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Serum Level of Antibodies Against Novel *Acinetobacter Baumannii* OmpA-selected Peptides in ICU Staff: Promise for the Future of Vaccine Development

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ABSTRACT

Extensively drug-resistant *Acinetobacter baumannii* is considered one of the most dangerous threats to global health, requiring novel therapeutic interventions. The outer membrane protein A (OmpA) is an immunogenic agent that triggers immune responses. The current study evaluated serum antibody levels against previously determined immunogenic OmpA peptides from *A baumannii* in ICU staff.

Serum samples were collected from 62 ICU staff members (representing the exposed group), healthy controls (representing the nonexposed group), and patients with systemic lupus erythematosus (SLE) (as controls for nonspecific antibody reactions). After excluding the cross-reactive antibodies via *Escherichia coli* lysate pretreatment, all the samples were assessed in the vicinity of *A baumannii* lysate by enzyme-linked immunosorbent assay (ELISA). All the positive samples were assessed for interaction with previously designed and selected peptides using ELISA. The protective potential of positive serum antibodies was surveyed in vitro using an opsonophagocytic study.

The most antibody positive samples against one of the dominant peptides were determined in the ICU personnel (75%). SLE serum samples did not react with candidate peptides. The strongest positive reaction was observed in serum treatment with one of the OmpA peptides (No. 5) with significant differences compared to other designed peptides. Our findings showed that ICU samples have substantially higher antibody levels than the nonexposed group; Positive samples show strong results in the opsonophagocytosiis assay.

This study demonstrates A baumannii colonization at human mucosal surfaces, especially in exposed healthy workers. Novel OmpA-derived peptides could be used to identify immunogenic

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vaccine candidates. Therefore, more studies are needed before this peptide and antibody levels are used in diagnosis, prevention, or treatment.

Keywords: *Acinetobacter baumannii*; ICU; Omp-A derived peptides; Serum antibodies

INTRODUCTION

Hospital-acquired infections are one of the main concerns in healthcare centers. The increased incidence of *Acinetobacter baumannii* infection has become a primary concern due to its rapid development, high mortality rates, and multidrug- and extensively drug-resistant strains.[1] This pathogen is the etiologic factor for a wide range of diseases, such as bacteremia, ventilator-associated pneumonia, urinary tract infections, meningitis, and surgical wound infections in hospitalized patients, especially in the intensive care unit (ICU).[2,3] Several studies on host immune reactions to *A baumannii* infection using mouse models have been carried out. Neutrophils and alveolar macrophages are essential in host defense against respiratory infections with *A baumannii*. Clinical and experimental reports show that, in addition to innate and specific cellular defenses, serum antibodies also play an important role in the control of the infection. Many attempts have been made to produce monoclonal antibodies to treat affected patients.[4,5,6,7]

Therefore, the need for non-antibiotic–based intervention strategies for this infection is critical. Vaccination or active immunization is one of the most effective strategies.[8] Currently, antibody-based immunotherapies are significantly used for prophylaxis and treatment of infections, autoimmune disorders, and malignancies,[9,10] and can provide protective immunity against *A baumannii*, so identifying an antigenic target for active and passive immunization is critical.[11]

So far, many studies have been conducted in this field, and 42 vaccine candidates have been introduced using reverse vaccinology and in silico techniques.[12] Furthermore, passive immunization with antibodies targeting outer membrane proteins (OMPs) has shown promising results.[1] OmpA appears to be a specific immunogenic protein among the many components of *A baumannii* antigens. This protein plays a crucial role in bacterial pathogenesis through biofilm construction, interaction with epithelial cells, induction of apoptosis, and complement inhibition and is an excellent candidate for a vaccine against this infection.[13] OmpA has already been demonstrated as a principal target of humoral immunity that can induce protective antibodies in a mouse bacteremia model.[14] Other components of *A baumannii*'s outer membrane, including outer membrane vesicles, have been studied for immunogenicity in mouse models and were shown to produce large amounts of antibodies against *A. baumannii*.[15] Due to such promising results, evaluation of immunogenicity against selected OmpA peptides concerning human antibodies is necessary. These antigenic factors can be used to design an antibody detection kit or may be helpful in therapeutic strategies.

In our previous study, we analyzed the dominant immunogenic peptides of OmpA by mass spectroscopy, which ultimately led to significant responses in our experimental models.[12,17,18] To complement the previous results, the reaction of human serum antibodies with peptides designed to identify an antigenic target was investigated for making a diagnostic kit or generating active or passive immunity. Additionally, we attempt to find out whether the serum antibodies of people in contact with this bacterium can react with these peptides. The results of this part of our research can be effective in finding a method to detect serum antibody levels in humans, in addition to therapeutic hopes.

