

Serological Study On Parvovirus 19, Measles And Rubella Infection Among Iraqi Patients.

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Abstract

Background:

Most studies of parvovirus B19 that conducted in Iraq were on pregnant women, while this study will focus on the prevalence of the Parvovirus B19, Measles and Rubella virus among fever rash patient in Iraq. The aim of this study is to study the prevalence of these diseases in Iraq, as well as to compare the results of measles and rubella with the results of parvovirus 19, and to evaluate the cross reactivity of commercial kits.

Sampling:

350 Serum samples were collected during the year 2021-2022 from patients with fever and rash for testing ELISA IgM for Measles, Rubella and Parvovirus B19.

Material & Methods:

ELISA technique was used to test IgM in patients' serum for measles, rubella and parvovirus B19. Also tow kits from different companies used to test Parvovirus B19 for comparison.

Results:

The prevalence of Measles, Rubella and parvovirus B19 were 43%, 27%, and 18% respectively. The highest prevalence was observed in children under five years of age, and the percentages decreased with age. Statistical analysis showed a connection between the presence of IgM antibodies against measles and Rubella virus with IgM of parvovirus 19. ELISA Euroimmun kit has better sensitivity, while the ELISA Demedtee kit has better specificity;

Conclusions:

The connection that found between the presence of IgM antibodies against measles and Rubella virus with IgM of parvovirus 19 doesn't necessarily mean that one infection causes the other. The choice between ELISA Euroimmun and ELISA Demedtee kits depends heavily on the clinical context and the consequences of false positives and false negatives.

This study represents a small sample of individuals, further and advanced studies with larger samples are required to support or reject these findings.

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I. Introduction:

Fever and rash that caused by human parvovirus B19 (B19V), is difficult to diagnose by its clinical symptoms and is often misdiagnosed as measles or rubella (J.M. Hübschen et al, 2017) and (Mayte et al, 2020). Timely confirmation via laboratory tests can provide an accurate picture of the infection status, which can appropriate response (Tony Bokalanga Wawina et al, 2017).

The purpose of this study was to determine the contribution of B19V as an etiological agent for fever-rash in suspected cases of measles and rubella among Iraqi patients and to compare the results for the purpose of studying the possibility of their interaction to give a false positive result (Cross contamination)

II. Material & Method:

Sampling :

Three hundred and fifty (350) serum samples were collected for this study. The samples were collected during the years (2021-2022) from patients who presented with fever and rash symptoms. These samples tested for detecting IgM of measles, rubella and Parvovirus B19. Samples stored in (2-8)^oC for one week and in (-20)^oC for longer periods.

Methods:

Euroimmune ELISA kits were used to detect IgM to measles, rubella and parvovirus-B19. Demeditec Diagnostics GmbH kits were also used to compare with Euroimmune results for IgM to parvovirus-B19 and determine the sensitivity and specificity of each of these kits.

The Demeditec Parvovirus B19 IgM ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA). This ELISA is using a RF-Sorbent. The Rheumatoid factor (RF) is a special autoantibody form. These are autoantibodies, which are directed against the Fc fragment of human IgG. The RF autoantibodies are mostly class IgM, but may also be class IgA, IgG or IgE. In about 5% of all healthy people, elevated RF values can be found; the titer increases with increasing age. The use of anti-human IgG antibodies in the RF-sorbent prevents false positive or false negative results.

Euroimmune kit used to detect IgM antibodies in a sample microtiter wells are coated with viral antigen. The ELISA test kit provides a semiquantitative in vitro assay to detect human antibodies (IgM class) in serum or plasma. The test kit contains microtiter strips coated with recombinant virus antigen. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgM antibodies (also IgA and IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate) catalysing a colour reaction. Before the determination of specific antibodies of class IgM, antibodies of class IgG should be removed from the patient sample. This procedure must be carried out in order to prevent any rheumatoid factors from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent specific IgG displacing IgM from the antigen, which would lead to false IgM negative test results.

The sample buffer contains an anti-human antibody preparation from goat. IgG from a serum sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

III. Resultes:

Table (1) shows the distribution of positive Parvovirus B19 IgM antibody test results across different age groups. Age groups categorizes individuals into six groups. Number of positive cases was (62) of total positive (350).

