

Development of an ELISA Based Method for the Detection of *Pemphigus vulgaris* Autoantibodies

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ABSTRACT

For the evaluation of autoantibodies in *Pemphigus vulgaris* (PV), an indirect ELISA assay was developed by using a semi-purified human epidermal skin extracted with approximately a molecular weight (M.W.) of 130-160 kDa. The evaluation of Pemphigus IgG, IgM and IgA autoantibodies in 75 patients and 50 control sera indicated that this indirect ELISA assay was a useful method for the detection of Pemphigus autoantibodies. Data of this study suggested that the mean OD (Optical Density) of specific IgG autoantibody was elevated in PV patients compared with the controls. The percentages of the total serum IgM and IgA antibodies were not statistically significant between patients and controls. *Iran. Biomed. J. 3 (3 & 4): 99-101, 1999*

Keywords: Pemphigus, Autoantibodies, ELISA

INTRODUCTION

Pemphigus vulgaris (PV) is an autoimmune disease characterized by erosions and blistering of mucous membranes of the skin. It has been suggested that an IgG autoantibody binds to a membrane associated antigen [1]. Since Buetner and Jordan [2] first reported skin autoantibodies in sera of PV patients, investigators attempted to characterize the skin antigen. Using immunofluorescent and light microscopy, Fukuyama *et al.* demonstrated that the Pemphigus antigen has a pattern of distribution that corresponds to the keratinocyte glycocalyx [3]. Hashimoto *et al.* [4] were able to block Pemphigus autoantibody binding to human epidermis by first incubating the tissue with concanavalin A, a lectin that has been shown to bind to the glycocalyx [5]. This revealed that the Pemphigus antigen was a membrane-associated glycoprotein. The PV antigen was recognized as a glycoprotein of approximately 130 kDa by immunoprecipitation and immunoblot analysis, and identified subsequently as desmoglein 3 (Dsg 3) by immunochemical evidence with anti-Dsg 3 monoclonal and polyclonal antibodies and PV sera. *Pemphigus foliaceus* (PF) antigen (the other subtypes of Pemphigus) was characterized as a

glycoprotein of approximately 160 kDa and identified as Dsg 1 [6]. Dsg 3 and Dsg 1 are known to be the members of cadherin super family of cell adhesion molecules [7]. To identify the autoantibodies against these antigens, the indirect immunofluorescent (IIF) technique has only been used in Iran. In this study, an ELISA assay was developed to detect the autoantibodies in the PV disease. In this method, a semi-purified human epidermal extract (with approximately M.W. of 130-160 kDa) was used as a source of antigen in which 75 PV patients were examined by this ELISA method.

MATERIAL AND METHODS

Patients. Serum samples from 75 patients with the clinical presentation of PV with an average age of 35.5 years were obtained in our laboratory. The selection of patients in this study was based on the presence of active form of disease and those of in remission (on treatment). Patients in active form of the disease were presented with extensive erosions and raw surface area all over the body with oral cavity involvement. Control individuals (n = 50) with no signs and symptoms of autoimmune and

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dermatological disease with an average age of 31 years were selected among Hospital laboratory personnel. Sera were stored at -20°C until use.

Antigen extraction. To extract the human epidermis from normal human skin, various techniques were used and compared. Our extraction method was based on the modified technique of Labib *et al.* [8]. Epidermis was removed by the immersion of the whole skin in 56°C water bath for 30 seconds. Epidermis fragments were then frozen and pulverized in liquid nitrogen. The resulting powder was homogenized with Tris-HCl buffer (0.0625 M, pH 6.8, 2% SDS) and centrifuged at 40,000g for 45 min. Finally, the supernatant was subjected to SDS-PAGE.

Sample preparation and SDS-PAGE. A 0.02 ml of concentrated diluent (0.188 M Tris-HCl buffer, pH 6.8, 6% SDS, 30% glycerol, 0.003% bromophenol blue, and 15% 2-ME) was added to the epidermal extract (0.005 to 0.03 ml) making up to the final volume of 0.06 ml distilled water. Samples were mixed and boiled for 3 min in a boiling water bath. The acrylamide concentration was 10%. Protein bands were visualized by Coomassie blue stain.

