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Original article

Prevalence of Cytomegalovirus infection among Egyptian patients with fever of unknown origin

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Background: Diagnosis of prolonged febrile illness of unknown origin (FUO) is challenging even with the advances in the diagnostic techniques. As common as the infection with cytomegalovirus (CMV) is, most health care providers would not suspect CMV infection as a cause of FUO unless mononucleosis syndrome is evident. The aim of this study is to investigate the rate of CMV infection among patients with FUO and shed light on IgG avidity as a diagnostic tool. **Patients and methods:** Two hundred and twenty three **(**223) immune competent patients with FUO were included in our study. They were subjected to all routine laboratory investigations, fever agglutinins, tuberculin and abdominal ultrasound along with IgG and IgM for CMV and IgG avidity test. **Results:** This study shows that the 92.8% of the overall studied population were positive for CMV IgG. However, only 74(33.2%) of the studied population was found positive for IgM. Only one patient had positive IgM with negative IgG. IgG avidity was high in almost all of them. Only 3 patients showed low IgG avidity denoting that they have primary infection. **Conclusion:** CMV infection was found to be the cause of 33.2% of prolonged febrile illness experienced by immuncompetent adults. Only 1.8% of patients had primary CMV infection and the majority of them had detectable IgG level and were diagnosed with primary infection depending mainly on IgG avidity test.

Introduction

Fever of unknown origin (FUO) defines a diverse range of febrile disorders that have fevers > 101 °F for > 3 weeks that remain undiagnosed after focused inpatient of outpatient FUO workup. Fever of unknown origins may be divided into four etiologic categories, i.e., infectious, neoplastic, non-infectious inflammatory conditions and miscellaneous other disorders. Since over 200 disorders may present as FUOs, the clinical diagnostic approach should be clue directed [1].

Cytomegalovirus (CMV), also known as human herpes virus 5, was first isolated in 1956. The name of this virus is derived from the fact that it causes enlargement of the infected cell resulting in the characteristic inclusion bodies seen on microscopy. Symptoms of CMV infection vary and depend on factors including the age and immune status of the patient. Transmission occurs via body secretions such as saliva, urine, tears, blood, or genital secretions. Cytomegalovirus has an incubation period of about 4 to 6 weeks [2].

Initial studies thus focused on detection of CMV IgM, due to its known utility as a transient marker of primary infection. It was shown that CMV

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IgM detection is a sensitive marker for primary CMV infection, but its specificity is relatively poor; only about 50% of CMV IgM-positive individuals have primary infection [3].

These disappointing findings for CMV IgM led to a search for a different laboratory assay that could be used to identify primary CMV infection with high specificity, as well as sensitivity. On assessing CMV IgG avidity showed that low CMV IgG avidity is both a sensitive and a specific marker of primary CMV infection [4]. Indeed, CMV IgG avidity is increasingly considered the "gold standard" for distinguishing primary from non-primary CMV infection [5].

Aim of the work

This study aims to investigate the prevalence of CMV infection among patients admitted to the fever hospital with fever of unknown origin. We also want to shed light on IgG avidity testing as a reliable method of differentiation between primary infection and viral reinfection/reactivation.

Patients and Methods

This study was conducted in tropical medicine department, Zagazig university hospitals and military fever hospital. It is a cross sectional study which included 223 patients, randomly selected from patients who were admitted to the hospital with prolonged febrile illness. The study included immunocompetent patients admitted to hospital with fever > 38.3 for more than 7 days of unknown origin after performing the routine investigations.

Exclusion criteria

Patients with less than 38.3 for less than 7 days, patients less 18 years, patients with end organ failure; patients with neutopenia and/or leucopenia or any evidence of immunesuppression, patients with history of autoimmune disorders and/or immunesupressive drugs, pregnant women and Patients with malignancy even during remission. All patients in the study were subjected to the following; detailed history taking including; personal data, presenting complaint, general symptoms and neurological symptoms, thorough clinical examination and the following laboratory investigations including; complete blood count, erythrocytic sedimentation rate, liver and kidney function tests, urine analysis and culture and blood culture, fever agglutinins (Widal and Brucella), tuberculin and routine chest X ray to exclude the possibility of tuberculosis along with routine pelvi-abdominal ultrasound examination. CMV IgG and IgG avidity were performed using the qualitative immunoenzymatic determination of specific antibodies based on the ELISA (Enzyme-linked Immunosorbent Assay) technique **(Novalisa, NovaTec Immundiagnostica GmbH)**

Principle of the test

Microplates are coated with specific antigens to bind to corresponding antibodies of the sample. After washing the wells to remove all unbound sample material, one well is incubated with avidity reagent and the corresponding well with washing buffer. The avidity reagent removes the low-avidity antibodies from the antigens whereas the high-avidity ones are still bound to the specific antigens. After second washing step to remove the rest of avidity reagent and low-avidity antibodies, a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a third washing step unbound conjugate is removed. The immune complex, formed by the bound conjugate, is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader [6].

