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A Possible Role for *Chlamydia pneumoniae* in Vaso-occlusive Crisis in Sickle Cell Disease

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Background: Vaso-occlusive crisis (VOC) is the most common complication in sickle cell disease (SCD); it causes a wide spectrum of end-organ damage, a process found to be mediated by inflammatory responses. Through activating endothelial and immune cells, *Chlamydia pneumoniae* (Cp) infection was postulated to be a factor in the morbidity of acute chest syndrome in sickle cell patients (SCP).

Objective: To provide serological evidence of a possible role of Cp in VOC in SCD by investigating the occurrence of Cp IgG and IgA antibodies in SCD patients compared to control subjects.

Design: Open Controlled Trial.

Setting: Bahrain Defense Force Hospital and Princess Al-Jawhara Center for Molecular Medicine, Arabian Gulf University Bahrain.

Method: Venous blood samples were collected from one hundred and twelve patients who had acute phase of VOC and from one hundred and twelve controls. Anti-Cp IgG and IgA antibodies were detected by using species specific Cp IgG and IgA enzyme immunoassay (EIA) kits, in both patients and controls sera. Parametric comparisons were performed using *t*-test.

Result: The results showed a significant difference in Cp IgG and IgA antibodies prevalence between patients and controls (*P<0.0001*). Dual Cp IgG and IgA seropositive were higher in patients than controls.

Conclusion: The study provided serological evidence of a possible role of Cp infection in VOC in the SCD.

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Sickle cell disease (SCD) causes chronic hemolytic anemia, a heightened susceptibility to infections, end-organ damage and intermittent episodes of vascular occlusion causing both acute and chronic pain[1]. SCD is a public health problem in many countries. It is well known in the Arabian Gulf countries such as Saudi Arabia, Bahrain and Oman[2-4].

The vaso-occlusive crisis (VOC) is the most common complication of SCD, which leads to frequent hospitalization. VOC lead to recurrent pain and a wide spectrum of end-organ damage[5]. Acute VOC in SCP may progress to stroke, embolism, cardiac infarction and death[6-8]. Red blood cells, vascular endothelium and leukocytes are involved in VOC[9]. Additionally, inflammatory responses are documented prior and post VOC in SCP; it is displayed as an increase in the number of the activated immune cells and the levels of cytokines[10-12].

It is possible that infection is involved in VOC, at least in the level of propagation of vaso-occlusion process[13]. *Chlamydia pneumoniae* (Cp) has been implicated in the morbidity of acute chest syndrome (ACS) in sickle cell patients[14]. Furthermore, Cp has been associated with several health problems such as atherosclerosis and stroke[15,16]. The role of Cp in the pathogenesis of vaso-occlusive crisis in sickle cell disease is not fully investigated and still controversial.

The aim of the present study is to evaluate a possible role for Cp in either initiating or promoting VOC in SCP through identifying certain serological markers.

METHOD

One hundred and twelve Hb-SS patients, 63 males and 49 females, presented with acute painful crises. Patients had no clinical manifestation of respiratory tract infection; therefore, there was no possibility of recent Cp infection. One hundred and twelve Hb-AA controls, 63 males and 49 females, who were matched for age and gender to the patients.

The control individuals were chosen from BDF hospital and Arabian Gulf University staff and their children. Excluded from the study patients who received blood transfusion, antiplatelet drugs, anti-inflammatory drugs, anti-sickling therapy, such as Hydroxyurea during the last four months or using analgesic during the five days preceding the study. In addition, subjects who had respiratory tract infection during last two months were excluded.

Informed consent was obtained from all the participants in the study. The study was approved by the ethics committee in BDF hospital. Venous blood samples (10 ml) were collected from patients and control into EDTA tubes and anticoagulant free tubes. Complete blood count (CBC), sickle cell test, total bilirubin and LDH tests were preformed. Sickle cell positive subjects were confirmed by Hemoglobin electrophoresis.

Sera were isolated from anticoagulant free tubes and stored at -70^{o}C until they were analyzed.

