Journal of Medical Microbiology and Infectious Diseases

JoMMID
ISSN: 2345-5349
eISSN: 2345-5330

Detection of Anti-IgGs against Heat Shock Proteins 27 and 20, HP91 Peptide, and HIV-1 Polypeptides in HIV-Positive and Negative Patients

Alireza Milani[1], Kazem Baesi[1], Elnaz Agi[2], Azam Bolhassani[1*]

[1]Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran; [2]Iranian Comprehensive Hemophilia Care Center, Tehran, Iran

ARTICLE INFO

Original Article

Keywords: HIV-1 proteins, Heat shock proteins, HP91 peptide, Antibody detection

Received: 25 Oct. 2020
Received in revised form: 11 Nov. 2020
Accepted: 29 Nov. 2020
DOI: 10.29252/JoMMID.8.3.113

***Correspondence**
Email: azam.bolhassani@yahoo.com; A_bolhasani@pasteur.ac.ir
Tel: +982166953311 Ext. 2240
Fax: +982166465132

ABSTRACT

Introduction: A simple and sensitive diagnosis method is needed to identify HIV infection in sera of untreated, treated, and drug-resistant patients. The purpose of this study is to determine whether heat shock proteins (Hsp)-27 and -20 and HP91 peptide along with HIV-1 polypeptides can serve as potential biomarkers to distinguish HIV infection in untreated, treated, and drug-resistant individuals compared to HIV-negative subjects. **Methods**: At first, human sera were obtained from 141 participants, including 20 naïve HIV-infected, 71 treated, 30 drug-resistant, 20 HIV-negative (healthy/control) individuals. The recombinant Hsp27, Hsp20, and five designed HIV-1 polypeptides were expressed in *Escherichia coli* and purified by affinity chromatography under denaturing or native conditions. Finally, the antibodies against these antigens were quantified in sera using ELISA. **Results**: Our data showed that HIV-infected patients significantly displayed higher serum levels of anti-Hsp27, anti-HP91, and anti-Nef-Tat-Gp160-P24, anti-Nef-Vpr-Gp160-P24, anti-Nef-Vif-Gp160-P24, anti-Nef-Vpu-Gp160-P24, and anti-Nef-Rev-Gp160-P24 polypeptide antibodies than healthy groups ($p < 0.05$), but not for anti-Hsp20. Moreover, the serum levels of antibodies against Hsp27, Hsp20, HP91, and HIV-1 polypeptides were not statistically significant between different groups of patients ($p > 0.05$). **Conclusion**: The levels of anti-Hsp27 and anti-HP91 antibodies in serum increased in HIV-1 seropositive subjects along with antibodies against five HIV-1 polypeptides suggesting their potential value as a diagnostic marker for HIV-1 infections.

INTRODUCTION

Human immunodeficiency virus-1 (HIV-1) is the primary cause of AIDS in the world. HIV encodes three significant genes (*gag*, *pol*, and *env*), four accessory genes (*vpr*, *vpu*, *vif*, *nef*), and two regulatory genes (*rev* and *tat*), which play an essential role in the replication and pathogenesis of the virus [1]. The high genetic variability of HIV-1 has led to some problems in the prevention, diagnosis, and treatment of AIDS [2]. Thus, scientists are trying to determine the conserved and immunogenic epitopes using bioinformatics tools in all populations to develop HIV therapeutic vaccine and diagnosis [3]. At present, HIV is diagnosed by various immunological and molecular techniques. The enzyme-linked immunosorbent assay (ELISA) is a standard method of HIV detection [4]. The diagnosis of HIV infection is usually assessed by serological detection of antibodies against HIV *gag*, *pol* and *env* structural proteins, especially P24 assay. However, some reports showed antibodies against other viral proteins (*e.g.*, Nef) among seronegative high-risk HIV individuals before developing antibodies to viral structural proteins [5]. On the other hand, the endogenous heat shock proteins (HSPs) as molecular chaperones are highly conserved among different species [6]. HSPs were classified based on molecular size, sequence similarities, location within the cell, and function. HSPs fall into six prominent families depending on their molecular weights, including HSP40, HSP60, HSP70, HSP90, HSP100, and small HSPs [6-8]. The Hsps play a significant role as biomarkers in infectious diseases. Among HSPs, small Hsps (~ 12-43 kDa), Hsp20 and Hsp27, are discriminated by the presence of a highly conserved sequence (80-100 amino acids) known as the "α-crystallin domain." The small HSPs were implicated in the pathogenesis of various autoimmune-mediated

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.8.3.113]

disorders and cancer [9-12]. The expression of HSPs up-regulates under environmental (*e.g.*, high temperature or oxidative stress), biological (*e.g.*, cell proliferation and differentiation), and pathological (*e.g.*, inflammation and tumor growth) stress conditions [13]. Moreover, virus interaction with HSPs showed essential roles in regulating viral infections, including cell entry and nuclear import, viral replication and gene expression, folding/assembly of viral protein, apoptosis regulation, and host immunity [14]. On the other hand, high-mobility group box 1 (HMGB1) is a proinflammatory molecule that translocates to the cytosol and is then secreted into the extracellular environment. Extracellular HMGB1 is actively secreted by innate immune cells (*e.g.*, macrophages, neutrophils, and monocytes), acts as a proinflammatory cytokine, and releases passively during cell injury or death [15]. The cytokine-inducing part of the HMGB1 molecule is among the first 20 amino acids of the B-box domain (aa 89-108) known as HP91 peptide [16]. As compared to normal healthy cells, a variety of solid tumor cells showed increased expression and secretion of HMGB1, especially in inflammation-associated cancers [15]. This study aimed to assess anti-Hsp27, anti-Hsp20, and anti-HP91 antibody levels in the sera of untreated, treated, drug-resistant individuals. We further evaluated whether the immunogenic HIV-1 polypeptides can serve as potential biomarkers alongside Hsp27, Hsp20, and HP91 peptide to distinguish untreated, treated, drug-resistant groups compared to the healthy/control group.