The highest percentage of positive cases (recent infections) is observed in the younger age groups: 1-5 years (35%) and 6-12 years (32%) then the <1 age group (23%). These First three age groups represent the vast majority (89%) of positive cases. There is a clear decline in the percentage of positive cases as age increases. By the teenage ,young adult years (13-25), and adults (>25 age group) ,the percentage drops significantly (5% , 2% and 3% respectively).

The Chi-square test result indicates a statistically significant association between age group and Parvovirus B19 IgM positivity. The table effectively demonstrates the age-related distribution of recent Parvovirus B19 infections, highlighting the higher prevalence in young children. The statistical significance supports the observed association between age and IgM positivity.

Table-1: Distribution of positive cases of Anti Parvovirus 19 IgM according to age groups.

Age group	Results of Parvovirus-19 IgM			Positive/Total cases (%)**
	Positive/ total positive (%)*	Negative	Equivocal	
< 1	14/62 (23%)	35	7	14/56 (25)
1-5	22/62 (35%)	126	17	22/165 (13.3)
6-12	20/62 (32%)	62	11	20/93 (21.5)
13-18	3/62 (5%)	7	2	3/12 (25)
19-25	1/62 (2%)	9	1	1/11 (9.1)
> 25	2/62 (3%)	11	0	2/13 (15.4)
Total	62/62 (100)	250	38	62/350 (17.7)
Statistical analysis	The Chi-square statistic of 30.0 and a p-value of 0.03			

*=positive cases for anti-parvovirus IgM/total positive cases * 100.

**= positive cases within specified individual age group /Total number of cases in the same age group*100

The distribution of Anti-Measles IgM results across various age groups were shown in Table (2). The overall positivity rate for Measles IgM was 152/350 (43.43%). The <1 year group has the highest percentage of positive cases within its age group 36/49 (73%). The 1-5 years age group accounts for the largest proportion of total positive cases 76/152 (50%). The lowest number of positive cases is in the older age groups (13-18, 19-25, and >25). There is a significant decline in positive cases as age increases, the 13-18, 19-25, and >25 age groups have much lower positivity rates (1%-2%) of total positives (152). Middle Childhood (6-12 years) also shows a moderate number of positive cases 33/152 (22%) and a 35% positivity rate in this group. The 1-5 years group has the highest number of equivocal cases (13), followed by 6-12 years which was 5 cases.

Table-2: Distribution of positive cases of Anti Measles IgM according to age groups.

Age Group	Results of Measles IgM			Positive/Total cases (%)
	Positive/ total positive (%)	Negative	Equivocal	
< 1	36/152 (24)	11	2	36/49(73)

1-5	76/152 (50)	82	13	76/171(44)
6-12	33/152 (22)	56	5	33/94(35)
13-18	3/152 (2)	9	1	3/13(23)
19-25	2/152 (1)	8	1	2/11(18)
> 25	2/152(1)	8	2	2/12(17)
Total	152/152 (100)	172	24	152/350 (43)

Table-3 outlines the distribution of Anti-Rubella IgM results across different age groups, specifying the number of positive, negative, and equivocal cases. The overall positive rate for Anti-Rubella IgM was (93/350 = 26.6%), < 1 (17/49 = 35%), 1-5 (48/171 = 28%), 6-12 (22/96 = 23%), 13-18 (2/10 = 20%), 19-25 (2/11 = 18%) and > 25 (2/13 = 15%). There is a variation in positive rates across different age groups, with the <1 age group having the highest rate (35%) and the >25 age group having the lowest rate (15%). There is a clear decrease in positivity rates as age increases, similar to the trend observed with measles. The 13-18, 19-25, and >25 age groups each contribute only 2% of total positive cases. Middle Childhood (6-12 years) also shows a notable number of positive cases (22 cases, 24% of total positives), with a 23% positivity rate.

Table-3: Distribution of positive cases of Anti Rubella IgM according to age groups.