Enzyme Immunoassay (EIA)

Cp IgG and IgA antibodies in patients and controls sera were measured by using species specific Cp IgG and IgA enzyme immunoassay (EIA) kits (Labsystems, Helsinki, Finland) according to the manufacturer's package insert. EIA test results were expressed as enzyme immuno-units (EIU). More than 45 EIU were considered as positive result for IgG while more than 12 EIU were considered as positive result for IgA. According to manufacture instruction, EIU approximately correspond to the inverted titers of the Labsystems's Cp microimmunofluorescence assay (MIF). For statistically differences, parametric comparisons were performed by using *t*-test, SPSS program, and $p<0.05$ was considered as a significant result.

RESULT

The characteristics and clinical data from patients and healthy subjects were shown in Table 1. The characteristics included age, gender, WBC, RBC, Hb, HCT, MCV, MCH, MCHC, platelets, total bilirubin and LDH. The data showed that the patients had Hb concentration and hematocrit value below the normal range and lower than the control group. Patients' white blood cells count was higher than those in the control subjects. However, the platelet counts of both groups were almost the same. Total bilirubin level in patients was higher than control group.

The prevalence of anti-Cp IgG/IgA in control and patients are shown in Table 2 and Figure 1. The result revealed that 60 patients (53.6%) were positive for anti-Cp IgG antibodies and had more than 65 EIU compared to 34 control subjects (30.4%), which had about 34 EIU. Prevalence of anti-Cp IgG revealed statistical significant difference between patients and control group ($p<0.0001$). Table 2 and Figure 1, showed significant increased level of Anti-Cp IgA antibodies ($p=0.0001$) in 52 patients (46.4%) who had more than 18 EIU compared to 28 control subjects (25%), who had about 9 EIU. Dual Cp seropositive, for both IgG and IgA, was detected in 41 patients (36.6%) compared to 19 controls (17%).

Table 1: Patients and Controls Data

Characteristics	Patients (n=112)	Controls (n=112)	*p value*
Age (years, range)	25.47 (8-52)	26.12 (5-54)	-
Gender (No & %)	49 F (43.75%) 63 M (56.25%)	48 F (42.9%) 64 M (57.1%)	-
WBC (SD) (4.4-9.6 ×10^3/µl)	8.94 (5.06)	6.04 (1.69)	*p<0.003*
RBC (SD) (4.2-6.3 ×10^6/µl)	3.95 (0.57)	4.79 (0.58)	*p<0.0001*
Hb (SD) (11.5-16.0g/dl)	9.4 (1.52)	12.87 (1.71)	*p<0.0001*
HCT (SD) (36.0-51.0 %)	28.20 (4.25)	37.06 (4.08)	*p<0.0001*
MCV (SD) (77-96 fl)	71.85 (7.70)	77.73 (7.12)	*p<0.0046*
MCH (SD) (30-34 pg)	24.26 (3.63)	26.99 (3.11)	*p<0.0037*
MCHC (SD) (32-36 g/dl)	33.11 (2.28)	34.68 (1.88)	*p<0.0066*
Platelets (SD) (150-450 ×10^3/µl)	253 (136)	267 (105)	*p<0.662*
Total Bilirurbin (SD) (0.0-1.1 mg/dl)	3.22 (2.37)	0.5 (0.2)	*p<0.0001*
LDH (SD) (100-190 u/l)	541 (289)	210 (105)	*p<0.0001*

Table 2: Prevalence of Anti-Cp IgG/IgA in Control Subjects and Patients

		Controls (n=112)	Patients (n=112)
IgG	Mean (EIU)	34.87	65.35
	Positive	34 (30.4%)	60 (53.6%)
	p value	*p<0.0001*	
IgA	Mean (EIU)	9.07	18.82
	Positive	28 (25%)	52 (46.4%)
	p value	*p<0.0001*	
Dual positive (IgG & IgA)		19 (17%)	41 (36.6%)