MATERIAL AND METHODS

Generation of HIV-1 polypeptides in large scale. Five polyepitope DNA constructs were designed using bioinformatics tools such as Nef-Tat-Gp160-P24, Nef-Vpr-Gp160-P24, Nef-Vif-Gp160-P24, Nef-Vpu-Gp160-P24, and Nef-Rev-Gp160-P24. Briefly, the reference sequences of HIV-1 proteins were obtained from the UniProt database (www.uniprot.org) and compared with the sequences recorded in the HIV Molecular Immunology Database. All immunization sequences recorded in the database were extracted from the Immune Epitope Database (IEDB). The peptide sequences were checked if they bind to class I and II MHC molecules by NET MHC database, and the selected epitopes were categorized. Processing (TAP transport and Proteasomal cleavage) of the selected epitopes were predicted using the IEDB database. The selected epitopes were compared with the previously reported sequences that had better scores, and finally, dominant epitopes were selected for each protein. Then, the selected epitopes were checked if they bind to B cells. Epitope Conservancy Analysis among HIV subtypes and population coverage was performed using the IEDB website. The interaction between the selected epitopes and MHCs was studied by the peptide-protein docking websites (3, 17-19). Finally, the peptide constructs were designed using the selected epitopes. According to the Qiagen protocol, all polyepitope DNAs were subcloned into a pET expression vector and expressed in the *E. coli* strain. The polypeptides were purified by affinity chromatography using Ni-NTA column (Qiagen), dialyzed, and assessed by NanoDrop spectrophotometry. All conditions of the generated polypeptides were briefly mentioned in Table 1. The sequences of five polypeptides were shown in Figure 1.

Preparation of HP91 peptide. The HP91 peptide (DPNAPKRPPSAFFLFCSE) with N-terminal biotin was synthesized and purified by Bio-Matik Co. (Canada; (20)). The peptide was dissolved in PBS 1X for ELISA.

Generation of the recombinant Hsp27 and Hsp20 proteins in large scale. The recombinant Hsp27 and Hsp20 proteins were previously provided by our group (16). Herein, we generated them on a large scale based on previous conditions, as shown in Table 2**.**

Sample collection. In a cross-sectional study, untreated, treated, and drug-resistant participants (test group) were collected from the Infectious Disease Department of Imam Khomeini Hospital (Tehran, Iran), and maintained in Biobank at Pasteur Institute of Iran. They were older than 18 years of age and included untreated (naïve/ infected) and treated by antiretroviral drugs (one NNRTI or one PI in combination with two nucleoside reverse transcriptase inhibitors (NRTI) for at least one year). Moreover, the National Institute for Medical Research Development (ethical code: IR.NIMAD.REC.1398.329) approved the study protocol, and informed written consent was obtained from all 141 participants before blood collection. We considered 71 treated, 20 naïve HIV-infected (untreated HIV-infected), 30 drug-resistant, 20 HIV uninfected (HIV-negative) individuals in each group. Baseline demographic and adherence data were collected through the physician interview. In brief, whole blood samples were collected in sterile EDTA-containing tubes, and after centrifugation, serum samples were aliquoted and immediately stored at -80°C.

Detection of IgG antibodies against proteins, polypeptides, and HP91 peptide. For detection of IgG antibodies against the recombinant proteins and polypeptides, 96-microwell plates (Nunc, Germany) were individually coated overnight at 4°C with 100µl of the recombinant (r) Hsp27 (3 µg/ml), rHsp20 (3 µg/ml), rNef-Vpu-Gp160-P24 (3 µg/ml), rNef-Vif-Gp160-P24 (3 µg/ml), rNef-Vpr-Gp160-P24 (3 µg/ml), rNef-Rev-Gp160-P24 (3 µg/ml), rNef-Tat-Gp160-P24 (3 µg/ml), and HP91 peptide (5 µg/ml). It should be mentioned that the dose of proteins/polypeptides/ peptides as an antigen, as well as the concentration of IgG antibody, were determined by titration on four samples of four groups and applied for all samples. The plates were then blocked with 1% BSA in PBS for 2 h at 37 °C and incubated with 100 µl of human sera diluted 1:100 in

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.8.3.113]

blocking buffer containing 0.05% (v/v) Tween 20 for 2 h at 37 °C. The bound human antibodies were detected by goat anti-human IgG conjugated to horseradish peroxidase (HRP, Sigma, 1:10000) and then the addition of 3,3′,5,5'-Tetramethylbenzidine (TMB, Sigma) as the substrate. The enzyme reaction was stopped by adding 50 µl of sulphuric acid (1M), and the absorbance was determined at 450 nm.

Table 1. Generation of HIV-1 polypeptides

DNA construct	DNA size	Polypeptide	Expression system	Cloning site	Conditions (temperature, OD_{600}, IPTG dose, time)	Polypeptide size	Purification conditions
pUC-nef-rev-gp160-p24	~ 888 bp	Nef-Rev-Gp160-P24	pET24a (+)/*Rosetta*	*Bam*HI/*Hind*III	(37°C, 0.6-0.7, 1mM, 3 h)	~ 33 kDa	Denaturing
pUC-nef-vpr-gp160-p24	~867 bp	Nef-Vpr-Gp160-P24	pET24a (+)/*Rosetta*	*Bam*HI/*Hind*III	(37°C, 0.7-0.8, 1mM, 4 h)	~ 32 kDa	Denaturing
pUC-nef-vif-gp160-p24	~867 bp	Nef-Vif-Gp160-P24	pET24a (+)/*Rosetta*	*Bam*HI/*Hind*III	(37°C, 0.7-0.8, 1mM, 16 h)	~ 32 kDa	Denaturing
pUC-nef-vpu-gp160-p24	~870 bp	Nef-Vpu-Gp160-P24	pET24a (+)/*Rosetta*	*Bam*HI/*Hind*III	(37°C, 0.7-0.8, 1mM, 16 h)	~ 32 kDa	Denaturing
pUC-nef-tat-gp160-p24	~831 bp	Nef-Tat-Gp160-P24	pET24a (+)/*Rosetta*	*Bam*HI/*Hind*III	(37°C, 0.7-0.8, 1mM, 3 h)	~ 31 kDa	Denaturing

Table 2. Generation of human Hsp27 and Hsp20 proteins

DNA construct	Cloning site	Protein	Expression system	Conditions (temperature, OD_{600}, IPTG dose, time)	Protein size	Purification conditions
pQE-hsp27 (Accession No: NM_001540)	*Nhe*I/ *Sal*I	Hsp27	pET23a (+)/*Rosetta*	(37°C, 0.6-0.7, 1mM, 4 h)	~ 27 kDa	Native
pQE-hsp20 (Accession No: NM_144617)	*Nhe*I/ *Hind*III	Hsp20	pET23a (+)/*Rosetta*	(37°C, 0.7-0.8, 1mM, 4 h)	~ 20 kDa	Native

Statistical analysis. Statistical analysis was performed using Prism 5.0 software (GraphPad). One-way ANOVA was used to analyze the differences in the levels of antibody production. For all analyses, $p < 0.05$ was considered statistically significant.

