Article published online: 2022-07-07

ARTICLE

Complement C3 and C4 Levels in Recurrent Aborting Women with or without Antiphospholipid and Anticardiolipin Autoantibodies

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Published: 20 October 2014
Ibnosina J Med BS 2014;6(5):213-218
Received: 20 December 2013
Accepted: 08 July 2014
This article is available from: http://www.ijmbs.org

Abstract

Background: Accumulating body of evidence suggests a role for the complement system in the etiology of abortion. **Objectives:** To evaluate levels of complements (C3, C4) in cases of abortion with or without circulating antiphospholipid (APL) and anticardiolipin (ACL) autoantibodies. **Patients and Methods:** A total of 103 women were enrolled in this case controlled study including 73 patients with a history of three or more abortion and 30 healthy parous women. For all, ACL, APL, C3, C4 levels were estimated. **Results:** The means and ranges of circulating complements (C3, C4) were as follows [C3 level: (90 ± 9; 39-115 mg/dl versus 109±16;95-138 mg/dl) in patients and controls respectively; C4 level: (18±10; 11-25 mg/dl; versus 36±7; 23-39 mg/dl) in patients and controls respectively]. The differences in the means between patients and controls were highly significant ($p \leq 0.01$). In the patients' group, according to the mean, the range of C4, C3 and the elevation of both ACL and APL, three subgroups (A, B, C) were recognizable. Group A included 21 aborting women with high levels of ACL and APL. Their complement levels (mean ± S.D; range) were as follows:[C3: (59.7±11.6; 39-65 mg/dl); C4: (14.7±5.2; 11-16 mg/dl)]. Group B included 34 aborting women with normal levels of ACL & APL. Their complement levels (mean ± S.D, range) were as follows: [C3: (88.6±19.3; 59-90 mg/dl) and C4:(18.4±7.3; 15-21 mg/dl)]. Group C included 18 aborting women with normal levels of AC & APL and their complement levels (mean ± S.D; range) were as follows:[C3: (102.7± 15.1; 90-115 mg/dl) and C4: (21.4 ± 5.8; 17-25 mg/dl)]. The differences between the means of C3 in these subgroups were highly significant ($p < 0.01$), while the differences between the means of C4 between B and C were not significant. **Conclusion**: Low C3 & C4 levels were detected in recurrent aborting women with or without autoantibodies (APL & ACL). These data may suggest a role for these complements in the pathogenesis of recurrent pregnancy loss.

Key words: Recurrent abortion, Complements, Autoantibodies

Introduction

There are various causes of abortion. Many factors such as hormonal, genetic and anatomic features, systemic hypertension, infection, diabetes, and hyperthyroidism are well established etiologies (1). Immunological factors are also involved in these cases (2). Elevated plasma levels of antiphospholipid antibodies (APL) have been reported to be one of these factors (3). The complement system is an essential component of the innate immune system, composed of over 30 proteins that act in concert to protect the host against invading organisms, initiate inflammation and tissue injury (4). Complement activation is at the core of a long list of disease pathologies. Altered complement regulation causes and may perpetuate complications of pregnancy (5). However the association between complement activation and pregnancy outcome has been poorly studied (6). The aim of this study was to examine the levels of C3 & C4 in cases of recurrent pregnancy loss with or without antiphosphlipid (APL) and anticardiolipin (ACL) autoantibodies.

Materials and Methods

Study protocol

This study was conducted between January 2010 and September 2013. The Ethics Committee of Hawler Medical University, Erbil, Iraq, approved the protocol. Informed consent was obtained from all participants. A total of 103 women were enrolled in this case-control study. The study included 73 patients who had abortion and 30 healthy matched controls groups. The strictly selected patients' group had a history of three or more recurrent early pregnancy losses. The exclusion criteria were history of uterine anomalies, positive chromosomal analysis, diabetes mellitus, thyroid disease, aspirin, heparin, antibiotic, corticosteroid intake, embryo anomalies, chronic systemic diseases including: lupus, autoimmune disease, hypertension, asthma and cardiopulmonary disease, patients with inter-current infections, acute illness, malignancy, and smoking. In addition, investigations for the hormonal assay and infections (CMV, rubella, toxoplasmosis) were negative. All participants were negative for C-reactive protein. The control group included 30 healthy non-pregnant women who had a history of previous healthy pregnancies (to exclude the possibility of physiological hypercomplementemia in case if the control were pregnant). The diagnosis was confirmed according to the clinical criteria and partial thromboplastin time (7,8).

Laboratory methods

A 5ml aliquot of blood was taken from each patient. The separated serum was stored at -30°C until analyzed for APL, ACL, C3, and C4. The detection of APL & ACL were conducted using ELISA technique as described by the manufacturer (Orgentec Diagnostika GmbH, Mainz, Germany). According to the manufacturer's instructions, the cut values of both APL & ACL were 10 GPL IU/ml. Determination of the C3 & C4 were done by radial immunodiffusion technique as described by the manufacturer (LTA s.r.I, Bussero, Milano, Italy). According to the manufacturer's instructions, the normal expected ranges for both C3 & C4 were: 90-155 mg/dl and 20-50 mg/ dl respectively.