MATERIALS AND METHODS

Serum Samples

The participants were examined in 3 separate groups containing ICU staff members (n=33), healthy controls (n=23), and systemic lupus erythematosus (SLE) patients (n=7). According to the following grouping:

Group1: ICU staff members were considered as exposed individuals regarding the high risk of bioaccumulation of *A. baumannii* in ICU (n=33). Group 2: nonexposed (healthy) individuals including housewives with no medical history of A baumannii infection or hospitalization in the ICU (n=23) as). Group 3: SLE patients were investigated for evaluating the possibility of cross-reaction of their autoantibodies to the bacterial antigens and selected peptides. The SLE patients were age and gender-matched with the

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participants in other groups. All of them had the main pathogenic factor including anti-dsDNA and they had not received any immunosuppressive drugs, which were the main inclusion and exclusion criteria.[19,20]

In order to take samples from all participants, written informed consent was obtained from patients and healthy individuals. Samples from these groups were evaluated for antibodies to both *A baumannii* and selected immunogenic peptides from the OmpA protein.

Bacterial Culture and Confirmation

Culture

A baumanii isolated from patients of a previous study,[12,13,14] was used. To obtain a pure colony, the bacteria were cultured and grown in Müller-Hinton agar medium, and finally, the colonies were confirmed by polymerase chain reaction (PCR). Briefly, a fresh culture of *A baumannii* and overnight cultured *E coli* at half McFarland (1.5×10^8 colony forming units [CFU]/ mL) was diluted with distilled water (1.5 times its volume) to decrease the bacterial content to 10^8 CFU/mL. The Bradford assay was used to determine the concentration of bacterial lysate protein.[18] The cultured bacteria were then confirmed by PCR of the 16S rRNA and *OXA-51* genes. The nucleotide sequence data reported in this study have been submitted to the GenBank sequence database and specified under the accession number: KY052003.1 for the *OmpA* gene.

***EColi* Culture and Lysate Preparation**

The intact bacteria were harvested after a 48-hour culture of the ATCC strain (25.922; American Type Culture Collection) in nutrient broth (Difco) to prepare a lysate from pure *E coli*. All the *E coli* bacteria that had grown were collected by centrifugation at 5000*g* for 20 minutes. The residual sediment was pelleted and washed 3 times in sterile phosphate-buffered saline (PBS) to prepare a 50%(v/v) stock suspension for serum adsorption.[21]

ELISA

Antigen Preparation

The bacterial lysate was used to prepare the antigen for the testing and evaluation of serum antibodies by enzyme-linked immunosorbent assay (ELISA). Briefly, an appropriate amount of fresh bacterial culture (10^8 CFU / mL) was prepared. To prepare the antigen, power density, and pulsing, we used a sonicator with the frequency of 55 kHz and time interval of 0.5 seconds between each voltage. The Bradford method was used to determine protein concentration.

Pre-adsorbed Serums

Since the presence of antibodies against the *Enterobacteriaceae* family can cause cross-reactions in this study, serum samples were exposed to *E coli* bacterial lysates to remove these antibodies and increase the accuracy and specificity of the test.[21,22,23] Since during this process, antibodies against *E coli* precipitate as immune complexes, the supernatant without *E coli* antibodies was used. Briefly, serum samples (0.5 mL) were incubated with *E coli* lysate (0.5 mL) at 37°C for 2 hours with gentle shaking. Then the above mixture was incubated overnight at 4°C. After incubation, the specific antibodies to *E coli* antigens were removed by centrifugation (400*g*, 10 min), and the supernatant was collected as residual serum used for anti-*A baumannii* and selected OmpA peptide reactions.

Detection of Positive Anti-*A Baumannii* Serum Antibodies

Serum samples from the 3 study groups were screened by ELISA using appropriate concentrations of *A baumannii* lysates measured in different serum sample dilutions (1:10, 1:20, and 1:100 serum dilutions; 2, 5µg/mL of bacterial lysates and serum dilution). ELISA was performed according to the protocol of a previously published work.[17,18] Briefly, 96-well plates were coated overnight at 4°C with the above concentration of bacterial lysates per well. The plates were washed and blocked by incubation in the presence of 200 µL of bovine serum albumin (0.5%) for 2 hours. After 3 washes, the plate was incubated with the appropriate dilution of serum samples containing high antibody titers against the selected peptides or serum samples from improved patients, confirmed by immunoblotting for anti-Acinetobacter, and developed with tetramethylbenzidine substrate.[17,18]

The remaining test steps were completed using horseradish peroxidase-conjugated anti-human IgG (DAKO/Lot: P021402128401). Absorbance was measured at 450/620 nm.[16]

The ELISA titer was calculated as the mean optical density (OD) of triplicate wells in relation to the last serum dilution for which the OD could be measured. This was done based on the cut-off level found in serum from the healthy group.

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Detection of Serum Reactive Antibody against OmpA-selected Peptides

According to a previous study,[17] five Omp-A protein-derived peptides were available. Its immunogenic properties have been proven by previous research. To investigate its antigenic properties in dealing with human sera, it was necessary to study the reaction of the human sera with these peptides. The test was performed with various serum dilutions (1:10, 1:50, and 1:100) and appropriate concentrations of peptides (2 and 3 µg/mL). The mean OD was calculated for the samples.

