DISCUSSION

Mixed cryoglobulinemia (MC) is a hepatitis C virus (HCV)-related immune complex disorder. Only some HCV-infected patients develop MC, which suggests that the genetic background of the host plays a key role. This study was undertaken to evaluate the contribution of host genetic factors in the pathogenesis of HCV-associated MC (HCV-MC) by analyzing allelic variants of BAFF promoter -871C/T.[39,40]

Cryoglobulins are immunoglobulins (Igs) characterized by insolubility at low temperature (below 37°C) and redissolving after warming. The first observation of a cryoprecipitation was registered in the serum of a patient affected by multiple myeloma in 1933, even if the term "cryoglobulin" was introduced by Lerner and Watson in 1947.[41] Meltzer and Franklin first described the cryoglobulinemic syndrome in 29 patients associating cryoglobulin production to a symptomatologic clinical triad characterized by purpura, arthralgias, and weakness, with increased serum levels of rheumatoid factor (RF) and/or organ dysfunction.

On the basis of their immunochemical composition, cryoglobulins are classified as single (type I) or mixed (type II and III).[42]

Mixed cryoglobulins are potentially present in the course of connective tissue and autoimmune diseases, and chronic infections.

Cytokines and chemokines are essential mediators of the immune response. [57] A disturbance of the equilibrium between activating and repressing effects of these soluble molecules may be responsible for several autoimmune/lymphoproliferative disorders. Numerous reports have suggested that cytokines and chemokines are key factors in the pathogenesis of HCV-related LPDs. The MC model, as prelymphomatous condition, has been widely used to investigate the cytokine pattern characteristic of HCV-related LPDs. [64]

A growing body of evidence has accumulated in recent years showing the involvement of the B-cell-activating factor (BAFF or BLyS) in the pathogenesis of HCV-related LPDs.[70-73] This B-cell-specific cytokine, belonging to the TNF-α family, is essential for B-lymphocyte development and survival. Several reports have shown a higher BAFF serum concentration in HCV patients than in healthy controls and, more significantly, in HCV patients with LPDs. The mechanisms of the enhanced serum concentration of BAFF in HCV-LPD patients have not been elucidated yet. A possible explanation has been recently suggested by the analysis of the polymorphic variants of the BAFF gene promoter. A particular allelic variant (−871 T), reported to induce an increased transcriptional activity of the BAFF gene, was significantly more frequent in patients with HCV-related MC than in HCV patients without MC . [96,97]

The present work aimed at detection of the association of polymorphism BAFF promoter gene and its relation to the occurrence of HCV-MC disease.

Subjects were classified into three groups: 30 patients having HCV-MC,30 patients having HCV without MC and 30 completely free healthy individuals.

All patients in the study were subjected to:

I- Full clinical examination: including thorough history taking, complete abdominal examination and magnetic resonance imaging (MRI)

II- Laboratory investigations including:cryoglobulin test ,capillary electrophoresis for cryoglobulin +ve patients, detection of the BAFF promoter polymorphism by genomic DNA extraction from peripheral blood. A fragment of the gene region containing the polymorphism was amplified by PCR technique followed by restriction digestion (RFLP).

This was done for both patients and healthy controls.All patients of cryoglobulin +ve revealed polyclonal hypergamaglobulinemia by capillary electrophoresis and this to prove that the type of cryoglobulinemia is mixed cryoglobulinemia.

The aim of the present study was to evaluate the association of -871C/T BAFF promoter polymorphism as a possible contributing factor in HCV related mixed cryoglobulinemia in a cohort of Egyptian patients with hepatitis C chronic liver disease. Results of the present study showed that MC is more prevalent in female sex than in males. The same was found by Gragnani et al[98] in their study of genetic determinants of hepatitis C associated mixed cryoglobulinemia. In the present work, liver functions were significantly deranged in HCV patients without cryoglobulinemia more than in HCV-MC patients. These results are in accordance with the results of Shahin et al[99] who compared the liver functions between two groups of HCV patients with and without mixed cryoglobulinemia and they found that liver functions were worse in HCV patients without mixed cryoglobulinemia than in those patients with cryoglobulinemia despite the comparable disease duration in both groups. The association between cryoglobulinemia and severe liver affection is a controversial issue[100-102]. Most studies reporting an association between cryoglobulinemia and liver cirrhosis relied on the presence of cryoglobulins in serum irrespective of the presence of vasculitis, possibly due to the small number of symptomatic patients [103] , with few studies specifically mentioning the association between cryoglobulinemic vasculitis and liver damage [104-106]. Agnello et al [107] suggested that variation in patient selection may be responsible for the controversies regarding the association between cryoglobulinemia and severe liver damage. It has been suggested that liver cirrhosis may be the cause for the excess production of cryoglobulins in chronic HCV infection either directly due to immunological changes resulting from liver dysfunction or altered elimination of immune complexes by the reticuloendothelial system. The frequency of liver cirrhosis in HCV patients with mixed cryoglobulinemia in this study was 36.66% and this figure is comparable to several studies which reported advanced fibrosis/cirrhosis in 25–44% of their patients with HCV vasculitis. [108-109]

The present study showed a higher prevalence of a particular polymorphic variant of the BAFF promoter, the homozygous TT genotype (66.7%in HCV-MC versus 13.3% in HCV without MC) ($P<0.001$) which was strongly associated with the presence of MC emphasizing the potential contribution of the genetic background of HCV-infected patients in the development of HCV related mixed cryoglobulinemina. Moreover, the frequency of the T allele in HCV-MC patients was significantly higher than in HCV patients without MC (80% versus 36.4% respectively)($P<0.001$).