Statistical analysis

Data are expressed as mean ± standard deviation or as percentage and ranges as appropriate. Student t tests were applied to explore differences between groups. $P < 0.05$ as the accepted level of significance.

Results

Elevated levels of ACL & APL (>10 I.U/ml) were noted in 21 women (28.8%). Table 1 illustrates the data on C3 and C4 levels in both patient and control groups. The differences in the means between both groups were highly significant. From the 73 recurrent aborting women, 21 women had a positive elevation of both ACL & APL, while the rest (52 women) had normal levels of both. Three subgroups of patient population were recognizable. These are designated as group A (recurrent aborters with high levels of ACL & APL), group B (recurrent aborters with normal levels of ACL & APL and group C (recurrent aborters with normal levels of ACL & APL).

Table 2 provides details of these subgroups and the corresponding C3 and C4 level data. The differences in the means of C3 in these subgroups were significantly different (A&B, A&C, B&C) (Table 2). The differences between the means of C4 in the same the subgroups A & B and A& C were also significant but the difference between subgroups B & C was not significant (Table 2).

Discussion

In the present study, circulating complement C3 and C4 levels in patients suffering from recurrent abortion with either positive or negative ACL & APL were significantly lower than those in the control healthy women. Additionally,

Table 1. The values of C3, C4 (in mg/dl) in patients and controls expressed as mean ± SD (range). The statistical significance of the differences between groups for each complement separately.

Complement Class	Patients (n=73)	Controls (n=30)	Statistical significance of the differences
C3	89.74 ± 8.6 [39-115]	109.2 ± 15.8 [95-138]	t=8.08; df= 101; p < 0.01
C4	18.08 ± 9.5 [11-25]	34.5 ± 6.3 [23-39]	t=8.7; df=101; p < 0.01

Table 2. Summary of data on C3 and C4 levels (mg/dl) in patient subgroups A, B and C. Data are presented as mean ± SD [range] for each variable per subgroup.

Groups	A	B	C
Number	21	34	18
Details (n)	High ACL & APL	Normal ACL & APL	Normal ACL & APL
C3 Data:			
Mean ± SD [Range]	59.7 ± 11.6 [39-65]	88.6 ± 19.3 [59-90]	102.7 ± 15.1 [96-115]
Statistical Analysis	A vs. B: t = 2.73, df = 53, p < 0.01	B vs. C: t = 2.96, df = 50 p < 0.01	A vs. C: t = 10.048, df = 37 p < 0.01
C4 Data:			
Mean ± SD [Range]	14.7 ± 5.2 [11-16]	18.4 ± 7.3 [15-21]	21.4 ± 5.8 [17-25]
Statistical Analysis	A vs. B: t= 2.024; df=53, p ≤ 0.05	B vs. C: t=1.5; df=50, NS.	A vs. C: t=3.8; df=37, p ≤ 0.01

C3 and C4 levels in patients suffering from recurrent abortion with high levels of ACL & APL were significantly lower than those in patients without these autoantibodies. Pregnancy clearly presents an immunological riddle in that the mother doesn't reject semi-allogenic fetus. Few alterations of this critical balance appear to occur during normal pregnancy, as most pregnancies proceed to term without major complications. However, disturbance in this delicate balance may play an important role in serious pregnancy-related disorders; most prominently in cases of recurrent pregnancy loss (9).

A fully active complement system deriving from the maternal circulation as well as from local production by various cell sources is present in the placenta (10). As fetal tissues are semi-allogenic and alloantibodies commonly develop in the mother, the placenta is potentially subjected to complement-mediated immune interface with a potential risk of pregnancy loss (11). Though activated complement components are present in the normal placenta, in successful pregnancy uncontrolled complement activation is prevented by three regulator proteins present on the trophoblast membrane such as decay accelerating factor (DAF), membrane cofactor protein (MCD) and CD59 (12). Experiments performed in a murine model of APS showed that activation of the complement cascade is required to induce pregnancy loss and thrombosis. Xu, *et al* (13) investigated the role of Crry, a complement protein that regulates the deposition of C3 and C4 in rodents, whose function is similar to

