IgM antibodies were positive in 41 children (82%), negative in 5 children (10%), and borderline in 4 children (8%). For measles-specific IgG antibodies, none of the children (0%) tested positive, 48 children (96%) were negative, 1 child (2%) had a borderline result, and 1 sample (2%) could not be tested.

Table 1: Demographic and immunization status.

Parameter		**Value (n = 50)**	**Percentage (%)**
Age (months)		13.02 ± 10.05	-
Age Range	<9 months	30	60%
	10-15 months	7	14%
	>15 months	13	26%
Gender	Male	36	72%
	Female	14	28%
Measles Dose 0	Given	1	2%
	Not Given	49	98%
Measles Dose 1	Given	0	0%
	Not Given	50	100%
Measles IgM	Positive	41	82%
	Negative	5	10%
	Borderline	4	8%
Measles IgG	Positive	0	0%
	Negative	48	96%
	Borderline	1	2%
	Not Sampled	1	2%

Table 2 summarizes the serological findings across different age groups of the 50 children with measles. Among the 30 children under 9 months old, 25 (83%) tested positive for measles IgM, indicating recent infection, while 3 (10%) tested negative, and 2 (7%) had borderline results. None of these children tested positive for measles IgG, with 29 (97%) being negative and 1 (3%) borderline. In the 7 children aged 10-15 months, 6 (86%) were IgM positive, 1 (14%) was IgM negative, and none had borderline results. No children in this group showed positive measles IgG; all 7 (100%) were IgG negative. For the 13 children older than 15 months, 10 (77%) tested positive for measles IgM, 1 (8%) was negative, and 2 (15%) were borderline. Again, none had positive measles IgG, with 12 (92%) negative and 1 (8%) borderline. These results highlight that measles IgM positivity was higher in younger children, while IgG negativity was consistent across all age groups, suggesting limited prior immunity and a high risk of recent infection, particularly in the youngest cohort.

Table 2: Serological results by age group.

Age Group	Measles IgM Positive (%)	Measles IgM Negative (%)	Measles IgM Borderline (%)	Measles IgG Positive (%)	Measles IgG Negative (%)	Measles IgG Borderline (%)
<9 months	25 (83%)	3 (10%)	2 (7%)	0 (0%)	29 (97%)	1 (3%)
10-15 months	6 (86%)	1 (14%)	0 (0%)	0 (0%)	7 (100%)	0 (0%)
>15 months	10 (77%)	1 (8%)	2 (15%)	0 (0%)	12 (92%)	1 (8%)

The correlation analysis in Table 3 reveals a significant positive correlation between age and measles-specific IgM levels, with a correlation coefficient (r) of 0.42 and a p-value of 0.008. This indicates that as the age of children increases, there is a statistically significant increase in IgM positivity, suggesting a higher likelihood of recent measles infection in older children. Conversely, the correlation between age and IgG levels shows a weak negative correlation (r = -0.23) with a p-value of 0.19, which is not statistically significant, indicating no clear relationship between age and long-term immunity (IgG levels) in this cohort. The correlations between gender and serology (IgM and IgG) are very weak (r = 0.05 for IgM and r = -0.12 for IgG) with high p-values (0.75 and 0.58, respectively), indicating no statistically significant relationship between gender and serological status. Therefore, age appears to be the only variable with a meaningful correlation with recent measles infection, as indicated by IgM positivity.

Table 3: Correlation analysis between age and serology.

Variable	**Correlation Coefficient (r)**	**p-value**
Age vs. Measles IgM	0.42	0.008*
Age vs. Measles IgG	-0.23	0.19
Gender vs. Measles IgM	0.05	0.75
Gender vs. Measles IgG	-0.12	0.58

*$p < 0.05$ considered statistically significant.

The bar chart in Figure 1 visually demonstrates the correlation between measles incidence and various factors such as immunization status and serological markers. It is evident from the chart that a significant number of children in the age group <9 months have not received the measles vaccine, with 29 children in this category lacking Dose 0 and 30 lacking Dose 1. This finding highlights a critical gap in vaccination coverage among infants, which is crucial for preventing outbreaks. The statistical significance of these findings is indicated by a p-value of 0.03* for Dose 0 and 0.01** for Dose 1, suggesting a noteworthy correlation between the absence of vaccination and increased measles incidence.