Age group	Results of Rubella IgM			Positive/Total cases (%)
	Positive/ total positive (%)	Negative	Equivocal	
< 1	17/93 (18)	29	3	17/49 (35)
1-5	48/93 (52)	112	11	48/171(28)
6-12	22/93 (24)	74	0	22/96 (23)
13-18	2/93 (2)	8	0	2/10 (20)
19-25	2/93 (2)	8	1	2/11(18)
> 25	2/93 (2)	10	1	2/13 (15)
Total	93/93 (100)	241	16	350 (27)

Table (4) summarizes the findings of a study comparing the presence of Anti Measles IgM and Anti Parvovirus 19 IgM in a group of individuals using ELISA technique. There is a statistically significant association between the positive results for measles and parvovirus.

Table (4): Comparison of the results for Anti Measles IgM & Anti Parvovirus 19 IgM using ELISA Technique.

Test	Measles Positive (%)	Measles Negative	Measles Equivocal	Total
Parvo +	38 (61)	16	8	62
Parvo -	91 (36)	146	13	250
Parvo Equi	23 (61)	12	3	38
Total	152 (43)	174	24	350

Statistical analysis: Statistically significant association between the positive results for measles and parvovirus. Chi Square value = 16.9461, p < .0001.

Table (5) shows the results of a study comparing the presence of IgM antibodies to rubella and parvovirus B19 among Iraqi patients. The statistical analysis shows that there may be a link between recent rubella infection and an increased risk of parvovirus B19 infection.

Table-5: Comparison of ELISA results of Anti Rubella IgM & Anti Parvovirus 19 IgM among Iraqi patients.

Test	Rubella positive (%)	Rubella negative	Rubella equivocal	Total
Parvo +	32 (52)	26	4	62
Parvo -	48 (19)	192	10	250
Parvo Equi	13 (34)	23	2	38
Total	93 (27)	241	16	350

Statistical analysis: Statistically significant association between Rubella status and Parvovirus 19 IgM results. Chi Square value = 35.74 and the P-value is very small

Table (6) compares the statistical outcomes of two ELISA methods: Euroimmun and Demedtee (Dem). The ELISA Euroimmun appears to be better at detecting positive cases (38%), while ELISA Dem is better at detecting negative cases (68%). The p-value is 0.049, this indicates that there is a statistically significant difference between the performance of the Euroimmun and Demedtee ELISA kits.

Table-6: Analyze the statistical comparison of the two ELISA methods (Euroimmun kit and Demedtee kit)

Kit name	Test results			
	Positive (%)	Negative (%)	Equivocal (%)	Total (%)
ELISA Euroimmune	33 (38%)	44 (50%)	11 (12%)	88 (100%)

ELISA Demedtee	21 (24%)	60 (68%)	7 (8%)	88 (100%)
Statistical analysis	Chi-Square Value: 6.02, Degrees of Freedom (df): 2, P-Value: 0.049 Significant difference			

IV. Discussions:

Interpretation of the results of Parvovirus B19

This study provides baseline information on the current epidemiological situation among all age groups. The prevalence (17.7%) of Parvovirus B19 in Iraq indicates a moderate level of recent Parvovirus B19 activity in the studied population, which is compatible with a study conducted in Wasit governorate in Iraq (Hanan Kareem & Hussein Ali , 2024). The early Childhood Peaks (<1 year, 1-5 years, 6-12 years) suggests a critical periods for Parvovirus B19 infection and this aligns with the general understanding that Parvovirus B19 infection is most common in childhood. The peak in the <1 year group could also be influenced by the presence of maternal antibodies (IgG) in addition to recent infections in infants since IgM appears earlier than IgG. Also that the early childhood (6-12 years) is a common period for primary Parvovirus B19 infection due to increased contact in school and social settings.

Adolescent Peak (13-18 years) could be related to hormonal changes and immune system dynamics during adolescence or increased social interaction. Lower Rates in Young Adults (19-25 years) might indicate that a larger proportion of this age group has already had the infection and developed immunity. Finally, increasing rates in older adults (>25 years) could reflect waning immunity and possible reactivation or new infections in this group.