RESULTS

Design of the polypeptide constructs. The Nef, Vpr, Vif, Vpu, Rev, Tat, Gp160, and P24 epitopes with the highest MHCI and MHCII binding level and specific binding to common HLA molecules in target populations were selected from bioinformatics analysis. The top immunodominant epitopes were selected to design the polypeptide constructs after checking by peptide-protein docking websites (Figure 1). It should be mentioned that the $P24_{8\text{-}151}$ was highly immunogenic and used as a long peptide.

Generation of the recombinant proteins and polypeptides. The recombinant human Hsp20 and Hsp27 proteins were successfully generated in the *E. coli* strain under native conditions. Moreover, five polypeptides were produced in the *E. coli* strain under denaturing conditions (Tables 1 & 2). The NanoDrop Spectrophotometer showed that the recombinant proteins and polypeptides had a concentration range of about 0.6-0.9 mg/ml. Figure 2 indicates the expression and purification of five recombinant polypeptides.

HIV-1 infection led to increased Hsp27 serum levels. The general influence of HIV-1 infection on Hsp27, Hsp20, HP91 serum levels was measured in three groups of untreated, treated, and drug-resistant subjects (test groups) compared to healthy subjects (control group). The untreated, treated, and drug-resistant subjects showed significantly higher levels of anti-Hsp27 antibody than the control group ($p < 0.05$). Moreover, no significant differences were found in serum levels of anti-Hsp27 antibodies in untreated, treated, and drug-resistant individuals ($p > 0.05$). In contrast, HP91 and Hsp20 did not show any significant differences between test and control groups. However, an increased level of anti-HP91 antibody was observed in untreated patients compared to the control group ($p < 0.05$).

The frequency of antibodies to Hsp27, Hsp20, and HP91 was determined in serum samples of untreated/Naïve (G1), treated (G2), drug-resistant (G3), and control (G4) groups. The mean absorbance values for Hsp27 were 1.017 ± 0.322 (G1), 1.0422 ± 0.399 (G2), 0.970 ± 0.502 (G3) and 0.668 ± 0.038 (G4). The mean absorbance values for Hsp20 were 0.872 ± 0.362 (G1), 0.833 ± 0.373 (G2), 0.836 ± 0.428 (G3) and 0.506 ± 0.105 (G4). The mean absorbance values for HP91 peptide were 0.306 ± 0.110 (G1), 0.258 ± 0.069 (G2), 0.262 ± 0.060 (G3) and 0.205 ± 0.049 (G4). The results indicated that these proteins and peptides could not

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.8.3.113]

determine the differences between test groups. Besides, the anti-Hsp20 antibody was not increased significantly in test groups than control groups, suggesting that Hsp20 cannot be considered a promising biomarker for determining untreated groups from healthy groups. Figure 3 shows the differences between the seroreactivities in all individuals against the recombinant Hsp27 and Hsp20 proteins and HP91 peptide. To determine the serum reactivity for each recombinant protein, a cut-off value was calculated by considering the mean absorbance values of control sera plus two standard deviations (mean ± 2SD). Overall, 80%, 60%, 40% of the naïve/untreated sera recognized the recombinant Hsp27, Hsp20, and HP91, respectively. Moreover, 75%, 51%, 20% of the treated sera recognized the recombinant Hsp27, Hsp20, and HP91, respectively. On the other hand, 66%, 49%, 35% of the drug-resistant sera recognized the recombinant Hsp27, Hsp20, and HP91, respectively.



Fig1. The polypeptide constructs generated in *E. coli*

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.8.3.113]

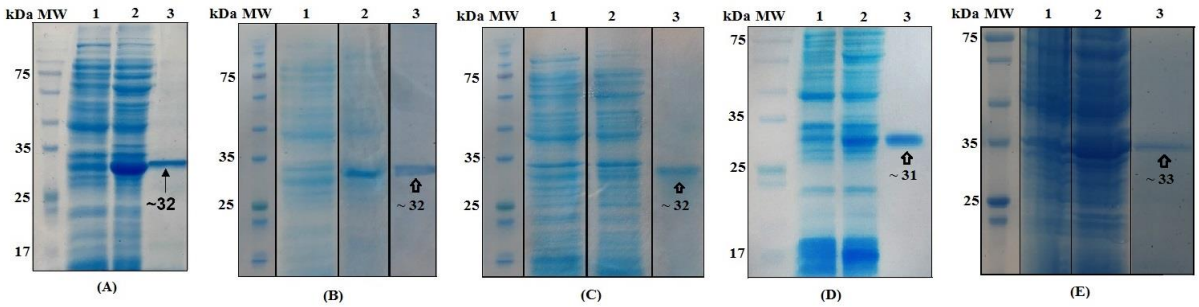


Fig 2. Expression and purification of the recombinant polypeptides in *E. coli* Rosetta strain. Lane 1, before IPTG induction (BI); lane 2, after IPTG induction (AI); lane 3, the purified polypeptides by affinity chromatography under denaturing conditions. A) rNef-Vpr-Gp160-P24, B) rNef-Vif-Gp160-P24, C) rNef-Vpu-Gp160-P24, D) rNef-Tat-Gp160-P24, E) rNef-Rev-Gp160-P24; MW, Molecular weight marker (Prestained protein ladder, 11-180 kDa, Fermentas).