Steps of the test

1. 100 µl standards/controls diluted samples were dispensed into their respective wells. Leave wells A1/A2 for the Substrate Blank.
2. The wells were covered with the foil supplied in the kit.
3. The wells were incubated for 1 hour ± 5 min at 37 ± 1 °C.
4. When incubation has been completed, the foil was removed, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Overflow from the reaction wells was avoided. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step! Note: Washing is important! Insufficient washing results in poor precision and false results.
5. 100 µl of Avidity Reagent were dipensed in wells B1, C1, D1, E1 etc., except for the

Substrate Blank well A1. Dispense 100 µl of Washing Buffer in wells B2, C2, D2, E2 etc., except for the Substrate Blank well A2.

6. The wells were incubated for exactly 10 min at 37 ± 1 °C.
7. Step 4 was repeated.
8. 100 µl Conjugate was dipensed into all wells except for the blank wells (A1/A2) and the wells were incubated for 30 min at room temperature (20...25 °C) and they were not exposed to direct sunlight.
9. Step 4 was repeated.
10. 100 µl TMB substrate solution was dispensed into all wells.
11. The wells were incubated for exactly 15 min at room temperature (20...25 °C) in the dark. A blue colour occurs due to an enzymatic reaction.
12. 100 µl Stop Solution were dispensed into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a color change from blue to yellow occurs.
13. The absorbance was measured at 450/620 nm within 30 min after addition of the Stop Solution [6].

Interpretation of results [7]

Low avidity IgG below 45% indicates a primary infection acquired in the past 2 months. Equivocal avidity between 45 and 55% indicates that precise statement about the time of infection cannot be made. High avidity IgG indicates past infection or reinfection.

Statistical analysis

Analysis of data was done using SPSS epi info version 16. Categorical data were represented as number and percentage and compared using chi square test. Numerical data were represented as mean and standard deviation. Normally distributed data were compared using t test otherwise with data with no normal distribution Mann Whitney test was used to compare data ranks.

Results

Table 1 represents a summary of the demographic, clinical and laboratory data of the overall studied population. **Table 2** shows that the 92.8% of the overall studied population were positive for CMV IgG as a marker of past infection. On the other hand, only 33.2% of the studied population were positive for IgM which is a marker of acute infection. Only one patient had positive IgM with negative IgG, the finding that suggests primary infection. All patients who had positive both IgG and IgM were tested for IgG avidity. IgG avidity was high in almost all of them. Only 3 patients showed low IgG avidity denoting that they have primary infection. This means that, among 74 (33.2%) patients with evidence of acute CMV infection, only 4 (1.8%) patients had primary infection (one patient diagnosed with IgM with negative IgG and 3 patients had positive IgG and IgM with low IgG avidity). It also shows that, IgG alone can detect one case (0.44%) out of four cases having primary infection while IgG detected the other 3 (1.3%) cases.

Table 3 represents a comparison between patients with CMV and other patients as regards the demographic, clinical and laboratory data. It shows that patients with CMV were significantly younger. The percentage of patients below 30 among was 70.2% vs 52.03% in other patients (p=0.02). The rural residence was also found to be significantly more frequent among patients with CMV (62.2% vs 8.1% among other patients p<0.001).

Table 3 shows also a comparison as regards the most important symptoms and signs experienced by patients in the study. It shows no significant differences between CMV patients and other patients as regards any of them except clinical moderate splenomegally, the sign which was found to be significantly more frequent among patients with CMV than other patients (21.6% vs 6.7% p=0.001). There were no significant differences between the two patients groups as regards any of the complications.

Comparison between patients with CMV and other patients as regards laboratory parameters reveals that patients with CMV had significantly higher differential lymphocytic count than other patients (mean= 44.8 vs 39.99 % of WBC's p= 0.03). They also had significantly higher direct bilirubin level than other patients (mean= 0.54 vs 0.42 mg/dl p=0.04). Otherwise there were no significant differences as regards any other laboratory parameter.

Table 1. Summary of the demographic, clinical and laboratory data of the studied population.