Bioinformatics Study of the Selected Peptides

According to a previous study,[17] the predicted peptides are shown in Table 1.

Analyses of Peptides Structure

The structural OmpA immunogenic peptides were predicted using the FOLD-PEP server (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/).[24] This server can predict the folding of peptides ranging in length from 5 to 50 amino acids.

Table 1. The predicted peptide sequences

Name	Sequence	Length	Position*	Tcell-MHCII Percentile Rank**
P18	YVLLGAGHYKYDFDGVNR	18	122-139	0.09
P16	LRVFFDTNKSNIKDQY	16	230-245	0.16
P27	VTVTPLLLGYTFQDSQHNNGGKDGNLT	27	24-50	0.2
P26	HLKPAAPVVEVAPVEPTPVAPQPQEL	26	197-222	0.23
P30	**ICLTGGG**LRVFFDTNKSNIKDQY**GGGTLCI**	30	18-33	0.06

*Numbers of the beginning and the end of the predicted peptides

**Low adjusted rank = good binders

Peptide-antibody Binding Evaluation

Using the HPEPDOCK server (http://huanglab.phys.hust.edu.cn/hpepdock/), 25 the prediction of the binding of the human IgG heavy-chain variable fragment to the peptide was investigated. This fragment with 116 amino acids was released in UniprotKB-P01825. There was a disulfide bond at position 41-114, and its identifier in the PDB databank is 7CZP. The docking result was evaluated by the PDBsum server (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/UploadPDBfile.pl).

In Vitro Complement Susceptibility and Opsonophagocytosis Assays

To measure opsonophagocytosis, we used the HL60 cell line and neutrophils.

The opsonophagocytosis test was performed using a serum sample with a high antibody titer in response to *A baumannii* proteins and a designed peptide from the OmpA protein. Sandoglobulin (MEDICA/CSL Behring; ATC: J06BA02) was used as a positive control with a dilution similar to that of the positive sera.

Four groups were used as controls: a) bacteria and cells (HL60 and neutrophils) without adding rabbit complement and serum; b) bacteria and serum sample without adding rabbit complement and cells; c) bacteria without adding rabbit complement serum and cells; d) bacteria with rabbit complement without adding serum and cells.

Cell Culture

We performed the opsonophagocytosis assay in both cell culture systems, including the HL60 cell lines and neutrophils obtained from healthy individuals to complete the test and ensure the results.

HL60 Cell Line and Differentiation

The differentiation method of HL60 cells has previously been described.[26,27] In brief, HL60 cell lines were incubated at 37°C in 5% CO_2 in RPMI 1640 (Irvine Scientific, Santa Ana, CA, USA) with 10% fetal bovine serum, 1% penicillin, streptomycin, and glutamine (GeminiBioProducts). At the confluency of 70% to 75%, the cells were differentiated by an 8-day coincubation of N, N-dimethylformamide 1 (1.55 µL). HL60 differentiation was confirmed morphologically by

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Giemsa staining and the nitro blue tetrazolium test. The HL60 cell lines were harvested, and 40 µL of cell suspension was added to microtiter wells (at 4×10^5/wells concentration).

Neutrophils

Neutrophils were separated from heparinized blood samples of the healthy participants using dextran sedimentation, [28] Neutrophils (1×10^6 per well) were suspended in Hanks' buffer. Suspension cells (40 µL) were then added to microtiter wells (1×10^6 per well). In this experiment, neutrophils from 2 different healthy individuals were used.

Opsonophagocytosis

Either the differentiated HL60 cells (40 µL; 4×10^5 per well) or neutrophils (40 µL; 1×10^6 per well) were added to bacteria (20 µL; 1000 CFU) round bottom wells at a ratio of 400:1 for HL60 (bacteria to HL60) and 500:1 (bacteria to neutrophils) with or without 20 µL of sera positive for OmpA-selected peptides (of different dilutions, 1:32 to 1:512) that were or were not heat-inactivated. 10 µL of rabbit complement solution was added to each well, and the plates were incubated for 45 min at 37°C. Sandoglobulin (Brand: CSL Behring) was used as positive control (this drug is used as intravenous immunoglubulin therapy). It has been used for opsonophagocytosis in the presence of opsonic antibodies against a large number of extracellular bacteria such as pneumococci and Haemophilus influenzae type b (Hib) among others. After incubation at 37°C with gentle shaking, 80 µL of the solution from each well was plated on Müller-Hilton agar and incubated at 37°C for 18 to 24 hours. The number of live and nonphagocytosed bacteria was calculated to determine the reaction rate. Viability was calculated as the ratio of CFUs in the presence of HL60 to CFUs in the absence of HL60.