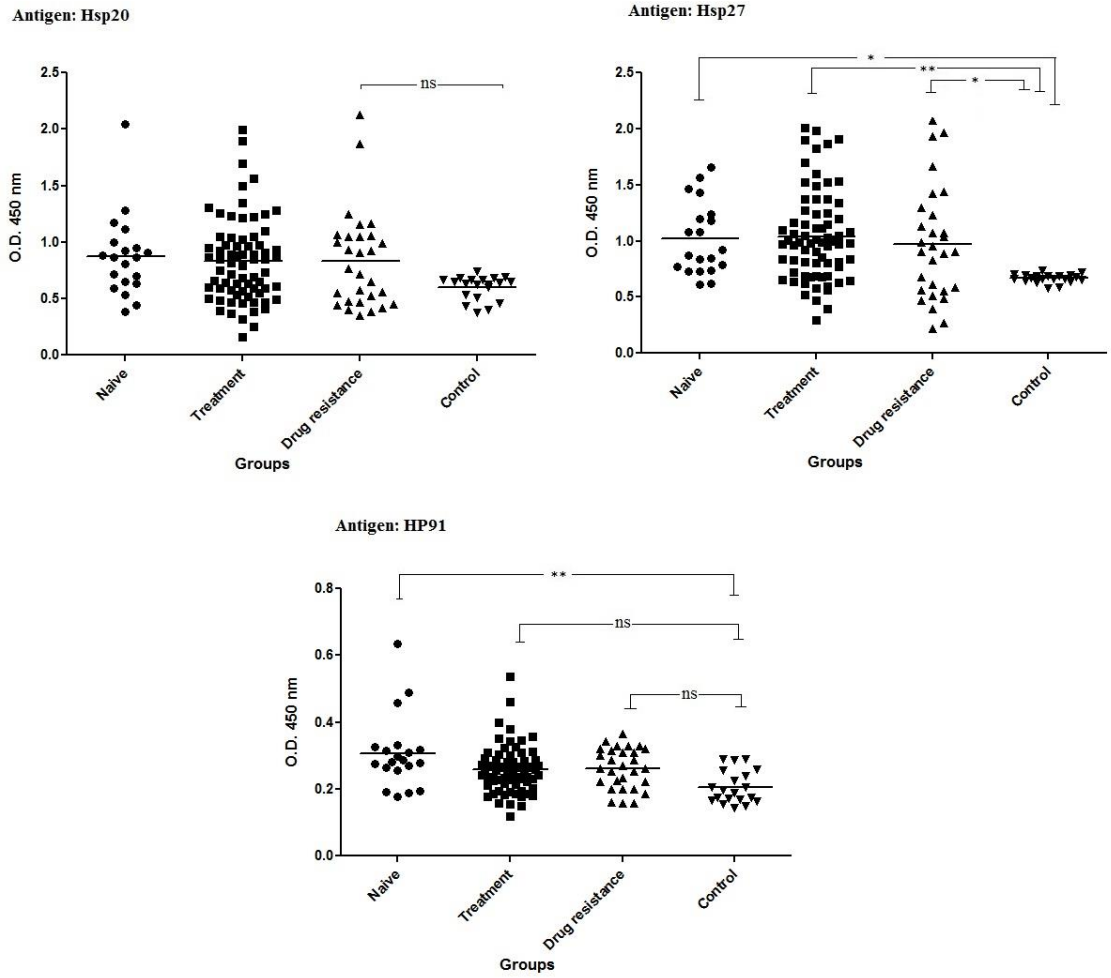


Fig3. Analysis of IgG antibody levels against rHsp20 (A), rHsp27 (B), and rHP91 (C) coating antigens in Naïve/ untreated treated and drug-resistant individuals as compared to healthy (control) group using ELISA. The horizontal line represents the mean value of optical density for each antigen.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.8.3.113]

The levels of anti-polypeptides antibodies did not vary between untreated, treated, and drug-resistant subjects. Our data showed significantly elevated HIV-1 antibodies against polypeptides in test groups' sera compared to the control group ($p < 0.001$). Furthermore, the serum levels of antibodies against all polypeptides did not significantly differ between untreated, treated, and drug-resistant individuals ($p > 0.05$). It seems that test groups can be diagnosed by these polypeptides versus control groups as a biomarker, but there is no difference between the three test groups.

The levels of antibodies to rNef-Tat-Gp160-P24, rNef-Vpr-Gp160-P24, rNef-Vpu-Gp160-P24, rNef-Vif-Gp160-P24, and rNef-Rev-Gp160-P24 was determined in serum samples of untreated/Naïve (G1), treated (G2), drug-resistant (G3), and control (G4) groups. The mean absorbance values for rNef-Tat-Gp160-P24 were 1.292 ± 0.230 (G1), 1.384 ± 0.269 (G2), 1.421 ± 0.258 (G3) and 0.703 ± 0.040 (G4). The mean absorbance values for rNef-Vif-Gp160-P24 were 1.150 ± 0.139 (G1), 1.179 ± 0.134 (G2), 1.165 ± 0.158 (G3) and 0.667 ± 0.047 (G4). The mean absorbance values for rNef-Vpu-Gp160-P24 were 1.196 ± 0.130 (G1), 1.224 ± 0.195 (G2), 1.238 ± 0.173 (G3) and 0.656 ± 0.077 (G4),. The mean absorbance values for rNef-Rev-Gp160-P24 were 0.832 ± 0.253 (G1), 0.784 ± 0.209 (G2), 0.688 ± 0.204 (G3) and 0.443 ± 0.128 (G4). The mean absorbance values for rNef-Vpr-Gp160-P24 were 1.100 ± 0.130 (G1), 1.049 ± 0.159 (G2), 1.047 ± 0.214 (G3) and 0.689 ± 0.076 (G4). Figure 4 shows the differences between the seroreactivities in all individuals against the recombinant polypeptides. A cut-off value was calculated to determine the serum reactivity for each recombinant polypeptide by considering the mean absorbance values of control sera plus two standard deviations (mean ± 2SD). Generally, 100%, 100%, 100%, 100%, and 65% of the naive sera recognized the recombinant Nef-Tat-Gp160-P24, Nef-Vpr-Gp160-P24, Nef-Vpu-Gp160-P24, Nef-Vif-Gp160-P24, and Nef-Rev-Gp160-P24 polypeptides, respectively. Moreover, 100%, 100%, 100%, 100%, and 70% of the treated sera recognized the recombinant Nef-Tat-Gp160-P24, Nef-Vpr-Gp160-P24, Nef-Vpu-Gp160-P24, Nef-Vif-Gp160-P24, and Nef-Rev-Gp160-P24 polypeptides, respectively. On the other hand, 100%, 100%, 100%, 100%, and 49% of the drug-resistant sera recognized the recombinant Nef-Tat-Gp160-P24, Nef-Vpr-Gp160-P24, Nef-Vpu-Gp160-P24, Nef-Vif-Gp160-P24, and Nef-Rev-Gp160-P24 polypeptides, respectively. The designed rev peptide was less recognized in different test groups.