	Studied group N=223	
Age		31.6 ± 12.2
Age groups	≤30	134 (60.1%)
	>30	89 (39.9)
Gender	Male	193 (86.5%)
	female	30 (13.5%)
Residence	Urban	165 (74%)
	rural	58 (26%)
Signs	Fever	223 (100%)
	Sore throat	171 (76.7%)
	Lymphadenopathy	123 (55.2%)
	Fatigue	223 (100%)
	Rash	11 (4.9%)
	Clinical splenomegally	26 (11.7%)
	Sonographic splenomegally	39 (17.5%)
Complications	Hepatitis	106 (47.5%)
	Pneumonia	3 (1.3%)
	Eye	2 (0.9%)
	CNS	0 (0%)
	Cardiac	0 (0%)
Hemoglobin (mg\dl)	12.9 ± 1.81	
RBCs (cellx10^6/µL)	5.05 ± 0.76	
WBCs (cellsx10^3/µL)	7.55 ± 3.29	
Neutrophil (% of WBC's)	51.7 ± 19.75	
Lymphocytes (% of WBC's)	41.6 ± 17.7	
Platelets (cells x10^3/µL)	214.1 ± 75.5	
ESR (mm/1st hour)	29.5 ± 22.8	
ALT(IU/L)	130.8 ± 138.7	
AST(IU/L)	87.8 ± 92.6	
Total bilirubin(mg/dl)	1.07 ± 0.94	
Direct bilirubin(mg/dl)	0.46 ± 0.44	
Urea(mg/dl)	28.3 ± 12.5	
Creatinine(mg/dl)	1.01 ± 1.62	

Table 2. Frequency of positive CMV IgG, IGM, and IgG avidity.

CMV	**Studied group N=223 N (%)**	
IgG	Positive	207 (92.8%)
	Negative	16 (7.2%)
IgM	Positive	74 (33.2%)
	Negative	149 (66.8%)
IgM positive with IgG negative		1(0.44%)
IgG avidity test (N=73)	High avidity	70(31.4%)
	Low avidity	3(1.3%)
Primary infection (IgG negative/ low avidity)		4(1.8%)

Table 3. Comparison between CMV patients and other patients as regards demographic, clinical and laboratory data.

			IgM Positive (n=74)	IgM negative n=149	test	*p*
Age	≤30		52(70.2%)	82(55.03%)	**4.79**	**0.02 S**
	>30		22 (29.8%)	67(44.9%)		
Gender	Male		65 (87.8%)	128(85.9%)	**0.16**	**0.69 NS**
	Female		9 (12.2%)	21(14.1%)		
Residence	Rural		46(62.2%)	12 (8.1%)	**75.2**	**<0.001 HS**
	Urban		28(37.8%)	137(91.9%)		
symptoms and signs	Fatigue		74 (100%)	149(100%)	-----	-----
	Sore throat		56 (75.7%)	115 (77.2%)	0.06	0.81 (NS)
	Lymphadenopathy		45 (60.8%)	78 (52.3%)	1.43	0.23 (NS)
	Rash		3 (4.05%)	8 (5.4%)	Fisher	0.67 (NS)
	Spleen enlargement	US	12 (16.2%)	27 (18.1%)	0.12	0.72 (NS)
		Clinical	16 (21.6%)	10 (6.7%)	10.7	0.001 **(HS)**
Complications	Hepatitis		37(50%)	69 (46.3%)	0.27	0.61 (NS)
	Pneumonia		1 (1.3%)	2 (1.3%)	Fisher	0.995 (NS)
	Eye		1 (1.3%)	1 (0.6%)	Fisher	0.83 (NS)
	CNS		0 (0%)	0 (0%)	------	----
	Cardiac		0 (0%)	0 (0%)	-----	-----
Hemoglobin (mg\dl)			12.8 ±1.97	12.99±1.73	0.597*	0.56 NS
RBCs (x10^6 cells/µL)			5.01 ± 0.67	5.1 ±0.81	0.61*	0.55 NS
WBCs(x10^3 cells/µL)			7.64± 3.4	7.51 ±3.3	0.114	0.91 NS
Neutrophil (% of WBC's)			48.8 ±20.3	53.2 ±19.4	1.5	0.134 NS
Lymphocytes (% of WBC's)			44.8± 16.7	39.99 ±17.9	2.18	0.03 **S**
Platelets (x10^6 cells/µL)			217.1± 68.4	212.6±78.9	1.03	0.302 NS
ESR (mm/1st hour)			28.1± 23.6	30.2 ±22.4	0.99	0.32 NS
ALT (IU/L)			154.9±166.5	118.9±121.4	1.28	0.201 NS
AST(IU/L)			104.5±112.4	79.5±80.2	1.28	0.2 NS
Total bilirubin (mg/dl)			1.25 ±1.4	0.98±0.56	1.65	0.09 NS
Direct bilirubin (mg/dl)			0.54± 0.54	0.42 ±0.38	2.05	0.04 **S**
Urea (mg/dl)			27.7 ±15.1	28.6±11.1	1.4	0.16 NS
Creatinine (mg/dl)			1.22± 2.8	0.91±0.22	0.99	0.33 NS

Discussion

Diagnosis of prolonged febrile illness of unknown origin is challenging even with the advances in the diagnostic techniques. Patients who were enrolled in our study had already spent a week in the hospital and despite all the routine investigations done, no cause was found.