Opsonophagocytosis methodd has been described previously.[26,27] In brief, *A baumannii* was cultured overnight in Müller-Hinton Agar at 37°C. Then 1 or 2 bacterial colonies were inoculated with physiological saline to the concentration of 0.5 McFarlane. Then, at the exponential (log) phase, the bacteria were washed twice with Hanks' buffer. Then 20 µL of suspended bacteria in Hanks' buffer was added into each well of a 96-well plate.

Bacterial Viability Assay

In order to determine the non-opsonized bacteria in the estimation of the killing of the serum antibodies after 12 hours and to determine by CFU calculation. CFU for all tubes were normalized to the average CFUs of the tubes with control serum, and the amount of killing was calculated (CFUs from the individual tube/average CFU of tubes with control serum).

Statistical Analysis

The calculated data are presented as mean±SD. The main score of the results was compared with the independent-sample t test, and the results are reported as the average standard error. Rock diagrams were used to determine the cut off. *p* values of less than 0.05 were considered statistically significant. The graphs were drawn using MS Excel 2010 (Microsoft Co., Redmond, WA) and analyzed in IBM-SPSS version 23.0 (IBM Corp., Armonk, NY, USA). The receiver operation characteristic curve (ROC curve) was applied to analyze the efficiency of diagnosing patients' disease stages. The data established from opsonophagocytosis were tested for significant differences using the Kruskal–Wallis test, followed by Dunn's multiple comparison test.

RESULTS

Peptide Folding and In Silico Interaction with Antibody Structure

The binding of designed peptides to human IgG heavy chain variable fragment (7CZP) was investigated using the HPEPDOCK server for each peptide, and 10 of the best conformation models to bind the antibody were predicted. The docking number is specified for all modes. A negative docking number indicates a lower energy level and greater stability for the peptide to bind to the antigen-binding fragment of the antibody. The docking number for all 5 peptides was in the same range from −180 to −219 (Figure 1), and the docking score was obtained at −214.555.

Based on the PROCHECK statistics on Ramachandran plot data, the most favored regions were 90.9%. From the 30 amino acids, the number of glycine residues was 6. G-factor scores of P30 in interaction with IgG heavy chain variable fragment are shown in Table 2.

Demographic Findings

The demographic characteristics of the participants are shown in Table 3.

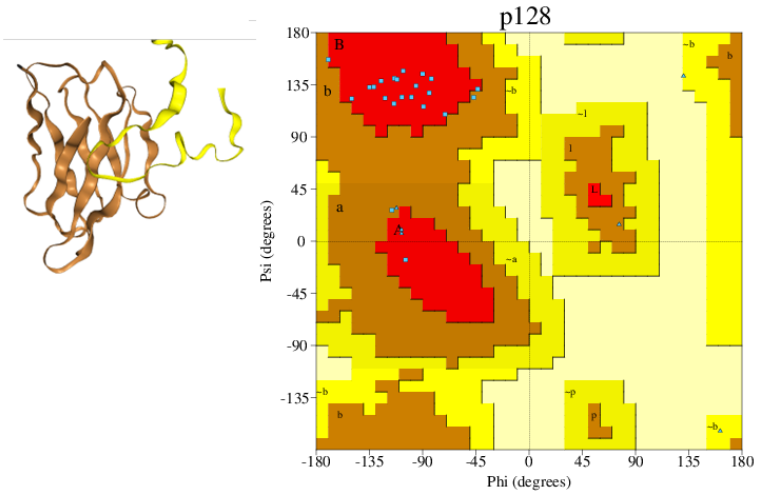


Figure 1. 3D structure of P30 in interaction with IgG heavy chain variable fragment according to HPEPDOCK (left). Ramachandran plot of the structure (right).

Table 2. G factor parameters

Parameter		Score	Average Score
Dihedral Angles	Phi-psi distribution	−0.50	
	Chi1-chi2 distribution		
	Chi1 only	−0.20	
	Chi3, chi4	0.31	−0.23
Main-Chain Covalent Forces	Main-chain bond lengths	0.64	
	Main-chain bond Angles	−0.30	
Overall Average			−0.04

Table 3. Demographic specifications of the study groups; SLE, systemic lupus erythematosus

Study Groups	Age Average	Sex (Female/Male)
Exposed	34±6	29/4
Nonexposed	30±4	9/13
Patients with SLE	32±13	7/0

Use of Optimum Cut-off Values of Laboratory Results

The cut off values computed by the ROC curve and the optimal cut off values were 0.74 for the non-exposed group (specificity, 73%; Sensitivity, 76%). The ROC curves are presented in Figure 2A. According to cut off values, of the 33 ICU nurses, 25 samples were positive, and 8 were negative. In the control group (n=22), samples from 6 (27%) of the participants were positive and 16 samples were negative (Figure 2B). The average OD level of positive samples was equal to 1.3±0.45.

