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DETECTION OF HUMAN PARVOVIRUS B19 AMONG PATIENTS WITH FEVER-RASH LIKE ILLNESS IN NORTH INDIAN POPULATION

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ABSTRACT: Introduction: The present study designed to investigate Human parvovirus B19 (HPV-B19) as an etiological agent of rash–fever ill patients confirmed negative for measles and rubella infection which is responsible for the same symptoms in North Indian population. **Materials and Methods:** Total 319, negative serum samples selected after measles/rubella IgM antibodies detection. The patients between the age of (4 to 52 years) with fever and rash-like illness were divided into four groups: 4-14, 15-25, 26-36 and >37 years old. The serological analysis was done by Indirect IgM ELISA and Real-time PCR used for molecular identification of HPV-B19 in this study. **Results:** The prevalence of HPV-B19 was found to be 29.4% by both methods in Measles and Rubella negative serum samples. We found a 29.4% positive result by HPV-B19 IgM ELISA and 7.5% positive by Real-time PCR against HPV-B19 infection. The results have been statistically significant (p= 0.0001*). The highest prevalence (38.5%) was found between 15-25 years of age group. **Conclusion:** The study was designed to find the frequency of HPV-B19 infection and observed that the patients with negative results for measles and rubella infection having the probability of HPV-B 19 infection in this region. The combined approach of the serological and molecular method confirms the contribution of HPV-B19 in the etiological role of fever-rash illness and provides the exact differential diagnostic approach for disease management.

INTRODUCTION: Human parvovirus B19 (HPV B19) is a commonly widespread human pathogen. It is DNA viruses, discovered in 1975 by Cossart and colleagues [1]. It is a small non-enveloped, linear, single-stranded DNA (ssDNA) virus of about 5.6 kb and 20-25 nm in diameter that belongs to the genus Erythro-parvovirus within the family Parvoviridae [2].

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The HPV B19 viral genome encodes three proteins: non-structural protein, NS1, and two viral capsid proteins, VP1 and VP2 [3]. On the basis of phylogenetic analysis of DNA sequences, HPV B19 subdivided into three genotypes: genotype 1 (B19-related viruses), genotype 2 (A6-related viruses) and genotype 3 (V9-related viruses) and two subgroups within genotypes 1 and 3 [2].

Generally, infections are caused by genotype 1. Due to differences in relative frequencies, population study, sample type, time, and geographic location, all the three genotypes co-circulate [4-6]. HPV B19 virus generally spreads in children with different age groups between the spring and winter season and at present in

symptomatic or asymptomatic. The infection occurs as a transient self-limiting illness besides this, HPV B19 is also present in acute form depending on the state of the immune system of the infected person.

HPVB19 infection act as an etiological agent for fever-rash illness among healthy individuals and known as the fifth disease in which the formation of antibody and virus is associated with cutaneous explosion and polyarthralgia. HPV B19 causes infection only in humans and is an important pathogen in patients with the hematologic disorder and immuno-compromised state *etc.* [7] Many researchers described the role of HPV B19 as the causative agent in patients with chronic anemia, on chemotherapy and hematological malignancies [8-11]. There is no vaccine available for human parvovirus B19, though many attempts have been made to develop one that targets against HPV B19. Till 2017, none of the human vaccines have been made against Parvovirus B19. The frequency for HPV B19 is increased with age incidence, 5-10% (aged 2-5 years) among young children, increasing up to 50% by age 15 years and 60% by age 30 years.

A small proportion of adults get an infection per year, resulting in the prevalence of a parvovirus approximately 90% in groups older than 60 years [12-16]. In childcare workers, an infection can be an occupational hazard with a rate of 20% reported in some studies. It was analyzed that the number of cases has been increased every 3-4 years. Peoples living in remote geographical locations have not been exposed to human parvovirus B19.

The current diagnosis of HPV B19 infection includes the detection of HPV B19-IgM antibodies and HPV B19-DNA from the blood by ELISA and Real-time PCR technique [17-19]. Although the IgM antibody against HPV B19 is generally detectable only for a few months, the IgG HPV B19 antibody persists for many years or life-long [20]. In contrast, only a few reports have been available on HPV B19 infection in patients from North India. Hence, the objective of this study was to find out the prevalence of HPV B19 infection among the patients who presented with fever-rash like illness and clinically diagnosed as negative for measles and rubella infection in the North Indian population.

The findings of this study will provide useful information for settings in which there is a recrudescence of rash-fever illnesses despite high vaccination coverage for measles and rubella in India.

MATERIALS AND METHODS:

Study Site: The present study is a cross-sectional hospital-based study. In this study, a total of 319 blood samples were taken from suspected patients of different regions of Uttar Pradesh, received at the Regional Reference Laboratory, Sanjay Gandhi Post Graduate Institute of Medical Sciences during the Measles and Rubella outbreak in 2015 to 2016. Once they were confirmed serologically negative for both infection, then those samples were stored at -80 °C for the diagnosis of HPV B19 infection.