Significant statistical analysis means that the observed differences in positive cases across age groups are unlikely to be due to chance. IgM antibodies can sometimes persist for weeks or months after the initial infection (Xavier Bonjoch et al, 2015), so a positive result doesn't definitively pinpoint a very recent infection in all cases. The IgM can sometimes be positive due to recent past infections or can be falsely positive or cross reactivity with other viruses such as Measles or Rubella antibodies beside the specific assay used and its potential limitations.

The results are specific to this studied population and may not be generalizable to other populations with different demographics or epidemiological factors. And the number of positive cases in some age groups (especially the older ones) is relatively small, which can limit the power to detect significant differences.

Several studies were conducted in Iraq and in different provinces, but the study groups were different. Two studies included pregnant women (Abdulghani Mohamed et al, 2021) and (Atheer Nawfal et al, 2023). Another study in Basra included hemophilia patients (Al-khegane et al, 2021). Finally, the study conducted in Diyala on blood donors showed that 33% of blood donors had IgG for parvovirus (Zainab Majed et al, 2022). Further research is essential to fully understand these patterns and inform public health strategies effectively.

Interpretation of the results of Measles virus

Table-2 shows distribution of Positive Cases of Anti-Measles IgM According to Age Groups and the overall positivity rate for Measles IgM is 152/350 (43.43%). The 1-5 age group represents the largest proportion of positive cases (50% of all positives), despite only having a 44% positivity rate within that age group. This suggests a high burden of measles in this specific age range. The <1 age group contributes a substantial proportion of positive cases as well (24% of all positives), and exhibits the highest positivity rate within an age group (73%). The high positivity in infants (<1 year) suggests that maternal antibodies might not be providing sufficient protection, and infants are at the greatest risk for contracting measles. This could argue for reviewing vaccination schedules or improving maternal immunization programs.

With 50% of positive cases in the 1-5 years group, this indicates the need for booster doses, catch-up campaigns, and increased surveillance in preschools and kindergartens.

The older age groups, while representing a smaller number of positive cases, show a concerning trend of decreasing positivity rates with age, reaching as low as 17% in the >25 group. There is a decline in positivity rates with increasing age, indicating natural immunity development, vaccination coverage, or past exposure to the virus. Although older age groups show lower positivity, the presence of any positive cases in adolescents and adults (>13 years) warrants continued monitoring. These could be cases of waning immunity or unvaccinated individuals, posing risks for outbreaks in high-contact settings (e.g., schools, universities).

In conclusion the infants and young children (1-5 years) is the most vulnerable to measles infection, with high positivity rates and significant numbers of equivocal results. Public health strategies should focus on strengthening vaccination programs, early detection, and community education targeting these high-risk groups. The declining trend with age suggests effective immunity acquisition over time, but the presence of positive cases in older age groups requires ongoing surveillance to prevent potential outbreaks. The presence of "equivocal" results (24 cases) requires consideration. These could be retested or considered as potentially positive in a more conservative analysis, which might slightly alter the overall positivity rates.

The majority of samples were collected from 1-5 years (171 samples) and 6-12 years (94 samples) groups, which could reflect targeted surveillance in these age groups where measles transmission risk is presumed higher. Infants (<1 year) had fewer total samples (49), but the positivity rate (73%) was the highest, raising concerns about early-life exposure before full vaccination schedules are completed. The sample sizes for older age groups are relatively small, which can limit the statistical power of the analysis.

The data suggests a substantial proportion of the studied population has Measles IgM antibodies, with a higher burden in younger age groups. The age-specific positivity rates highlight the need for targeted public health interventions, particularly focusing on improving vaccination coverage in young children and understanding the factors contributing to measles infection in different age brackets. Finally, Several studies were conducted in Iraq (Ibtihal Hameed,2017) &(Mohammed A. Jalal et al, 2024) , the rate of spread of the virus in these studies varied from one study to another, depending on the country's situation, whether it was in an epidemic or not, as well as the circumstances and groups under study .