Analysis of Antibody Reaction to *A. baumannii* Lysate Antigens in Positive Samples

Initially, the serum from both the exposed and nonexposed groups were treated with E coli lysate to remove cross-reactive antibodies. Comparison between the OD of pre-adsorbed and non-adsorbed samples in both the exposed and non-exposed groups showed that the OD of the pre-adsorbed group was significantly lower than that of the non-adsorbed group (0.97±0.56 vs. 1.38±0.69 nm, respectively, for all samples) (p=0.001). These results for the exposed group in the pre-adsorbed and non-adsorbed state were equal to 1.68±0.61 vs. 1.18±0.56, respectively. These results for the non-exposed group in the pre-adsorbed and non-adsorbed states were equal to 0.68±0.36 vs. 0.94±0.55, respectively. These findings indicated that the elimination of cross-reactive anti-E coli could reduce false positive results with *A baumannii* lysate and ultimately increase the specificity of the test. These findings are shown in Figures 3A and 3B.

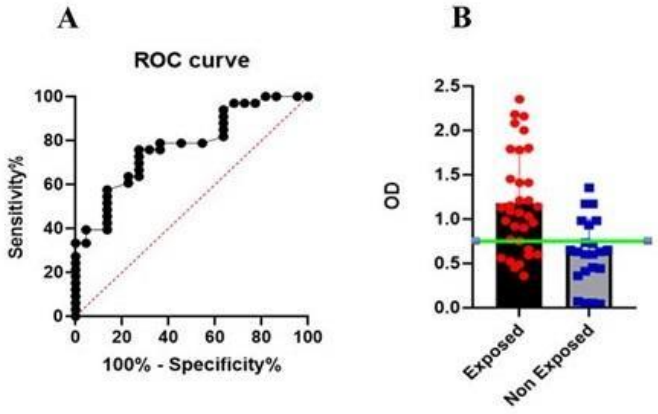
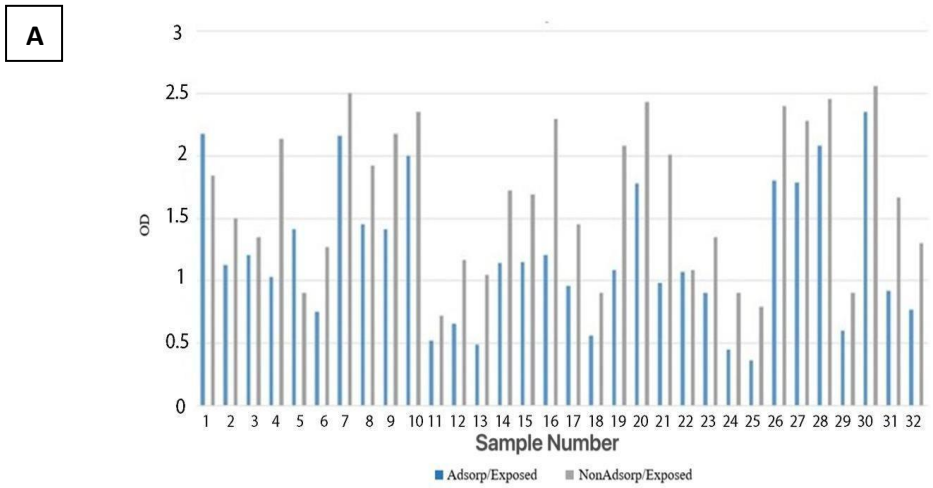


Figure 2. Calculating the cut-off values using the ROC curve. Serum antibody levels against *Acinetobacter baumannii* in the exposed and nonexposed groups with the cut-off value of 0.74 (according to the ROC curve) shows that the number of antibodies was higher in the exposed group (not significant). The average OD of positive samples was equal to 1.33±0.45. OD, optical density; ROC, receiver operating characteristic.



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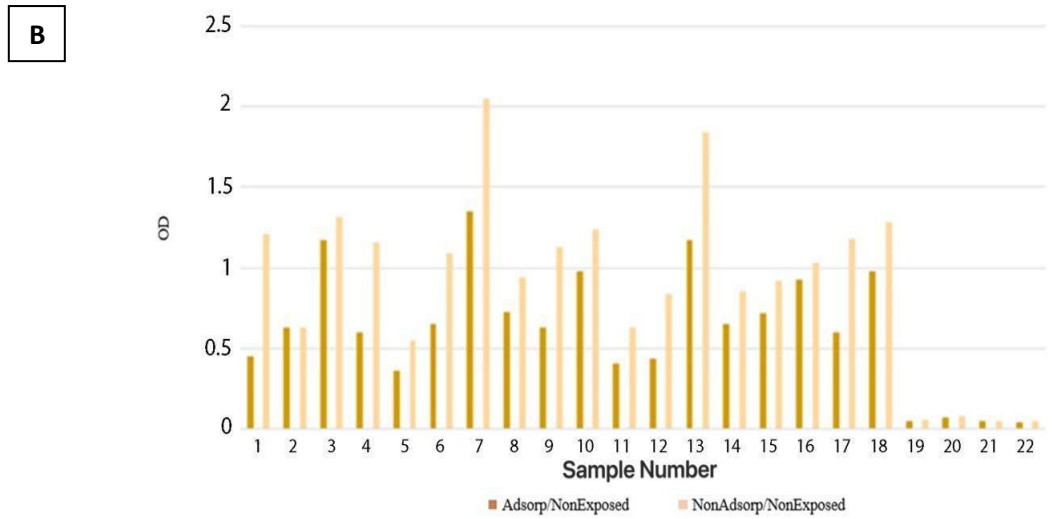