Collection of Clinical Specimens: Patients with clinical signs of high-grade fever with any two of the following symptoms like rash and conjunctivitis were enrolled for the study. Furthermore information used in this study for evaluation are those which were collected by health professionals during the time of Measles and Rubella outbreak. Total 319, serum samples were collected and divided into four age groups 4 to 14, 15 to 25, 26 to 36 and >37 years. Aliquots of serum samples were stored at -80 °C for further investigation.

Enzyme-Linked Immune Sorbent Assay (ELISA): Anti-measles and anti-rubella IgM antibodies in serum samples were tested using the IgM ELISA kit (Siemens, Germany). The diluted sera added to the assay plate coated with antigen and incubated at 37 °C for 1 h. After washing, conjugate was added for 1 h at 37 °C and at last substrate was added for 30 min in dark at room temperature as per the kit manufacturer's instructions. The absorbance was measured at 450 nm/620 nm respectively, using an ELISA reader (Finstruments, Multiskan Model, Lab systems Finland, Type-347). The kit includes negative and positive controls and results are based on a sample optical density ratio.

All negative samples for measles-rubella infection, tested for Human Parvovirus B19 using IgM ELISA kit (DRG, Germany) according to the manufacturer's directions and the optical value was measured by ELISA reader at 450nm/620nm while

result was determined by cut off values which always run in duplicate.

Real-Time Polymerase Chain Reaction: Viral DNA from serum samples was extracted through commercially available QIAamp viral DNA kit (Qiagen) according to the manufacturer's instructions. Real-time polymerase chain reaction for HPV B19 was done by using HPV B19 real-time PCR kit (Shanghai, China), which contains B19 reaction mix, PCR enzyme mix, nuclease-free water, internal control, B19 positive control. Real-Time PCR has been carried out under following condition: 1 cycle at 37 °C for 2 min, 1 cycle at 94 °C for 2 min and 40 cycles at 93 °C for 15 sec with 60 °C (fluorescence measured) for 1 min. Thermal cycling was performed using (ABI 7500 standard) Real-time PCR instrument, and the result was analyzed through the output of the threshold cycle (Ct) value.

Statistical Analysis: Data was analyzed using Graph Pad Prism version 6.02 (Graph Pad Software. CA). Chi-square test was performed to evaluate the significant difference between various age group by both methods to diagnosis of HPV B19 infection applied in this study.

Ethical Consideration: Ethical approval for the use of blood specimens was granted by the Ethics Committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, IEC code-2018-76-PhD-103 and written informed consents were obtained from all patients.

RESULTS: Human parvovirus B19 was detected in 94 samples out of 319 patients with fever- rash-like illness. Of the 319 serum samples tested for HPV B19, 94 (29.4%) samples were positive by ELISA and 24 (7.5%) samples were positive by real-time PCR having statistically significant **(**p= 0.0001*) **Table 1**.

TABLE 1: COMPARISON OF ELISA AND REAL-TIME PCR RESULTS FOR HUMAN PARVOVIRUS B19 INFECTION IN FEVER–RASH ILL PATIENTS

Fever–rash ill patients N=319	Human parvovirus B19 positive	Human parvovirus B19 negative	P-value
Real Time PCR	24 (7.5%)	295 (92.5%)	
ELISA	94 (29.5%)	225 (70.5%)	< 0.0001*

#Value of $P \leq 0.05$ was considered as significant and marked with an asterisk (*)

All the 24 real-time PCR positive samples were reactive against IgM antibody for HPV B19 by ELISA. The frequency of positive cases defined age group is statistically significant by both the methods as shown in **Fig. 1**.

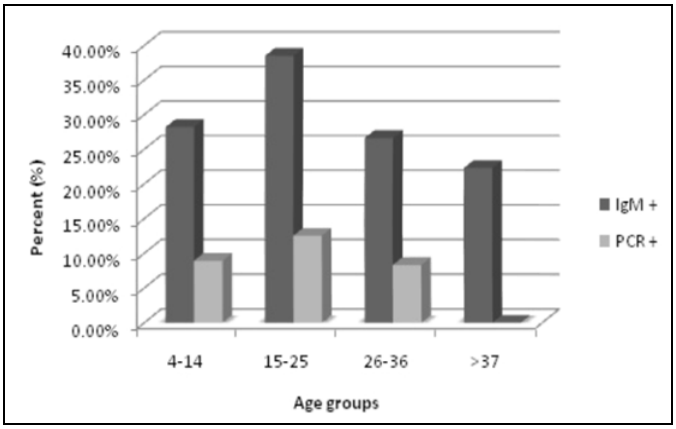

FIG. 1: COMPARISON BETWEEN TWO DIAGNOSTIC METHODS FOR THE DETECTION OF HPV B19 INFECTION IN DIFFERENT AGE GROUPS

This result confirmed the involvement of HPV B19 as an etiological agent. The proportion of samples positive for HPV B19 in males was 59.6% (56/94), and in females was 40.4% (38/94) **Fig. 2**.