DISCUSSION

According to the published reports, there is no sensitive assay of viral immunogenic polypeptides, heat shock proteins, and HP91 peptide as biomarkers in human sera to diagnose HIV-1 infection in different groups. In this study, the serum levels of antibodies against the five designed polypeptides, two small heat shock proteins (Hsp27 and Hsp20), and HP91 peptide were assessed to discriminate untreated/Naïve, treated, and drug-resistant individuals compared to the control group (HIV-uninfected adults) using indirect ELISA. This study used 141 serum samples from a well-characterized cross-sectional study in Tehran, Iran. The role of Hsps as biomarkers were evaluated primarily concerning cancer and inflammatory diseases. Serum Hsp70 level showed to be a useful biomarker for monitoring inflammatory processes in multiple sclerosis (MS; **[6]**). On the other hand, decreased plasma Hsp70 level combined with increased classical circulating biomarkers carcinoembryonic antigen (CEA) and carbohydrate antigen (CA 19-9) were associated with diagnosis of lung cancer in the early stages (I & II; **[13]**). Plasma concentrations of HSP70 increase with the progression of heart failure (HF); thus, it might act as a potential screening biomarker for early diagnosis of HF, as well **[21]**. The serum levels of both anti-HSP60 and anti-HSP70 were significantly enhanced in patients with Gram-negative bacterial infections compared to patients with Gram-positive and fungal infections, indicating their potential use as additional diagnosis and prognosis markers in infections **[22]**. Moreover, anti-HSP90 IgGs were related to the articular prognosis in rheumatoid arthritis (RA) patients **[8]**. The data indicated that HSP90α was released into the serum by cell damage due to acute rejection (AR) in organ and tissue transplantation. It was a potential biomarker to detect AR in kidney recipients **[23]**. Other findings showed plasma Hsp90α as a biomarker for liver cancer diagnosis and evaluating the therapeutic efficacy of liver cancer patients under surgery or interventional therapy **[24]**.

The levels of small Hsps (Hsp20, Hsp22, and alpha-B Crystallin) and markers of cytotoxic immune response simultaneously increased in serum and peritoneal fluid, and especially in exosomes from patients with ovarian cancer **[12]**. The serum soluble Hsp27 may function as a specific biomarker for monitoring chemotherapy response in patients with ovarian cancer **[25]**. A previous study indicated that patients with metastatic breast cancer did not have significantly higher hsp27 levels than cancer patients without metastases using Western blot analysis. Indeed, the mean hsp27 levels in cancer patients were higher than in the control patients; but, 66% of the breast cancer patients showed hsp27 within the normal range indicating low sensitivity **[26]**. Low Hsp27 serum level was associated with carotid atherosclerosis and oxidative stress as well **[10]**. Hsp27 was also implicated in chemotherapy resistance in breast cancer and leukemia and was associated with the acquisition of drug-resistant phenotypes **[11]**. Strong expression of Hsp27 protein in melanoma metastases could predict complete response to bevacizumab monotherapy in patients. In contrast, other multiple angiogenesis markers showed no relationship with

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.8.3.113]

treatment response **[27]**. On the other hand, other studies were performed to evaluate the levels of HMGB1 and its significant peptide, HP91, as biomarkers in cancer and inflammation diseases. For example, a study demonstrated the deregulation of HMGB1 and peroxiredoxin-6 (PRDX6) serum level in West Nile virus (WNV) -infected patients as a candidate biomarker distinguishing disease severity **[28]**. Some studies focused on the potential biomarker role of HMGB1 in predicting and monitoring therapy in various human cancers **[29]**. HMGB1 was also a common biomarker and potential target for traumatic brain injury (TBI), neuroinflammation, epilepsy, and cognitive dysfunction **[30]**.