Figure 3. Comparison between the OD of pre-adsorbed and non- adsorbed samples A) in the exposed groups and B) non-exposed groups. The data illustrates that the OD of the pre-adsorbed group was significantly lower than that of the non-adsorbed group in both exposed (mean±SD: 1.68±0.61 vs. 1.18±0.56; *p*=0.001) and non-exposed (0.68±0 .36 vs. 0.94±0.55; *p*=0.001) samples.
OD: Optical density.

Antibody-peptides Reaction Analysis in Positive Samples to Selecting OmpA Peptides

After cross-reactive antibody elimination, the presence of anti-designed peptides was investigated in the positive serum samples in response to *A baumannii* lysates. According to the determined cut-off, the ELISA results from positive serum samples with bacterial lysate showed that only peptide 5 had a significant reaction with positive serums. Accordingly, this peptide seems the most immunogenic compared to others. There were no significant differences between the reaction to the *A baumannii* lysate or P30 in the pre-treated samples. Therefore, all serum samples positive to *A. baumannii* lysate were able to detect peptide 5. There was no difference between the mean of OD levels in each reaction (p=0.948). Other peptides did not induce a significant response (Figures 4). Negative reaction in the pre-adsorption state to P5 was also confirmed by testing the samples against P30 (as a whole extract of *A. baumannii)* (p=0.956).

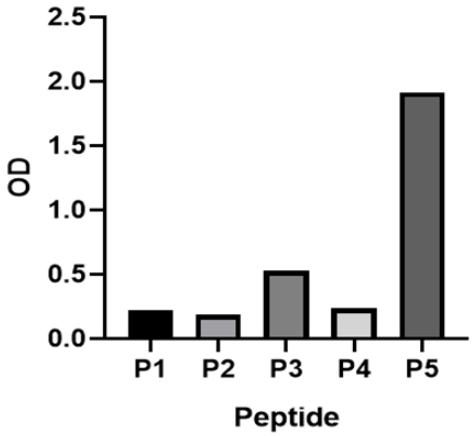

Figure 4. Assessment of 5 immunogenic peptides of the OmpA protein designed from A. baumannii with positive sera in terms of the presence of antibodies against bacterial lysates showed that peptide number 5 (P5) induced the highest response in terms of the presence of antibodies. The other peptides did not induce a significant response.

Pretreatment with *E coli* in Response to Peptides

There was no significant difference in the reaction to peptides between the *E coli*-pretreated samples compared with the non-pretreated samples (OD=1.331 vs. 1.331 nm; p=0.975).

Serum Antibody Reaction of SLE Patients to Peptide 5

There was no difference between the serum of the SLE patients with positive serum related to *A baumannii* lysate in reaction to P30 (p=0.929).

Opsonophagocytosis Assay

The opsonophagocytosis assay was performed using 9 serum samples that reacted with A baumannii lysate and peptide No. 5 (designed from the OmpA protein of this pathogen) which showed a high antibody titer and neutrophils from two different individuals in two groups (Group 1 with 4 samples and Group 2 with 5 samples), whose results were analyzed separately. This test was also performed using 4 positive samples and the HL60 cell line.

Serum samples were measured as normal human serum and heat-inactivated serum to assess the opsonization power of antibodies in the presence and absence of complements.

We also found that serum dilution plays a role in the process of killing bacteria with opsonophagocytosis, since in the serum dilution of 1:256, antibodies had the greatest ability to destroy bacteria with the help of neutrophils in the presence and absence of complements, and cultured bacteria in this dilution showed the lowest CFU numbers. The results showed that opsonophagocytosis was increased in the presence of complements compared to when the serum was inactivated. However, the results showed that decomplemented sera are also capable of opsonophagocytosis. The opsonophagocytosis results using the HL60 cell line are shown in Figure 5A; the opsonophagocytosis results using the neutrophils of the first person are shown in Figure 5B and those of the second person are shown in Figure 5C.