ELISA method. A 1/200 dilution of Ag (epidermal extract) in carbonate-bicarbonate buffer was coated into a micro-ELISA plate. After overnight incubation at 4°C, it was washed 3 times by PBS-Tween 20. Blocking step was achieved using 5% skimmed milk. After washing, patient's and control's sera at a dilution 1/50 in PBS-Tween 20 were added and incubated at 37°C for 90 min. The plates were washed and subsequently incubated with peroxidase labeled goat anti-human IgG, IgM or IgA (Dako, Denmark) for 60 min. After incubating and washing, substrate solution (3.4 mg/ml orthophenylen diamine dihydrochloride, OPD in citrate buffer) was added and the enzymatic activity was stopped using 12.5% of sulfuric acid. The plates were analyzed at 490 nm using an ELISA spectrophotometer.

RESULTS AND DISCUSSION

The indirect ELISA assay described in this study was developed to detect the Pemphigus autoantibodies by using a semi-purified human

160 kDa

130 kDa

65 kDa

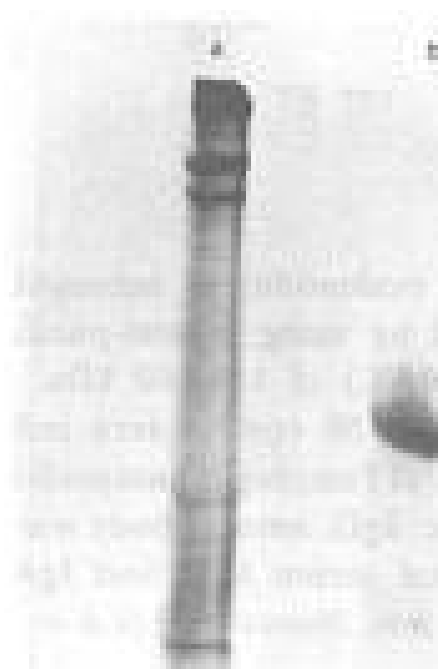


Fig.1. SDS-PAGE analysis of semi-purified epidermal extract (lane a), control protein (lane b).

epidermal extract with an approximately MW of 130-160 kDa (Figure 1).

The evaluation of Pemphigus IgG, IgM and IgA autoantibodies in 75 patients and 50 control sera indicate that this indirect ELISA assay is a reliable and reproducible method for the detection of Pemphigus autoantibodies. Data of this study indicate that the mean OD of specific IgG autoantibody was found to be elevated in PV patients compared with controls (Table1). No significant differences were detected statistically in the percentages of total serum IgM and IgA autoantibodies between patients and controls (Table 2). The PV was characterized by the presence of autoantibodies against epidermal cell membrane glycoproteins. It is a rare, however, potentially fatal blistering disease that affects the skin and mucous membrane. The role of autoantibodies on the pathogenesis of PV has been recently investigated using the IIF technique [9]. Since this technique lacks the sensitivity and specificity for the detection of autoantibodies in patients with early, localized disease, and also in patients under treatments. In this study, the reactivity of PV and PF sera with a semi-purified human epidermal extract as the source of antigen was examined by an ELISA assay.

Table 1. The mean and SD of the optical density (OD) of IgG autoantibody in patients and controls.

Parameter	Patients (n= 5)		Controls (n = 50)		p value
	Mean	SD	Mean	SD	
IgG	0.468	0.391	0.274	0.133	0.001

Table 2. The mean and SD of OD of IgM and IgA autoantibodies in patients and controls.

Parameter	Patients (n= 36)		Controls (n = 20)		p value
	Mean	SD	Mean	SD	
IgM	0.415	0.222	0.333	0.122	0.134
IgA	0.095	0.093	0.076	0.092	0.540

Our data indicate that the epidermal antigen is a major antigenic target of IgG autoantibodies produced by both PV and PF patients with early, localized disease and in patients under treatment. These results are consistent with previous finding [10] that ELISA assay can be a reliable technique to detect the bullous pemphigoid and PV autoantibodies. The detected autoantibodies described in our ELISA technique, carry IgG isotype predominantly. This study supports the relationship between the pathogenesis and well-defined antigen-antibody system in the PV patients. However, the development of an ideal ELISA system is required using recombinant proteins to facilitate the detection of PV antigen in the future.

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