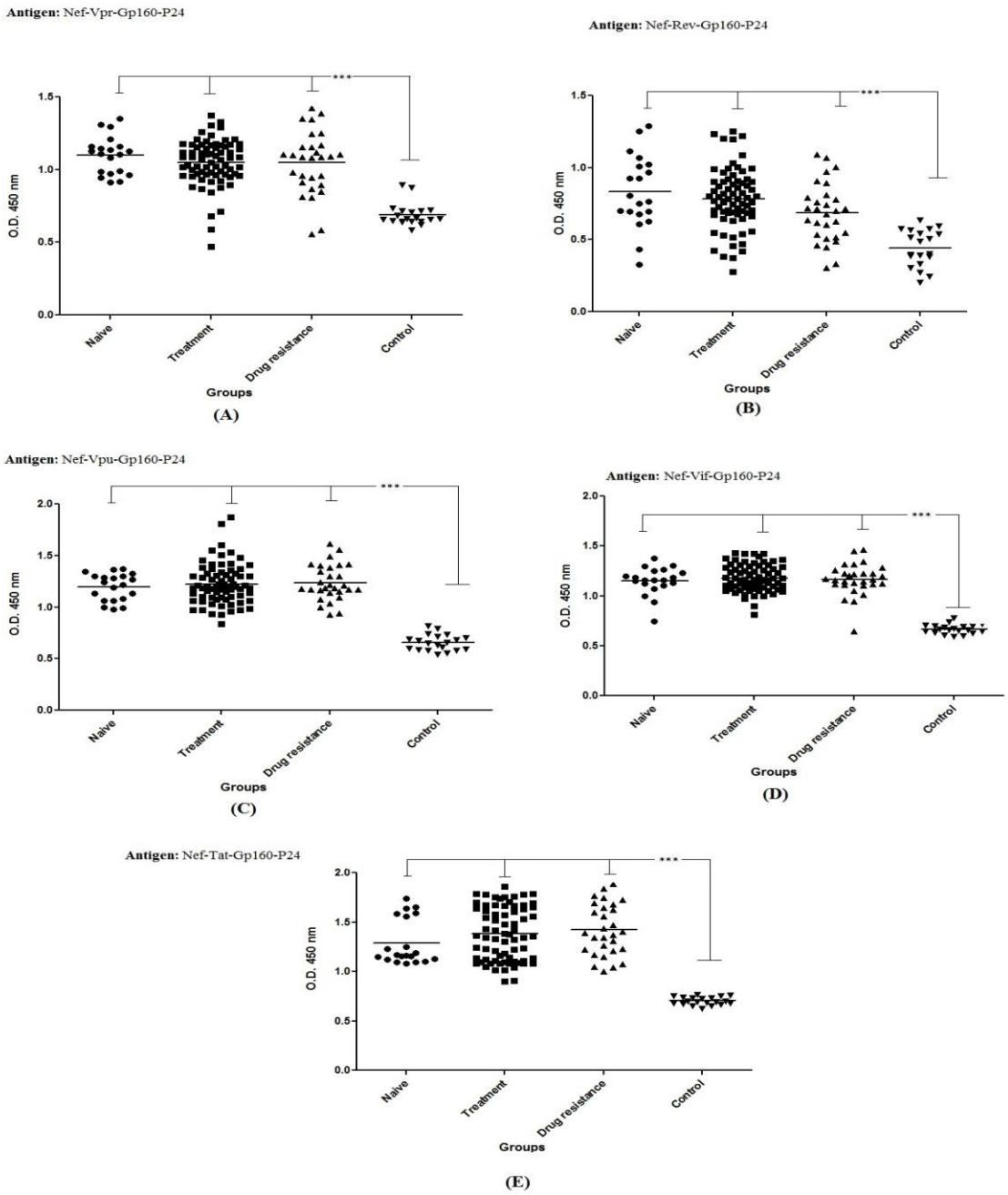


Fig 4. Analysis of IgG antibody levels against rNef-Vpr-Gp160-P24 (A), rNef-Rev-Gp160-P24 (B), rNef-Vpu-Gp160-P24 (C), rNef-Vif-Gp160-P24 (D) and rNef-Tat-Gp160-P24 (E) coating antigens in Naïve/ untreated, treated and drug-resistant individuals as compared to healthy (control) group using ELISA. The horizontal line represents the mean value of optical density for each antigen.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.8.3.113]

Our study was the first evaluation of anti-Hsp27, anti-Hsp20, and anti-HP91 antibodies in three groups: patients with HIV infections, patients treated with anti-HIV drugs, and drug-resistance patients compared to healthy groups. Our results showed significant production of anti-HSP27 antibody in all groups compared to healthy groups, but there was no significant production of antibodies against this protein. Also, the anti-HP91 antibody could be detected significantly in the untreated group compared to the healthy group. However, HSP27 showed an increased response in test groups, but it was high in all subjects (untreated, treated, and drug resistance). A study showed significant differences in the IgG antibody levels to Hsp60 and Hsp70 in HIV- infection. While no differences between HIV-infected and HIV-seronegative subjects in the anti-Hsp60 levels were found, HIV patients had significantly higher serum levels of anti-Hsp70 antibodies than the HIV-seronegative controls [31]. Another study confirmed this result, but it was observed that serum concentrations of anti-Hsp70 antibodies significantly decreased upon highly active antiretroviral therapy (HAART), while $CD4^+$ cell counts significantly enhanced [32]. The studies showed that Hsp27, Hsp40, Hsp60, and Hsp70 incorporate into the membrane of HIV virions. Among HSPs, Hsp70 was the most abundant Hsp associated with HIV virions leading to an increased immune response against this protein. It was suggested that HAART administration blocked HIV replication, thus inhibiting high expression of Hsp70 and noticeably decreased viral load [33]. Similarly, Anraku *et al.* (2012) showed that circulating Hsp60 levels were increased in HIV patients and decreased by antiretroviral therapy [34]. Hsp70-binding protein-1 (HspBP1) was a co-chaperone that inhibited the Hsp70 activity. HIV-positive patients showed a significant increase in HspBP1 and anti-HspBP1 serum levels compared to uninfected individuals. HspBP1 and anti-HspBP1 negatively correlated with CD4 counts. In the acute phase, HspBP1 was significantly increased 15 days after HIV infection [35]. Our previous study showed that overexpression of Hsp27, similar to Hsp20, significantly decreased herpes simplex virus-1 (HSV-1) replication, but it did not affect the replication of human immunodeficiency virus-1 (HIV-1) and hepatitis C virus (HCV) *in vitro*. Indeed, Hsp20 was identified as a novel anti-HCV, anti-HSV, and anti-HIV agent, but Hsp27 was efficient in suppressing HSV infection [36]. Another study indicated that initial increases in Hsp27 and Hsp70 were down-regulated during productive stages of viral synthesis *in vitro* [37]. Our present clinical study indicated that Hsp20 did not have a considerable change between test groups and the control group. In contrast, anti-Hsp27 antibodies were significantly increased in test groups as compared to the control group. Besides, HAART administration did not decrease the levels of Hsp27 in human. It was reported that women exposed to both human papillomavirus (HPV)-16 and -18 infections showed higher antibody responses than women exposed to HPV-16 or HPV-18 against HPV E7 and Hsp27, but not against L1, Hsp20, and HP91peptide. These data indicated that the anti-HP91 antibody was generated in HPV-exposed women significantly higher than HPV-uninfected women; however, the responses were poor compared to HPV E7 and L1-specific antibodies **[16]**. In this study, we showed that Hsp20 was not a major marker for the diagnosis of test groups from the control group. In contrast, the anti-HP91 peptide was higher in HIV-1 infected patients than in the control group. However, HP91 peptide did not show any difference in treated and drug-resistant subjects compared to a healthy group. Other results represented that HMGB1 levels were increased in patients with Crimean-Congo hemorrhagic fever virus (CCHFV), Dobrava virus (DOBV), or Puumala virus (PUUV) infections. Moreover, the concentration of HMGB1 was higher in patients with severe disease progression [38]. A study showed that the release of HMGB1 occurred not only from necrotic cells, but also from apoptotic cells during HIV-1 infection *in vitro* [39]. The data showed higher plasma levels of HMGB1 in HIV-1-infected patients compared to HIV-negative healthy controls [40]. This result was obtained in our experiment using HP91 peptide derived from HMGB1. In the current study, antibody detection against five designed HIV-1 polypeptides was performed along with Hsp27, Hsp20, and HP91peptide. Our results showed high levels of IgGs against each polypeptide compared to control groups. Interestingly, the level of anti-Nef-Rev-Gp160-P24 antibody was lower in treated and drug resistant groups than naïve groups compared to other polypeptides; however, these findings were not statistically significant. In contrast, the level of anti-Nef-Tat-Gp160-P24 antibody was higher in treated and drug-resistant groups than naïve groups as compared to other polypeptides, but these responses were not statistically significant. These newly designed polypeptides were used to diagnose HIV-positive patients compared to HIV-negative patients for the first time. However, there are few studies for the evaluation of HIV polypeptides/peptides in diagnosis. For example, a study indicated that 34 individuals infected with HIV-1 had antibodies reacting with synthetic peptides of the Nef protein (6-23 mers) [41]. Moreover, the C-terminal 13-mer Vpu peptide-specific antibody-dependent cellular cytotoxicity (ADCC) was associated with effective immune responses against HIV among Elite controllers [42]. The importance of some endogenous molecules and HIV-1 immunogenic peptides was determined in the diagnosis of HIV-1 infections. It was shown that serum levels were high in both the patients treated with drugs and the drug-resistant individuals.