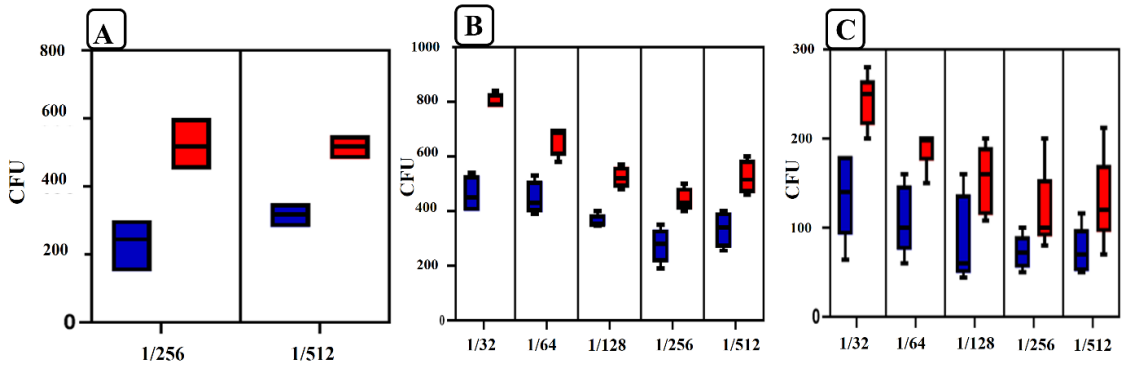

Figure 5. Opsonophagocytosis was higher in the presence of serum (Blue) and heat-inactivated complement (Red) serum. The use of the HL60 cell line (A) and neutrophils of the 2 healthy different individuals (B and C) show that opsonophagocytosis was superior in the presence of complements compared to inactivated serum. However, the results showed that decomplemented sera are also capable of opsonophagocytosis.

DISCUSSION

A baumannii is currently considered the most important antibiotic-resistant infection in clinical medicine. Due to the lack of effective antibacterial therapies for these fatal infections, identifying alternative therapeutic approaches is critical for this healthcare issue (29). Therefore, vaccination for the prevention of such infections and antibody-based immunotherapy might help minimize the burden of this pathogen.[8]

Numerous studies have investigated the role of the immune response, especially antibodies against *A baumannii*.[29,30] Specific antibodies can neutralize toxins

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and activate complements and phagocytosis. Currently, passive immunity with antibody administration as antibacterial, antiviral, and anti-inflammatory factors has been widely applied for infectious, autoimmune, and tumor immunotherapy.[1,31] Recently, the important role of serum levels of specific antibodies in protection against *A baumannii* has also been considered.[15,32] Our previous research on ICU nurses and healthy subjects presented that 8 out of 20 nurses had antibodies against *A baumannii* at serum dilutions of 1:50, and that all controls had negative serum antibody levels.[33]

Promising animal studies have shown that immunization with *Acinetobacter baumannii*-OmpA produced high antibody response against *A baumannii*.[34] In a recent work by Mehdinejadiani et al,[17] 5 OmpA-derived peptides were perfused into 3-week-old C57BL/6 mice; then the opsonophagocytic activity in the serum of immunized animals and cytokine production of splenocytes were evaluated. The results showed that activated spleen cells in P127 peptide-immunized mice represented enhanced interferon-gamma levels compared with the control groups. Moreover, the study demonstrated a proper killing activity of the antibodies produced against *A baumannii* in a dose-dependent manner.

These findings suggest that antigenic agent identification for active and passive immunization is necessary. Among various antigenic peptides, OmpA is one of the most important ones involved in bacterial survival, biofilm formation, immune response stimulation, inflammation, apoptosis, and antimicrobial resistance. OmpA is highly conserved (>80%) among other strains and has little similarity with the human proteome; therefore, humoral immune induction by this peptide makes it an ideal vaccine candidate.

OMPs are usually located on the cell surface and are easily detectable by antibodies. Therefore, the identification of its immunogenicity will be of great importance.[16,34] In addition, anti-OmpA antibodies enhance immunity by increasing opsonophagocytosis.[16]

Previous studies have identified the OmpA antigenic peptide as a potential immunogen candidate.[35] In an earlier study, we identified the OmpA sequence, and its immunogenic peptides were determined by online B and T cell prediction software.[16,17] Then, 5 selected immunological peptides with Al $(OH)_3$ as adjuvant were injected into C57B/6 mice subcutaneously.[16] Results of this study demonstrated that both peptides 5 and 2 had an important role in antibody production.[16]

According to the promising results of the abovementioned study, we decided to investigate the immunogenicity of these peptides in relation to human antibodies. These antigenic peptides may be useful as detection kits or in treatment strategies for patients with *A baumannii* infection. Accordingly, serum samples from ICU nurses who are in long-term contact with patients and the main sources of this infection were used.