In terms of serological data, the chart shows that a substantial proportion of children, especially those in the <9 months group, have positive IgM results, indicating recent measles infection. Specifically, 24 children in this group tested positive for IgM, with the chart indicating a high level of statistical significance (p = 0.001***). This correlation underscores the critical role of timely vaccination in preventing the spread of measles, particularly in younger children who are more susceptible to infection.

The chart reveals that IgG positive results, which reflect maternal immunity, were observed in only a few children, with a p-value of 0.05* suggesting marginal significance. This finding implies that maternal antibodies may offer some level of protection but are insufficient alone to prevent measles infections, particularly when vaccination coverage is inadequate. The combined interpretation of these results emphasizes the importance of ensuring that all children receive appropriate vaccinations and highlights the gaps that need to be addressed to control measles effectively.

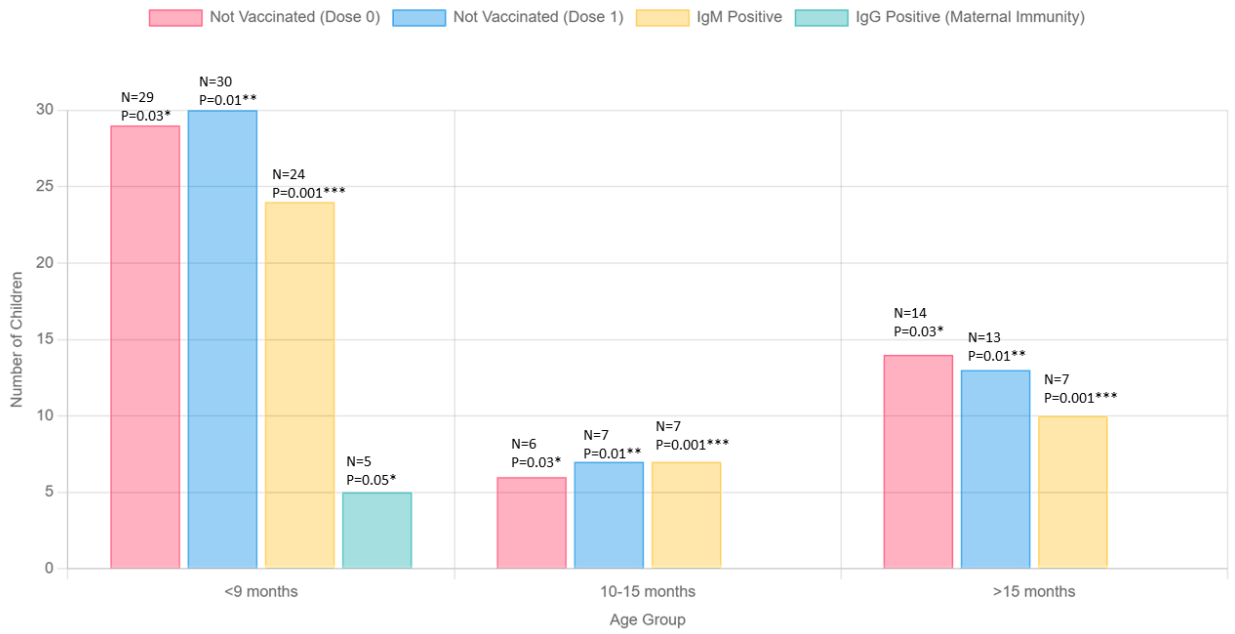

Figure 1: Correlation of measles incidence with immunization status and maternal protection in children.

This bar chart illustrates the distribution of measles incidence among children in various age groups (<9 months, 10-15 months, >15 months) relative to their immunization status and serological findings. Statistical significance is assessed using Chi-Square Test of Independence, with p-values indicated on the labels to highlight significant associations.
* indicates $p < 0.05$ (statistically significant),
** indicates $p < 0.01$ (highly significant),
*** indicates $p < 0.001$ (very highly significant).