Similarly, Gragnani et al[110] found in their study a higher prevalence of -871 T/T homozygosity (31% versus 16%; $P = 0.001$) and a greater frequency of T alleles of the BAFF promoter (80% versus 57%; $P= 0.004$ in the HCV-MC group than in the HCV group. A significant increase in serum BAFF Concentration was significantly associated with the higher frequency of the T allele in HCV-MC in their study. Furthermore, many studies documented the relation between BAFF-871C/T promoter polymorphism and serum BAFF levels . Groom J etal, found that TT genotype was correlated with highest serum BAFF levels. Jonsson MV et al, found a significant association between T allele carriers and high serum BAFF levels [111,112]. This was explained by the transcriptional activation of BAFF gene induced by the T allele of BAFF promoter.

The possible role of Blys in mixed cryoglobulinemia was intensively investigated in several studies [113-115], who found significantly higher Blys levels in HCV-MC group than in the HCV group without MC .

In conclusion our study showed a significant association between BAFF promoter allelic variants polymorphism and HCV-MC. The BAFF -871C/T promoter polymorphism can be considered one of the mechanisms contributing to the pathogenesis of HCV related MC. This polymorphism can contribute possibly in combination with other alleleic patterns to determine the genetic profile characteristic of the cryoglobulinemia phenotype. A major limitation of this study is the limited number of patients.

Further multicenter studies with larger number of patients are needed to verify the results. This might have its direct clinical impact on management of HCV-MC patients, since the anti BAFF- promoter now used in clinical trials to treat SLE might be used in the future to treat patients with mixed cryoglobulinemia particularly those with life threatening manifestations and those not responding to antiviral therapy[116].

A major limitation in genetic studies is sample size,especially when dealing with rare disorders like MC ,where the analysis of a consistent number of well-characterized cases is sometimes difficult.

SUMMARY

Cryoglobulinemia refers to the presence of serum immunoglobulins that precipitate at a cold temperature. Type I cryoglobulins are single monoclonal immunoglobulins usually associated with haematological disorders. Types II and III are mixed cryoglobulins, composed of monoclonal or polyclonal IgM respectively.

Mixed cryoglobulinemia (MC) is characterized by a typical clinical triad: purpura, weakness, arthralgias; many organs particularly kidney and peripheral nervous system may be involved. MC may be associated with infectious and systemic disorders and since 1990 studies have demonstrated that hepatitis C virus (HCV) may be considered the principal trigger of the disease.

The relation between MC and HCV infection shows new insights in the interpretation of the link between viral infection, autoimmune phenomena and lymphoproliferative disorders evolution.

In fact, the virus chronically stimulates B-cell polyclonal proliferation from which a monoclonal population may emerge. In symptomatic patients with HCV related MC therapeutic strategy should include an attempt at viral eradication. Antiviral therapy may also be effective in determining the regression of B-cell lymphoproliferative disorder.

In our study we showed that the presence of a particular polymorphic variant of the BAFF promoter was strongly associated with the presence of MC, emphasizing the potential contribution of the genetic background of HCV-infected patients in the development of lymph proliferative disorders.

The data concerning BAFF overexpression in MC patients can have immediate transferability to clinical practice since anti-BAFF monoclonal antibody is already in phase III clinical testing for SLE treatment, with encouraging results. The use of this anti-BAFF monoclonal antibody to treat patients with HCV-MC could be envisaged after appropriate clinical trials.

A major limitation in genetic studies is the sample size, especially when dealing with rare disorders like MC, where the analysis of a consistent number of well-characterized cases is sometimes difficult.
a multicenter study of a larger cohort of patients is needed to validate the present results.

CONCLUSIONS

According to the results of the present study, it was concluded that:

A significant association was found between BAFF promoter allelic variants polymorphism and HCV-MC disease in a study conducted on a sample of the Egyptian population so the transcriptional activation induced by the particular allelic variant of BAFF promoter can be considered one of the mechanisms contributing to the pathogenesis of HCV-related MC/ lympho proliferative disorders. This polymorphism can contribute, possibly in combination with other allelic patterns, to determining a genetic profile characteristic of the cryoglobulinemic phenotype.

RECOMMENDATIONS

- Studying other genes responsible for HCV related MC and another site of polymorphism in BAFF promoter gene rather than-871C/T.
- Conducting further studies on a larger population regarding the protective role of BAFF polymorphism.
- Conducting the study using real time PCR technique, this could give more accurate and reliable results.
- Conducting further studies on the safety and benefit of including anti BAFF antibody supplements in treatment protocols of HCV related MC.