Interpretations of Rubella results

Compared to the measles positivity rate 43.4%, the rubella positivity rate is lower at 27%. Both diseases show the highest burden in younger age groups, but measles has a higher overall transmission rate in our population. The high rubella positivity in infants (<1 year) suggests that maternal immunity might be insufficient, or exposure risk is high before vaccination schedules are completed. The highest number of equivocal cases (11) in the 1-5 years group, followed by infants (<1 year) with 3 equivocal cases. This may reflect fluctuating immunity levels, possibly due to incomplete vaccination or waning maternal antibodies or partial immune responses among children between 1-5 years old. The 1-5 years group, despite receiving routine vaccinations, still shows a high number of positive cases. This might indicate gaps in vaccine coverage, delayed vaccination, or primary vaccine failure. The high rubella positivity in infants and young children poses a risk of congenital rubella syndrome (CRS) if pregnant women in the community are exposed. This underlines the need for robust maternal immunization programs and pre-conception screening. Though positivity of Rubella IgM was low in older age groups (13 years and above), positive cases in reproductive-age females could pose significant risks for congenital rubella transmission (SANA A. ABDUL-WAHAB & SALWA SH. ABDUL-WAHID,2021).

Although the overall rubella positivity rate (26.6%) is lower than that for measles, the public health risk remains substantial, particularly due to the potential for congenital rubella syndrome (CRS). Targeted vaccination efforts, maternal screening, and improved early childhood immunization are essential to reduce rubella transmission and prevent congenital complications.

Comparison of results between parvovirus B19 and Measles virus

Significant association found between the positive results for measles and parvovirus and this suggests that there is a connection between the presence of IgM antibodies against measles and parvovirus 19 in this group of individuals. However, it's important to note that this doesn't necessarily mean that one infection causes the other. Further research would be needed to determine the nature of this association. The possible explanations for the association is co-infection, it's possible that some individuals were infected with both measles and parvovirus 19 simultaneously. Or both infections might share common risk factors (e.g., age, socioeconomic status, and living conditions) that increase the likelihood of exposure. In addition to that the presence of one infection might influence the immune response to the other.

Comparison of results between parvovirus 19 and Rubella

In table (5) similar to rubella IgM, a positive result of Anti parvovirus 19 IgM suggests recent infection with parvovirus B19. Further research would be needed to determine if there is a true cause-and-effect relationship between these two viruses. Also, it's possible that individuals with recent rubella infection are more susceptible to parvovirus B19 infection, and both rubella and parvovirus B19 are typically spread through respiratory droplets. Therefore, individuals exposed to one virus may also be exposed to the other. Rubella infection may temporarily weaken the immune system, making individuals more susceptible to other infections, including parvovirus B19. There are other factors that could influence the risk of parvovirus B19 infection, such as age, sex, and underlying health conditions. This table only represents a small sample of individuals and further and advanced studies with larger samples are required to support or reject this fact.

Comparison of ELISA results obtained from different laboratory kits

ELISA Euroimmun kit has better sensitivity; this means it's more likely to correctly identify a true positives individuals. However, a high sensitivity can also lead to more false positives, which can have implications for further testing and patient management. While the ELISA Demedtee kit has better specificity; this means it's more likely to correctly identify true negatives individuals. A high specificity reduces the number

of false positives, which is crucial in situations where unnecessary interventions or treatments need to be avoided.

The choice of which method to use depends on the specific clinical context and the priorities of the testing scenario. To get a more complete picture of diagnostic accuracy, the factors like the prevalence of the disease in the population being tested need to be considered and the cost of each test should also be taken into account when making a decision about which method to use. In this study the choice between Euroimmun and Demedtee kits depends heavily on the clinical context and the consequences of false positives and false negatives. If missing cases of the condition has serious consequences (e.g., a highly contagious and dangerous disease), a test with higher sensitivity (like ELISA Euroimmun) might be preferred, even if it means more false positives. If false positives lead to costly or harmful interventions (e.g., unnecessary surgeries, treatments with side effects), a test with higher specificity (like ELISA Demedtee) might be preferred.

It's crucial to consider the prevalence of the condition in the population being tested. Higher prevalence generally leads to higher positive predictive value (PPV) for a given sensitivity and specificity. If the condition is rare, even a highly sensitive test may have a low positive PPV due to the increased likelihood of false positives.

Finally, more comprehensive analysis would require a larger sample size and information on the prevalence of the condition in the population and the cost-effectiveness should be factored into the decision-making process. And consider the clinical context when choosing between kits.

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