In summary, the recombinant Hsp20 and Hsp27 proteins and HIV-1 immunogenic polypeptides were

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.8.3.113]

successfully generated in the *E. coli* strain. The serological studies showed that HIV-positive patients had higher antibody responses than HIV-1 negative patients against Hsp27, HP91, and five HIV-1 polypeptides (Nef-Vif-Gp160-P24, Nef-Vpr-Gp160-P24, Nef-Vpu-Gp160-P24, Nef-Tat-Gp160-P24, and Nef-Rev-Gp160-P24). Furthermore, no significant difference was observed in seroreactivities between test groups (untreated, treated, and drug-resistant subjects). The serum levels of the anti-Hsp20 antibody did not change in the test group compared to the control group. However, it is required to use the synthetic immunogenic peptides of Hsp27 and five polypeptides for serological assay. Moreover, it should be studied why anti-Hsp27 and anti-polypeptides antibodies are detected clearly in the sera of treated and drug resistance individuals.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

ACKNOWLEDGEMENTS

Authors acknowledge the financial support by NIMAD "National Institute for Medical Research Development" for experimental works (Grant No. 987674).

REFERENCES

1. Chiozzini C, Toschi E. HIV-1 tat and immune dysregulation in AIDS pathogenesis: A therapeutic target. Curr Drug Targets. 2016; 17 (1): 33-45.

2. Esparza J. What has 30 years of HIV vaccine research taught us? Vaccines. 2013; 1 (4): 513-26.

3. Davoodi S, Bolhassani A, Sadat SM, Irani S. Design and *in vitro* delivery of HIV-1 multi-epitope DNA and peptide constructs using novel cell-penetrating peptides. Biotechnol Lett. 2019; 41 (11): 1283-98.

4. Arya S, Lal P, Singh P, Kumar A. Recent advances in diagnosis of HIV and future prospects. Indian J Biotechnol. 2015; 14: 9-18

5. Bahraoui E, Benjouad A, Sabatier JM, Allain JP, Laurian Y, Montagnier L, et al. Relevance of anti-Nef antibody detection as an early serologic marker of human immunodeficiency virus infection. Blood. 1990; 76 (1): 257-64

6. Lechner P, Buck D, Sick L, Hemmer B, Multhoff G. Serum heat shock protein 70 levels as a biomarker for inflammatory processes in multiple sclerosis. Mult Scler J Exp Transl Clin. 2018; 4 (2): 1-9.

7. Santos-Junior VA, Lollo PCB, Cantero MA, Moura CS, Amaya-Farfan J, Morato PN. Heat shock proteins: Protection and potential biomarkers for ischemic injury of cardiomyocytes after surgery. Braz J Cardiovasc Surg. 2018; 33 (3): 291-302.

8. Hayem G, De Bandt M, Palazzo E, Roux S, Combe B, Eliaou JF, et al. Anti-heat shock protein 70 kDa and 90 kDa antibodies in serum of patients with rheumatoid arthritis. Ann Rheum Dis. 1999; 58 (5): 291-6.

9. Milani A, Basirnejad M, Shahbazi S, Bolhassani A. HSP roles as biomarkers and antigens in bacterial and viral infections. JoMMID. 2016; 4 (1): 1-7.

10. Jaroszyński A, Jaroszyńska A, Zaborowski T, Drelich-Zbroja A, Zapolski T, Dąbrowski W. Serum heat shock protein 27 levels predict cardiac mortality in hemodialysis patients. BMC Nephrology. 2018; 19 (1): 359.

11. Vidyasagar A, Wilson NA, Djamali A. Heat shock protein 27 (HSP27): Biomarker of disease and therapeutic target. Fibrogenesis Tissue Repair. 2012; 5: 7.

12. Wyciszkiewicz A, Kalinowska-Łyszczarz A, Nowakowski B, Kaźmierczak K, Osztynowicz K, Michalak S. Expression of small heat shock proteins in exosomes from patients with gynecologic cancers. Sci Rep. 2019; 9 (1): 9817.

13. Tang T, Yang C, Brown HE, Huang J. Circulating heat shock protein 70 is a novel biomarker for early diagnosis of lung cancer. Dis Markers. 2018; 2018: 1-8.

14. Bolhassani A, Agi E. Heat shock proteins in infection. Clin Chim Acta. 2019; 498: 90-100.

15. Wang Y, Jiang Z, Yan J, Ying S. HMGB1 as a potential biomarker and therapeutic target for malignant mesothelioma. Dis Markers. 2019; 2019: 1-10

16. Ramezani A, Aghakhani A, Soleymani S, Bavand A, Bolhassani A. Significance of serum antibodies against HPV E7, Hsp27, Hsp20 and Hp91 in Iranian HPV-exposed women. BMC Infect Dis. 2019; 19 (1): 142.

17. Khairkhah N, Namvar A, Kardani K, Bolhassani A. Prediction of cross-clade HIV-1 T-cell epitopes using immunoinformatics analysis. Proteins. 2018; 86 (12): 1284-93.

18. Namazi F, Bolhassani A, Sadat SM, Irani S. Delivery of HIV-1 polyepitope constructs using cationic and amphipathic cell penetrating peptides into mammalian cells. Curr HIV Res. 2019; 17 (6): 408-28.

19. Kardani K, Hashemi A, Bolhassani A. Comparison of HIV-1 Vif and Vpu accessory proteins for delivery of polyepitope constructs harboring Nef, Gp160 and P24 using various cell penetrating peptides. PLoS One. 2019; 14 (10): e0223844.