As common as the infection with CMV is, most health care providers would not suspect CMV infection as a cause of FUO unless mononucleosis syndrome is evident. Most health care providers think of CMV infection/reinfection as trivial self-limiting condition especially in healthy adults and hence the possibility of CMV infection in cases of FUO might not be carefully investigated and might be over looked.

The current study included 233 patients with FUO. After exclusion of malignancy, autoimmune disorders, neutropenia, patients with evidence of tuberculosis, patients with positive Widal or brucella agglutinins, results show that acute CMV infection was evident in nearly one third of patients (33.2%). Only four patients were proved to have primary CMV infection (1.8%). Only one of them had undetectable CMV IgG while the remaining three had detectable IgG and were detected only by IgG avidity. It is also worth noticing that 92.8% of patients in our study were found to have detectable IgG for CMV denoting past infection. This agrees with **Abdel Hamid et al.** who found the seroprevalence of CMV in Egypt ranges between 92% and 100% in different age groups [8].

The mean age of our studied population was 31.6 ± 12.2 with a range of 12-80 years. In the present study 60.1% of the studied patients were ≤ 30 years. Middle aged patients were the most represented among FUO cases, these results are in line with the demographic composition of Egypt. We also found that patients with CMV infection were significantly younger than other patients with fever in our study. This disagrees with **Forte et al.** who said that elderly people are more liable to episodes of reactivation than young adults owing this to the changes of senility in the immune system that he defined as immunosenescence [9]. However, the study by **Turner et al.** found that young adults suffering from CMV reactivation exhibit some changes in their immune system similar to the senility related changes. This may explain this predominance of young age among patients with CMV infection in our study [10]. Another thing is that young adults are more liable to reinfection with new CMV strains. Moreover, a study by **Cook,** found that sepsis and systemic inflammatory response can predispose to CMV reactivation [11]. This can lead us to assume that may be some of these young patients had another cause of systemic inflammation and that CMV reactivation was a result rather than a cause.

Regarding gender distribution among our studied population, males represented 193 (86.5%) and females represented 30 (13.5%) of them. This can be explained by the fact that in females, it is more likely to suspect the presence of an autoimmune condition more than in males, this may result in many males remaining undiagnosed and hence included in our study. This disagrees with **Van Boven et al.** who said that women were more liable to CMV reactivation/reinfection than men [12]. The cause of this debate is that the study of **Van Boven** included pregnant women and that pregnancy itself can lead to CMV reactivation while in our study pregnant women were excluded.

Comparing patients with CMV infection to other patients in our study as regards residence revealed that rural residence was found to be significantly more frequent among them. This agrees with **Styczenski** who said that the risk of CMV infection rises to 95% in developing countries due to over-crowdedness and lack of infection control measures.[13]

Of all the clinical manifestations encountered among studied population fatigue was the most frequent symptom found in 100% of patients followed by sore throat and lymphadenopathy seen in 76.7% and 55.2% successively, while the least common was rash which is found in 4.9%. This agrees with **Kano et al.** who said that cutaneous manifestations are rare in CMV reactivation in adults [14].

Comparison between patients with CMV infection and other patients revealed that clinical splenomegally was significantly more frequent in CMV patients than other patients. Splenomegally is most probably a reaction to the persistent viremia.

As regards complications in our study we found that hepatitis was the most frequent complication of febrile illness followed by pneumonia and eye affection and that none of these complications showed any significant differences in frequency among patients with CMV. This agrees

with **Styczynski** who said that CMV reactivation is often associated with hepatitis.[13]

Comparison between patients with CMV infection and other patients in the study as regards laboratory parameters revealed that those patients had significantly higher lymphocytic differential count although the total leucocytic count showed no significant difference. This agrees with **Labalette et al.** who said that patients with CMV infection experience increased lymphocytic count.[15]

Conclusion

After exclusion of malignancy, autoimmune disorders, tuberculosis, typhoid and brucella, CMV infection was found to be the cause of 33.2% of prolonged febrile illness experienced by immuncompetent adults. Only 1.8% of patients had primary CMV infection and the majority of them had detectable IgG level and were diagnosed with primary infection depending mainly on IgG avidity test.

We can also conclude that splenomegally and lymphocytosis were more frequent among patients with CMV infection than in other febrile patients and that the most common complication encountered in patients with CMV infection was hepatitis.

Author contribution

All authors have actively and effectively participated in this research. Dr. Zeinhom was responsible for data collection, dr, Jouda performed the statistical analysis and interpretation of data along with drafting the manuscript. Dr. Gad participated in the laboratory work and last but not least dr. Enakib was responsible for the concept and the final revision before submission.

Conflict of interest

We declare that we have no conflict of interest.

Financial disclosures: nothing to declare.

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