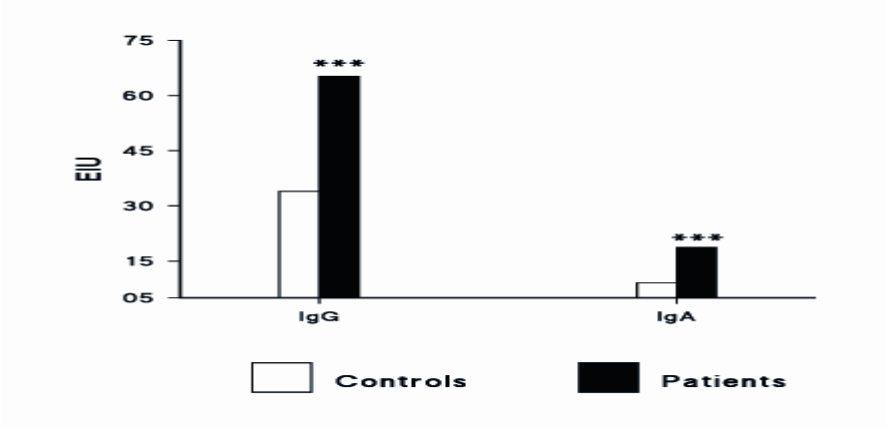

Figure 1: The Micrograph Illustrates the Occurrence of Anti-Cp IgG/IgA Antibodies in Control Subjects and Patients. Bars Indicate Mean of Antibody Concentration in EIU

DISCUSSION

Sickle cell patients are commonly infected by several infectious agents such as *Haemophilus influenzae, Streptococcus pneumoniae, Neisseria meningitidis* and *Salmonella*. Cp was found to be the most prevalent pathogen in 14 SCP who had ACS[14]. Chlamydial infection is known as chronic or persistent infection, which causes progressive tissue damage resulting in functional impairment of the infected organ[17]. Serological markers were the first evidence of associated the Cp infection, especially IgA and IgG, with asymptomatic carotid atherosclerosis, increased intima-media thickness and stroke[18-20].

This study revealed an increase of Anti-Cp IgG and IgA antibodies levels in sickle cell patients compared to controls. Anti-Cp IgA has been suggested as a marker of chronic or persistent Cp infection because of its biological half-life in serum is less than a week, compared to 23 days for IgG[21]. Dual positivity of both IgG and IgA (with high titers) increases the possibility of association of Cp with VOC.

RBCs, vascular endothelium and leukocytes have critical role in vaso-occlusive events[9]. It has been thought that RBCs has a critical role in activating and damaging the vascular endothelial cell, which in turn increases the expression of VCAM-1, E-selectin and ICAM-1 when they are exposed to sickle cells compared to normal RBC[22].

Cp is able to infect and multiply in endothelial cells, smooth muscle cells, monocytes/macrophages and lymphocytes *in vitro*[23,24]. Increased adherence of sickle cells to vascular endothelial cells and formation of oxygen radicals by endothelial cells have been documented[25]. This adhesion is influenced by abnormalities of RBCs membrane and plasma factors[22]. Degree of adherence of RBCs is associated with clinical severity of SCD[26]. Activated or damaged endothelial cells contribute to both micro-vascular and macro-vascular complications[22].

CONCLUSION

This study provides serological evidence of a possible role of Cp infection in VOC of SCD which further support the notion that Cp infection is involved in the morbidity of acute chest syndrome and possibly other severe complications in sickle cell patients.

Further studies to isolate directly the bacterial DNA by PCR from sickle cell patients with severe complications are currently planned to provide a direct evidence for the role of Cp infection in VOC of SCD.

REFERENCES

1. Mehta SR, Afenyi-Annan A, Byrns PJ, et al. Opportunities to Improve Outcomes in Sickle Cell Disease. Am Fam Physician 2006; 74(2): 303-10. Review.
2. Serjeant GR. The Geography of Sickle Cell Disease: Opportunities for Understanding its Diversity. Ann Saudi Med 1994; 14(3): 237-46.
3. Rajab KE, Issa AA, Mohammed AM, et al. Sickle Cell Disease and Pregnancy in Bahrain. Int J Gynaecol Obstet 2006; 93(2): 171-5.
4. Al-Riyami A, Ebrahim GJ. Genetic Blood Disorders Survey in the Sultanate of Oman. J Trop Pediatr 2003; 49 (Suppl 1): i1-20.
5. van Beers EJ, van Tuijn CF, Mac Gillavry MR, et al. Sickle Cell Disease-Related Organ Damage Occurs Irrespective of Pain Rate: Implications for Clinical Practice. Haematologica 2008; 93(5): 757-60.
6. deVeber G. Arterial Ischemic Strokes in Infants and Children: An Overview of Current Approaches. Semin Thromb Hemost 2003; 29(6): 567-73.
7. Hawley DA, McCarthy LJ. Sickle Cell Disease: Two Fatalities Due to Bone Marrow Emboli in Patients with Acute Chest Syndrome. Am J Forensic Med Pathol 2009; 30(1): 69-71.
8. Fitzhugh CD, Lauder N, Jonassaint JC, et al. Cardiopulmonary Complications Leading to Premature Deaths in Adult Patients with Sickle Cell Disease. Am J Hematol 2010; 85(1): 36-40.
9. Bains SK, Foresti R, Howard J, et al. Human Sickle Cell Blood Modulates Endothelial Heme Oxygenase Activity: Effects on Vascular Adhesion and Reactivity. Arterioscler Thromb Vasc Biol 2010; 30(2): 305-12.
10. Booth C, Inusa B, Obaro SK. Infection in Sickle Cell Disease: A Review. Int J Infect Dis 2010; 14(1): e2-e12.
11. Asare K, Gee BE, Stiles JK, et al. Plasma Interleukin-1beta Concentration Is Associated with Stroke in Sickle Cell Disease. Cytokine 2010; 49(1): 39-44.
12. Musa BO, Onyemelukwe GC, Hambolu JO, et al. Pattern of Serum Cytokine Expression and T-cell Subsets in Sickle Cell Disease Patients in Vaso-Occlusive Crisis. Clin Vaccine Immunol 2010; 17(4): 602-8.