In the first step, in the bioinformatics study, the binding of designed peptides to human IgG heavy chain variable fragment (7CZP) was investigated; docking scores for all 5 peptides ranged from −180 to −219.[36]

In the experimental step, some samples were treated with *E coli* lysate to remove cross-reactive antibodies. The results showed that pretreatment with these antigens removes cross-reactive antibodies. Then, the sera positive in response to *A baumannii* were screened, and ELISA was performed for the 5 candidate peptides. The results showed that despite the positive number of some nonexposed individuals, there is a remarkable diversity between the ICU staff and the control group. The results from peptide 5 treatment were consistent with our previous study; this peptide had the greatest role in the C57BL/6 immunization and protective antibody production.[16]

There was no significant difference in the anti-P30 detection between the *E coli*-pre-treated and non-treated samples. This finding indicates that the serum antibody to *A baumannii* is specific toP30, and this peptide has a strong antigenic structure.

Due to the polyclonal activity of their lymphocytes, we had to prove that the serum of SLE patients lacks antibodies with cross-reactivity with peptides. This study intended to confirm the specificity of the antibodies related to the samples under investigation.[19,20] Our results also showed that despite exposure to infected sites, patients with SLE had a positive reaction to the candidate peptide.

This finding provokes two hypotheses: 1) these patients had antibodies, just like nonexposed individuals with weak responses to the selected peptides; 2) These patients have a positive cross-reaction with this peptide due to an impaired humoral immune response–the latter is more controversial; it can be concluded that this peptide may not be very specific for some samples, which can cause problems in the preparation of diagnostic kits. Minimizing cross-reactivity could optimize these kits, but this hypothesis should be

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investigated further with more samples from patients with autoimmune diseases.

Protective factors, including the level of serum antibodies and complements, are critical in the opsonization and phagocytosis processes. Since *A baumannii* is a conditioned pathogen and rarely invades host cells, the specific antibody production to enhance opsonophagocytosis and subsequently reduce bacterial survival can be an effective approach to design vaccines.[35]

Opsonophagocytosis is the primary host defense mechanism against bacterial infections and can be used as a measure of functional antibodies.[26] *A baumannii* is one of the most frequently isolated gram-negative bacteria in neutropenic patients.[37] Increased neutrophil counts were seen in the bronchoalveolar lavage fluids of mice after 24 hours of pulmonary infection. Decreased neutrophils using monoclonal antibodies are associated with reduced survival in mice.[38] We used human neutrophils and the HL60 cell line for the opsonophagocytosis assay.

In this study, to determine the opsonophagocytosis assay mediated by OmpA anti-sera, positive samples were evaluated using human neutrophils as well as the HL60 cell line. The HL60 cell line can proliferate in suspension and differentiate into multiple cell lineages.[26]

The results of in vitro opsonophagocytosis assay may indicate the antibacterial function of anti-peptide antibodies (against selective peptides in the present study) generated in *A baumannii*-exposed individuals.

Many studies have been conducted in the field of antibody-based immunotherapy on the role of antibodies in preventing fatal infections caused by this bacterium, including approaches in the production of monoclonal antibodies. Recently, monoclonal antibodies have been produced against the same peptides whose immunoreactivity has been investigated in this report. These studies show that these peptides have a high immunogenicity. This needs further confirmation with opsonophagocytosis assays.[6,7]

After determination of serum reactive samples against designed peptides, we used bioinformatics tools to predict the binding of the human IgG heavy-chain variable fragment to the peptide was investigated. The antigen-binding energy to human antibodies (docking) was very similar in all five designed peptides. Because we selected the best sequences of peptides using a bioinformatics assay, this docking number similarity was not unexpected. However, P30 with the ICLTGGGLRVFFDTNKSNIKDQYGGGTLCI sequence had the highest reaction with human serum antibodies. In addition, this peptide has a suitable spatial for antibody detection due to the presence of 3 glycine amino acids among its primary and terminal sequences. This peptide has more leucine and isoleucine amino acids compared to other peptides, which effectively stimulate the humoral immune system.

Different parts of *A baumanii* cell wall contents– especially OmpA, which is the major cell wall protein– have attracted increasing attention for the design of therapeutic solutions. Undoubtedly, the immunogenic potencies of these antigens, primarily based on the peptides derived from them, can lead not only to the ideas of vaccine production or therapeutic monoclonal antibodies but also to the serum levels of antibodies in exposed people. Our findings showed that not only do the ICU staff, due to frequent exposure to *A baumannii*, have high serum antibodies, but also nonexposed individuals have low antibodies.

These findings could indicate an increasing spread of bacterial colonization at human biotic levels. However, proof of this theory requires a much larger number of exposed samples. Because this study demonstrated the presence of serum antibodies specific for the OmpA-derived peptide, more comprehensive studies of these peptides could provide a suitable laboratory kit to determine the safety of people at risk or hospitalized, especially in the ICU.

STATEMENT OF ETHICS

In order to take samples from all participants, written informed consent was taken from patients and healthy subjects. All participants in the study were administered in agreement with the ethical standards of the Ethics Committee at Shahid Beheshti University of Medical Sciences (IR.SBMU.MSO.REC.1397.650) and with the revised Helsinki Declaration in 2000. Samples from these groups were evaluated for antibodies to both *A. baumannii* and selected immunogenic peptides from the ompA protein.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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