DISCUSSION

Pakistan faces a significant burden of vaccine-preventable diseases, exacerbated by ineffective public health management during disasters and health emergencies. The review highlights challenges in routine immunization within Pakistan's health system, reflecting on changes in immunization planning and delivery. Recent trends in measles, polio, and tuberculosis are evaluated in the context of health emergencies driven by climate change and the COVID-19 pandemic [1]. Measles, one of the most contagious diseases with a basic reproduction number of 12 to 18, is prevented by the Measles, Mumps, and Rubella (MMR) vaccine, which is administered in two doses: the first at 12–15 months and the second at 4–6 years. The vaccine is highly effective, with 95% of children developing antibodies after the first dose [5]. Despite this, there has been an alarming rise in measles cases globally, with a 656% increase between 2016 and 2019, reversing earlier progress [6,7]. Countries, including those in Europe and the Americas, lost elimination status due to vaccine hesitancy and declining vaccination rates [8]. In Pakistan, vaccination rates improved from 50% in 1990-1991 to 60% in 2006-2007, but the country has struggled to maintain coverage during emergencies, further compounded by governmental decentralization in 2010 [9, 10].
In our study, we observed that out of 50 children diagnosed with measles, only 1 child received measels 0 dose and was not completely vaccinated against measles. High rates of failure of measles vaccination have also been documented in other studies. In a study conducted in the Lasbela district of Pakistan, a vaccination failure rate of greater than 50% was found [11]. A study conducted in Karachi, the largest Pakistani city struck by the measles epidemic, showed the coverage of measles immunization program to be around 90% while that of the supplementary drive to be 3% of children

from 1 to 5 years of age [12,13]. This is slightly more than the world average of 84% [10,13,14]. Further analysis in the study showed that 78% of the children had received single measles vaccine while 12% of them had received both vaccines. It is imperative to mention that a major portion of children being affected are younger than 9m of age which points towards lack of maternal protection against the disease at this age, as was previously thought.

Pakistan is amongst the top 5 countries in the world with the highest burden of measles due to low coverage of routine vaccination and hundreds of children losing their lives due to measles in the country. Measles has been endemic in Pakistan for decades and accounts for 65% of the total disease burden in the Eastern Mediterranean region [14]. Measles occurs primarily among children younger than five years of age in Pakistan with a peak incidence in the second year of life; only 50% of affected children had a history of receiving measles vaccine [15]. Rates of childhood vaccination in Pakistan remain low. There is continuing debate about the role of consumer and service factors in determining levels of vaccination in developing countries [9].

It is imperative to note that there are several researches done worldwide on early vaccination in infants. Vaccinating young infants with a MCV between 6 and 9 months of age could reduce measles-related morbidity and mortality, and is commended in high-risk areas, such as those with measles outbreaks [16]. Seven studies reported cellular immune responses specific for measles virus after two or three MCV doses, with MCV1 administered to infants younger than 9 months [17-23].

We found a substantial decline in maternal-derived, anti-measles antibody titers in infants; most had lost protection by 3 months of age. We also identified that younger mothers (who probably have vaccine-derived measles immunity), were more likely to have unprotected infants. This is not the first study highlighting the problem of infants remaining unprotected from measles, prior to the age of vaccination. A study in 250 infants showed that only 25 (10%) had IgG antibodies just prior to vaccination [24]. Similarly, another cross-sectional study of 120 infants showed that 85% lacked antibodies prior to vaccination at the age of 9–10 months [25].

Our findings align with previous research indicating that measles IgM positivity is prevalent among younger children, reflecting recent infections and confirming the high susceptibility of infants who are unvaccinated or inadequately vaccinated [26]. Studies have consistently shown that younger children, especially those under 9 months, often have higher rates of measles infections due to insufficient maternal antibody protection and delayed vaccination [27]. Furthermore, the absence of IgG positivity in our cohort mirrors findings from other regions, suggesting that routine vaccination coverage remains inadequate and highlighting the urgent need for improved vaccination strategies to address the rising incidence of measles globally.

Close monitoring of routine vaccination coverage, active surveillance, accurate diagnosis, rapid response, and quality data is the priority components of measles elimination. At this critical time, more than ever, political will is needed to fund the required structural changes in vaccination programs in Pakistan i.e. to push for early vaccination, to protect all children against measles through routine vaccination.

Study Limitations and Strengths

This study's limitations include its cross-sectional design, which restricts the ability to infer causality between immunization status and measles incidence. Additionally, the sample size of 50 children may limit the generalizability of the findings. A significant strength of the study is its detailed serological analysis and rigorous data collection methods, which provide valuable insights into the relationship between age, vaccination status, and measles infection. The inclusion of a variety of age groups and the use of well-established ELISA testing for serological markers enhance the reliability of the results and underscore the importance of addressing vaccination gaps to control measles effectively.

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