13. Ramakrishnan M, Moisi JC, Klugman KP, et al. Increased Risk of Invasive Bacterial Infections in African People with Sickle-Cell Disease: A Systematic Review and Meta-Analysis. Lancet Infect Dis 2010; 10(5): 329-37.
14. Dean D, Neumayr L, Kelly DM, et al. Chlamydia Pneumoniae and Acute Chest Syndrome in Patients with Sickle Cell Disease. J Pediatr Hematol Oncol 2003; 25(1): 46-55.
15. Vainas T, Sayed S, Bruggeman CA, et al. Exploring the Role of Chlamydia Pneumoniae in Cardiovascular Disease: A Narrative Review. Drugs Today (Barc) 2009; 45 (Suppl B): 165-72.
16. Bandaru VC, Boddu DB, Laxmi V, et al. Seroprevalence of Chlamydia Pneumoniae Antibodies in Stroke in Young. Can J Neurol Sci 2009; 36(6): 725-30.
17. Kuo CC, Campbell LA. Chlamydial Infections of the Cardiovascular System. Front Biosci 2003; 8: e36-43.
18. Bucurescu G, Stieritz DD. Evidence of An Association between Chlamydia Pneumoniae and Cerebrovascular Accidents. Eur J Neurol 2003; 10(4): 449-52.
19. Lindsberg PJ, Grau AJ. Inflammation and Infections As Risk Factors for Ischemic Stroke. Stroke 2003; 34(10): 2518-32.
20. Wolf SC, Mayer O, Jurgens S, et al. Chlamydia Pneumoniae IgA Seropositivity is Associated with Increased Risk for Atherosclerotic Vascular Disease, Myocardial Infarction and Stroke in Dialysis Patients. Clin Nephrol 2003; 59(4): 273-9.
21. Boman J, Hammerschlag MR. Chlamydia Pneumoniae and Atherosclerosis: Critical Assessment of Diagnostic Methods and Relevance to Treatment Studies. Clin Microbiol Rev 2002; 15(1): 1-20.
22. Brown MD, Wick TM, Eckman JR. Activation of Vascular Endothelial Cell Adhesion Molecule Expression by Sickle Blood Cells. Pediatr Pathol Mol Med 2001; 20(1): 47-72.
23. Takaoka N, Campbell LA, Lee A, et al. Chlamydia Pneumoniae Infection Increases Adherence of Mouse Macrophages to Mouse Endothelial Cells in Vitro and to Aortas Ex Vivo. Infect Immun 2008; 76(2): 510-4.
24. Sessa R, Nicoletti M, Di Pietro M, et al. Chlamydia Pneumoniae and Atherosclerosis: Current State and Future Prospectives. Int J Immunopathol Pharmacol 2009; 22(1): 9-14.
25. Frenette PS. Sickle Cell Vaso-Occlusion: Multistep and Multicellular Paradigm. Curr Opin Hematol 2002; 9(2): 101-6.
26. Hebbel RP, Boogaerts MA, Eaton JW, et al. Erythrocyte Adherence to Endothelium in Sickle-Cell Anemia. A Possible Determinant of Disease Severity. N Engl J Med 1980; 302(18): 992-5.