20. Saenz R, Futalan D, Leutenez L, Eekhout F, Fecteau JF, Sundelius S, et al. TLR4-dependent activation of dendritic cells by an HMGB1-derived peptide adjuvant. J Transl Med. 2014; 12: 211.

21. Li Z, Song Y, Xing R, Yu H, Zhang Y, Li Z, et al. Heat shock protein 70 acts as a potential biomarker for early diagnosis of heart failure. PLoS ONE. 2013; 8 (7): e67964.

22. Bleotu C, Chifiriuc MC, Pircalabioru G, Bertesteanu SVG, Grigore R, Ruta SM, et al. Significance of serum antibodies against HSP 60 and HSP 70 for the diagnostic of infectious diseases. Virulence. 2014; 5 (8): 828-31.

23. Maehana T, Tanaka T, Kitamura H, Fukuzawa N, Ishida H, Harada H, et al. Heat shock protein 90α is a potential serological biomarker of acute rejection after renal transplantation. PLoS ONE. 2016; 11 (9): e0162942.

24. Fu Y, Xu X, Huang D, Cui D, Liu L, Li J, et al. Plasma heat shock protein 90alpha as a biomarker for the diagnosis of liver cancer: An official, large-scale, and multicenter clinical trial. EBioMedicine. 2017; 24: 56-63.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.8.3.113]

25. Stope MB, Wiegank L, Weiss M, Diesing K, Koensgen D, Burchardt M, et al. Drug-induced modulation of heat shock protein HSPB1 in an ovarian cancer cell model. Anticancer Res. 2016; 36 (7): 3321-7.

26. Fanelli MA, Cuello Carrión FD, Dekker J, Schoemaker J, Ciocca DR. Serological detection of heat shock protein hsp27 in normal and breast cancer patients. Cancer Epidemiol Biomarkers Prev. 1998; 7 (9): 791-5.

27. Schuster C, Akslen LA, Straume O. Expression of heat shock protein 27 in melanoma metastases is associated with overall response to Bevacizumab monotherapy: Analyses of predictive markers in a clinical phase II study. PLoS ONE. 2016; 11 (5): e0155242.

28. Fraisier C, Papa A, Almeras L. High-mobility group box-1, promising serological biomarker for the distinction of human WNV disease severity. Virus Res. 2015; 195: 9-12.

29. Richard SA. High-mobility group box 1 is a promising diagnostic and therapeutic monitoring biomarker in Cancers: A review. AIMS Mol Sci. 2019; 5 (4): 183-241.

30. Paudel YN, Shaikh MF, Chakraborti A, Kumari Y, Aledo-Serrano A, Aleksovska K, et al. HMGB1: A common biomarker and potential target for TBI, neuroinflammation, epilepsy, and cognitive dysfunction. Front Neurosci. 2018; 12: 628.

31. Füst G, Beck Z, Bánhegyi D, Kocsis J, Bíró A, Prohászka Z. Antibodies against heat shock proteins and cholesterol in HIV infection. Mol Immunol. 2005; 42 (1): 79-85.

32. Kocsis J, Prohászka Z, Bíró A, Füst G, Bánhegyi D. Elevated levels of antibodies against 70 kDa heat shock proteins in the sera of patients with HIV infection. J Med Virol. 2003; 71 (4): 480-2.

33. Gurer C, Cimarelli A, Luban J. Specific incorporation of heat shock protein 70 family members into primate lentiviral virions. J Virol. 2002; 76 (9): 4666-70.

34. Anraku I, Rajasuriar R, Dobbin C, Brown R, Lewin SR, Suhrbier A. Circulating heat shock protein 60 levels are elevated in HIV patients and are reduced by anti-retroviral therapy. PLoS One. 2012; 7 (9): e45291.

35. Ceccin ADF, Souza APD, Hilário GT, Muller DM, Romão PRT, Rodrigues Junior L. Hsp BP 1 and anti-Hsp BP 1 levels in the serum of HIV-infected individuals are associated to the disease progression. J Appl Microbiol. 2019; 127 (2): 576-85.

36. Vahabpour R, Soleymani S, Roohvand F, Zabihollahi R, Bolhassani A. *In vitro* anti-viral effects of small heat shock proteins 20 and 27: A novel therapeutic approach. Curr Pharm Biotechnol. 2019; 20 (12): 1011-7.

37. Brenner B, Wainberg M. Heat shock protein-based therapeutic strategies against human immunodeficiency virus type infection. Infect Dis Obstet Gynecol. 1999; 7 (1-2): 80-90.

38. Resman Rus K, Fajs L, Korva M, AvšičŽupanc T. HMGB1 is a potential biomarker for severe viral hemorrhagic fevers. PLoS Negl Trop Dis. 2016; 10 (6): e0004804.

39. Naghavi MH, Nowak P, Andersson J, Sönnerborg A, Yang H, Tracey KJ et al. Intracellular high mobility group B1 protein (HMGB1) represses HIV-1 LTR-directed transcription in a promoter- and cell-specific manner. Virology. 2003; 314 (1): 179-89.

40. Nowak P, Barqasho B, Sönnerborg A. Elevated plasma levels of high mobility group box protein 1 in patients with HIV-1 infection. AIDs. 2007; 21 (7): 869-71.

41. Greenway AL, Mills J, Rhodes D, Deacon NJ, McPhee DA. Serological detection of attenuated HIV-1 variants with nef gene deletions. AIDs. 1998; 12 (6): 555-61.

42. Madhavi V, Wines BD, Amin J, Emery S, Lopez E, Kelleher A, et al. HIV-1 *Env*- and *Vpu*-specific antibody-dependent cellular cytotoxicity responses associated with elite control of HIV. J Virol. 2017; 91 (18): e00700-17.

Cite this article:

Milani A, Baesi K, Agi E, Bolhassani A. Detection of anti-IgGs against heat shock proteins 27 and 20, HP91 peptide, and HIV-1 polypeptides in HIV-positive and negative patients. J Med Microbiol Infect Dis, 2020; 8 (3): DOI: 10.29252/JoMMID.8.3.113

[Downloaded from jommid.pasteur.ac.ir on 2026-11-13]

[DOI: 10.29252/JoMMID.8.3.113]