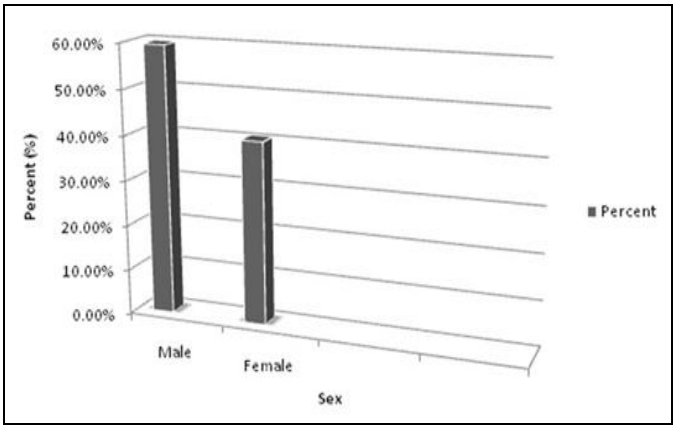

FIG. 2: PERCENTAGE OF HPV B19 INFECTION AMONG MALE AND FEMALE

The gender-specific difference was not statistically significant for HPV B19 (p= 0.15) **Table 2**.

To determine the association of the proportion of HPV B19 with age, the serum samples were divided into four age groups: 4 to 14 years, 15 to 25 years, 26 to 36 years, and >37 years.

Although, all 94 (29.4%) HPV B19 positive samples were observed in patients age group 4 to >37 years, among them, the highest numbers of positive were observed in the age group of 15 to 25

years. Out of 94 ELISA positive samples, 22 patients fall in-between age group of 4 to 14 years; 37 positive in age group of 15 to 25 years; 16 were found in age group of 26 to 36 years and 19 cases positive in patients who are above >37 yrs for HPV B19 as shown in **Fig. 3**.

There was no statistically significant difference between the age groups (p= 0.10) showed in **Table 2**. The patients of >37 years of age were least affected group which indicates the increased seroprevalence of protective IgG antibodies specific for HPV B19 among such individuals.

TABLE 2: HUMAN PARVOVIRUS B19 (HPV-B19) DETECTED IN SERUM SAMPLES FROM PATIENTS 4 TO >37 YEARS OF AGE WITH FEVER–RASH ILLNESSES IN NORTH INDIA

Characteristics of fever–rash ill patients N=319	Fever-rash ill patients with human parvovirus B19 infection N=94	Fever–rash ill patients without human parvovirus B19 infection N=225	P-value
Gender			
Male	56 (59.6%)	114 (50.7%)	0.15
Female	38 (40.4%)	111 (49.3%)	
Age group in year			
4-14 years	22 (23.4%)	56 (24.9%)	
15-25 years	37 (39.4%)	59 (26.2%)	0.10
26-36 years	16 (17.0%)	44 (19.6%)	
>37 years	19 (20.2%)	66 (29.3%)	

