Sequence variation of latent membrane protein 2A (LMP2A) gene from Epstein-Barr virus epitope cytotoxic T-lymphocyte (CTL) related to human leucocyte antigen-A24 (HLA-A24) in peripheral blood sample and cytobrush nasopharyngeal cancer patients

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ABSTRACT

Epstein-Barr virus (EBV) infects lymphocyte B and triggers latent phase in the host so that it causes nasopharyngeal carcinoma (NPC). Latent membrane protein 2A (LMP2A) epitope CTL-HLA-A24 is a target for recognition by cytotoxic T lymphocytes (CTL). The change in the epitope could influence the latency of particular EBV in the host due to its ability to evade immune surveillance mediated by CTL. The study aimed to determine the sequence variation of LMP2A epitope CTL-HLA-A24 gene from the peripheral blood samples and cytobrush of the NPC patients. Case-series study was conducted with total 16 cytobrush samples from NPC patients. DNA isolation, polymerase chain reaction (PCR) and gene sequencing were performed in this study. From cytobrush samples of NPC patients, it was found the changes of base sequence variation of LMP2A gene from GGC>GGA, CCA>CCC, TGC>TCC, GGT>GGC and TCT>ACT. CCA>CCC and TGC>TCC variations were found in epitope associated with HLA-A2 where there was a change of epitope sequence from TYGPVFMCL to TYGPVFMSL caused by missense mutation. The change of base sequence caused amino acid alteration from cysteine to serine. Whereas the variation of CCA>CCC did not change the sequence of amino acid proline so that the epitope was unaffected. In epitope associated HLA-A2 (CLGGLLTMV), there was a change in base sequence from GGT to GGC, but there was no change in amino acid and still as glycine. There were some new variations: in the upstream sequence of LMP2A from GGC>GGA which is silent mutation and the other variation is in downstream sequence of LMP2A from TCT>ACT which is missense mutation. These sequence variations of LMP2A gene found in this research were GGC>GGA, CCA>CCC, TGC>TCC, GGT>GGC and TCT>ACT. In our research, we found another variation compared the previous research. The variation was in the upstream sequence of LMP2A from GGC>GGA which is silent mutation and the other variation is in the downstream sequence of LMP2A from TCT>ACT which is missense mutation.

ABSTRAK

Epstein Barr Virus (EBV) menginfeksi limfosit B dan laten didalam tubuh host sehingga menyebabkan karsinoma nasofaring (KNF). Gen latent membrane protein 2a (Lmp2a) epitop CTL-HLA-A24 merupakan target cytotoxic T lymphocytes (CTL). Perubahan epitop tersebut dapat mempengaruhi latensi EBV dalam inang karena menghindari sistem imun yang dimediasi oleh CTL. Penelitian ini bertujuan untuk menentukan variasi sekuen gen LMP2A epitop CTL-HLA-A24 dari sampel darah tepi dan cytobrush penderita KNF. Rancangan penelitian Case-series digunakan pada penelitian ini. Sebanyak 16 sampel cytobrush penderita KNF dilakukan isolasi DNA, pemeriksaan polymerase chain reaction (PCR) yang dilanjutkan dengan sekuensing. Pada hasil sekuen sampel cytobrush penderita...
INTRODUCTION

Nasopharyngeal carcinoma (NPC) is an epithelial tumor located on the surface of the nasopharynx. NPC is a malignant tumor that mostly invades the head and neck. This carcinoma is rare in some countries in the world, especially in Europe and North America. However, many are distributed in a particular ethnic and geographic area. The incidence of NPC is high in some areas of southern China, especially in Canton, Guangzhou that is 30-80/100,000 per year. NPC cases in Indonesia continue to increase over time. In Yogyakarta Special Region, the incidence of NPC is increased each year.

Epstein-Barr virus (EBV) is proven to be responsible for the occurrence of NPC and commonly associated with the progression of the disease. EBV is the main virus that caused mononucleosis infection, mainly found in nasopharyngeal tumour cells but not all the lymphocytes. Besides virus, there are other risk factors that could cause NPC such as ethnic, smoking, genetic and gender. EBV infects nearly 95% percent of world population and not only caused cancer diseases.

EBV enters the human body by saliva through oropharynx mucosa and infects B cell residing in the submucosa by CD21-Gp350 bond between them. After that, EBV enters B cell's cytoplasm and disassembles its body and let its genetic material to enter the nucleus. EBV can undergo a latent phase after infecting B cell and reside in the body of the host for all the time. EBV-infected B cells will produce latent antigens such as Latent Membrane Protein (LMP)1 and LMP2A. LMP1 is detected approximately 65 and 35% in NPC patients in mRNA and protein levels, whereas LMP2A is found in more than 95% of NPC samples in mRNA levels and 50% in protein level, respectively.