human DAF and MCD. The investigators found that Crry deficiency resulted in embryonic developmental arrest and embryonic lethality due to complement deposition and placental inflammation. Holer et al (11) investigated the role of complement in APS using a mouse model in which passive transfer of human IgG containing APL (APL-IgG) induced fetal loss. They found that inhibition of the complement cascade *in vivo* prevents fetal loss and growth retardation, whereas C3 null mice were resistant to fetal injury induced by APL autoantibodies. They showed that in mice treated with APL-IgG, C3 placental deposition was markedly increased in comparison with C3 deposition in placentas of normal pregnant mice treated with control human IgG. Pierangeli and coworkers employed a model of induced thrombosis in mice to show that mice deficient in C3 and C5 were resistant to endothelial activation and thrombosis induced by APL (14). Holers and coworkers proposed a 2 hit hypothesis: first, APL antibodies target the placenta specifically and second cause platelets and endothelial cell activation inducing a pro-coagulant state (11). However this is not sufficient to cause fetal loss or growth retardation as the complement activation increases the production of mediators such as C3a, C5a, and C5b-9 MAC, which promote further platelets and endothelial cell activation leading to inflammation, tissue damage and finally fetal loss (15). Tissue factor (TF), which is the major cellular initiator of the coagulation protease cascade plays an important roles in both thrombosis and inflammation (16). Blockade of TF activity with a specific monoclonal antibody and experiments in low TF expressing mice showed less inflammation (fewer neutrophils and less C3 deposition) in deciduas and embryos survival in APL-treated mice, indicating that TF is a pro-inflammatory molecule in this model (17,18). Neutrophils from APL-treated mice do over express protease activated receptor 2 (PAR2) molecule and tissue factor with higher rate of fetal demise (19). By contrast, mice expressing deleted cytoplasm domain of TF (involved in signaling) show a normal neutrophil activation profile and are protected by APL-induced pregnancy loss. Mice lacking the PAR2 receptor showed a reduced inflammation on deciduas and less C3 deposition obtaining a reduced rate of fetal losses. The association between complement activation and pregnancy outcome had been poorly studied in addition there is little knowledge about the detailed mechanisms of complement activation in patients with APL or complement relations to the pathogenesis of APL-related complications (6,20). A novel role for complement as an effector in the pathway leading to pregnancy loss had been suggested (21). However, the clinical significance of complement is unclear in patients with unexplained pregnancy loss (22). Levels of C3 and/or C4 in unexplained habitual aborters with high anticardiolipin values or subclinical autoimmune diseases were significantly lower than in cases with explained habitual abortion (23-25). B2GP1, which is the antigenic target of APS, belongs to the complement control protein superfamily and shares homology with the membrane cofactor protein MCP/CD46 (26). Therefore, it was speculated that APS antibodies against B2GP1 cross react with MCP resulting in a functional deficiency of MCP which in turn leads to uncontrolled complement activation (15). Consequently, it is of interest to know that heparin, the standard anticoagulant treatment used to prevent obstetric complication in patients with APS has been shown to have anti-complementary effects (27). Even in the absence of detectable anticoagulation, heparin prevented antiphospholipid antibody induced pregnancy loss and inhibited systemic complement activation (28). Girardi proposed that, the murine and human findings suggest that there is a recognition of the fetal tissues by immune mechanisms that trigger complement fixation and in the presence of excessive complement activation or in the absence of complement inhibition, the fetus is at risk for injury (27). Although formation of pathogenic antibodies with activation of the classical pathway may have a role, this mechanism fail to characterize the majority of cases with unexplained recurrent pregnancy loss. It is however possible that complement activation is also involved in cases of APL-negative recurrent abortion (25,27,29). In antibody independent mouse models, it had been demonstrated that complement activation is a required event in the pathogenesis of placental and fetal injury (30). Asymptomatic partial primary complement deficiencies should be taken into consideration as a possible explanation for the low levels of complement factors observed in some of these habitual aborters. However in a subgroup of women with unexplained recurrent pregnancy loss who had hypocomplementemia, in the absence of known causes of complement consumption, might also suggest activation of maternal complement complement factors (29). The correct development of fetomaternal vasculature requires a balance between vascular endothelial growth factor (VEGF) and soluble VEGF. VEGF promotes placental formation and invasiveness through two receptors (VEGF-R1 and R2). The alternative splicing of VEGF-R1 generate soluble VEGF-R1 known as a potent antiangiogenic factor that selectively bind to circulating VEGF and prevent the physiological interaction with its receptor. Excess soluble

VEGF-R1 is shown to inhibit placental cytotrophoblast differentiation and invasion. Moreover elevated levels of this inhibitor have been detected in placenta of women with preeclampsia. Using a particular mouse model of recurrent fetal demise, some authors found that complement activation products from both classical and alternative pathways stimulate monocytes to synthesize soluble VEGF-R1 which sequesters free VEGF with consequent abnormal placentation and growth retardation (27,30,31).

In conclusion, this study demonstrates that C3 & C4 levels are low in cases of abortion with or without high levels of APL & ACL. Based on this study, searching for and detecting levels of C3 & C4 may be of benefit for improving prenatal care of pregnant women. Whether low levels of C3 & C4 is the cause or effect of pregnancy loss in the women cannot be explained by such study. In this regard, further prospective studies with a larger number of patients are encouraged to establish whether the complement system might be involved in mechanisms of recurrent abortion independently of the presence of detectable serum autoantibodies.

Acknowledgements

The authors would like to express their thanks to the staff of the Maternity Hospital, Erbil City, Iraq for their outstanding support during the conduct of the study.

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