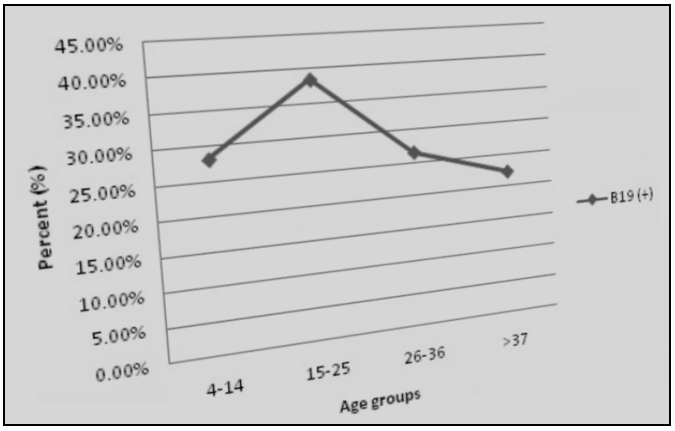

FIG. 3: DISTRIBUTION OF THE POSITIVE RESULTS FOR HPV B19 IgM ANTIBODIES IN DIFFERENT AGE GROUPS

DISCUSSION: The study revealed an overall frequency of HPV B19 infection in Measles and Rubella negative on the basis of Indirect IgM ELISA and Real-Time PCR assay. The symptoms of all three viruses (Measles, Rubella and HPV B19) are quite similar. In our study, we observed that parvovirus infections were more prevalent during the measles/rubella outbreak season. On the basis of Serological and Molecular investigation; we observed that out of 319 cases, 94 were positive and had a history of fever-rash illness, indicates an active immune response against HPV B19 infection. In our study more males were affected than females with the predominant age group between 15-25 years of age. The highest number of cases was observed in this age group due to their low immunity against this virus. A significant statistically positive correlation was noted in the present study between ELISA and Real-time PCR methods. Despite vaccination campaign conducted by Government of India against measles and rubella high frequency of positive cases were observed. The clinical symptoms of Measles and Rubella virus infections may be altered. In addition, some clinicians may have no chance to see the clinical course of these diseases, which are now rare in some regions. Furthermore, infection with different viruses may occur at the same time which causes febrile rash-illness disease. Hence, it is no surprise that many cases of suspected measles are actually caused by HPV B19 infection [21-22]. These were the reasons necessitating the significance of differential diagnosis, which lead us to design this study.

Laboratory diagnosis of HPV B19 infection occurs by detecting (IgM/IgG) specific antibodies and by molecular techniques like - Real-time PCR for viral DNA purpose. The combined use of both the techniques confirmed the etiological participation of HPV B19 among cases with fever-rash in north Indian region. The group of patients reveals the picture of acute HPV B19 infections having positive results by Indirect IgM ELISA and Real-time PCR method. In most of the cases, IgM antibodies can be measured at the time of appearance of rash and that provides confirmation of recent infection of the virus which remains measurable for 2-3 months after infection by ELISA method while, IgG generally present from the seventh day of illness and long-lasting, provides

confirmation for infection in the past. Assessment of time of infection based on the results of molecular techniques may be difficult because it has been observed that B19 virus DNA can prevail at low levels in immune-competent patients for up to 6 to 40 months post-infection. On the other hand, immuno-compromised patients cannot produce a satisfactory amount of antibodies, so analysis of viral DNA presence is necessary to document recent infection [5, 23-24]. In general, if laboratory confirmation is needed, IgM immunoglobulin testing from serum is suggested for most of the individuals and viral DNA testing for immune-compromised patients and in a plastic crisis.

The group of individuals shows the convalescent period with a negative result for IgM, and positive result for PCR, where IgM antibodies against HPV-B19 cannot be detected and HPV- B19 DNA is still present in the serum samples before the onset of illness. In this study we observed the combination of result *i.e.* positive HPV-B19-IgM and negative HPV B19-PCR. The use of other serological markers for laboratory diagnosis such as HPV B19-IgG antibodies, the avidity test for IgG as well as HPV-B19-DNA detection is useful approach for the testing of immunodeficient persons, who may not be able to produce specific IgM antibodies or when the virus is only present in different body parts. The real-time PCR assay was later used to screen clinical samples for the presence and infection [5, 23-24] titter of parvovirus. These findings are also confirmed by other researchers [25].

In many studies, Real-time PCR has been found to be a useful complementary method for IgM detection. On the other side, it is known that Real-time PCR could produce false-positive or false-negative results due to contamination and genetic diversity. The Real-time PCR method used in this study is found to be capable of detecting B19 DNA from widely varied geographic locations and archival sources. This suggests that the primers anneal to a relatively conserved region of the genome. In this study, by Real-time PCR method reported infection more specificity and less sensitivity of HPV B19 infection reported. Overall, in our study we obtained more specificity and lower sensitivity. The reason behind this can be the choice of PCR as the method to determine discordant results of the ELISA test to establish true-negative and true-positive results. Some authors conclude that it is hard to understand serological and molecular results, a supplementary assay such as anti-B19V IgG avidity must be applied [26-27]. This is especially important in the diagnosis during pregnancy because, given the long persistence of B19 DNAemia, supplementary measurement of VP1 IgG avidity improves the precision of diagnosis and the management of pregnant women affected by the B19 virus.

CONCLUSION: The laboratory examination is done by combining both the serological (HPV B19-IgM ELISA), and molecular method like Real-time PCR for the confirmation of HPV-B19 showed the association with moderate to severe fever-rash illness in North Indian population. The better knowledge of the epidemiology of parvovirus B19 may help clinicians with the differential diagnosis of HPV B19 clinical manifestations. The ELISA test for anti-B19 IgM detection shown good parameters for the diagnosis with a high specificity should be used and PCR is of limited value for diagnosis because detection of HPV-B19DNA can be analyzed before the rash onset. In conclusion, we recommend that all individuals with fever-rash illness should be screened for human parvovirus B19 infections because early diagnosis and appropriate intervention will help in proper management of these cases.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this paper.

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