LMP2A is responsible for the latent phase of EBV-infected B cell by inhibiting or disrupting B cell's signal transduction, keeping the virus in the latent phase/persistence. It is immunogenic and acts as the target antigen of cytotoxic T lymphocytes (CTLs) in NPC. CTL detects the virus by recognizing antigen presented by class I MHC (major histocompatibility complex) in surface of target cell. EBV-infected cells will express latent antigens and showed them in the surface in the form of epitope-class I HLA that may cause T cell response (CTLs). Stevens et al. have identified so far seven epitopes targets by CTL in LMP2A, whereas Khana et al. reported that CTL recognizes recombinant LMP2A epitope associated with HLA-A2 in tumour cells having
disturbance in protein TAP regulation, so that this virus isolate evading immune response through the change in amino acid chain in CTL's epitope.\textsuperscript{13}

Epitope part of LMP2A associated with CTL-HLA A24 gene has TYGPVFMLC amino acids arrangement. Previous study found that a change in that region in NPC patients could affect the recognition site of the epitope so that CTL could not recognize the new epitope, thus allowing EBV to avoid the immune surveillance of CTLs.\textsuperscript{9} HLA–A24, HLA–A11 dan HLA-A2 type are found in NPC patients resides in south east Asia and caucasian.\textsuperscript{14} Prior study found that out of nine people (healthy or infected with EBV), HLA-A24 showed the highest number, followed by HLA-A11 and HLA-A2. Native Indonesians with HLA-A24 have high probabilities of NPC.\textsuperscript{15}

NPC cases in Indonesia continue to increase due to most patients diagnosed at the late stage, so treatment failure occasionally occurs. To minimize the number of cases and treatment failure, early screening needs to be undertaken. Never theless, the study of genetic profile in patients with NPC remain scarce. Therefore, we aimed to find LMP2A-CTL epitope-HLA A24 gene in the peripheral blood and cytobrush of NPC patients, and also peripheral blood of healthy people. In addition, the sequence variation of LMP2A-CTL epitope-HLA A24 gene from those samples will be determined.

**MATERIALS AND METHODS**

**Design and samples**

This is a Case Series design towards the occurrence of sequence variation of *Lmp2a*-CTL epitope-HLA A24 gene from the samples of cytobrush NPC patients. Peripheral blood and cytobrush were obtained from the NPC patients at ear-nose-throat (ENT) polyclinic in Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

**Procedure**

DNA isolation and PCR were performed at Laboratory of Molecular Biology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta and sequencing was done at Genetika Science Indonesia – Laboratorium 1st Base.

Method used in DNA isolation is Booms method using kit. NucliSENS® Lysis Buffer, NucliSENS® automated isolation reagent (wash buffer, eluent buffer, silicone), aseton, etanol 70\%, H\textsubscript{2}O steril, HPLC water, platinum taq DNA polymerase (include buffer, MgCl\textsubscript{2}), 10 mM dNTP mix, Ultra Pure DNAs/RNAse, trisma base, 100bp DNA ladder (marker), lyophilized primer 100 nmol gen lmp2a, ddNTP set Bio Chemika, GF-1 PCR Clean Up Kit, 100bp DNA Ladder, loading buffer, agarose gel electrophoresis, TAE buffer, ethidium bromide.

PCR was performed with 1 \( \mu \)L DNA in a 24 \( \mu \)L total reaction mixture containing primer 10pmol 1\( \mu \)L for each forward and reverse, buffer 10x 2.5\( \mu \)L, MgCl\textsubscript{2} 50nm 1.5\( \mu \)L, dNTP mix 10nm 0.2\( \mu \)L, Taq DNA Polymerase 0.1 \( \mu \)L. The primer used for LMP2A was 5'-CAT TCT TGC TAT CCT GAC CG 3'(forward primer) and 5'-CTC CTC ACT TCC CAG TGT AAG G-3'(reverse primer).\textsuperscript{9}

The amplification protocol was one cycle at 95\(^\circ\)C for 5min, followed by 35 cycles of PCR with denaturation temperature 94\(^\circ\)C for 1min, annealing temperature 58\(^\circ\)C for 50 sec, extension temperature 72\(^\circ\)C for one min, final extension 72\(^\circ\)C for 5 min, 4\(^\circ\)C. Result product has 324 bp. PCR product then under went electrophoresis using 2.5% agarose gel with ethidium bromide to see the LMP2A gene band.

The PCR reaction product was extracted and purified. Sequencing was performed to see variation of base sequence coding LMP2A epitope associated with CTL-HLA A24. Sequencing consists of purification, cycle sequencing and precipitation.
Statistical analysis

The analysis of the LMP2A epitope CTL-HLA A24 gene and its variation was performed by descriptive analysis.

RESULTS

Sixty-four subjects were involved in the study and divided into 32 NPC patients and 32 healthy individuals (TABLE 1). Most of NPC patients were in the late stage (Stage IV).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NPC patients (%)</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 32)</td>
<td>(n = 32)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21 (65.6)</td>
<td>21 (65.6)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (34.4)</td>
<td>11 (34.4)</td>
</tr>
<tr>
<td>Age (year)</td>
<td>50.9 ± 13.9</td>
<td>49.0 ± 14.9</td>
</tr>
<tr>
<td>Stage TNM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1 (3.1)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1 (3.1)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>4 (12.5)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>26 (81.3)</td>
<td></td>
</tr>
</tbody>
</table>

Amplification LMP2A gene from peripheral blood sample of NPC patient

Thirty-two peripheral blood samples of NPC patients are amplified according to the prior PCR optimization. DNA band emerged in this experiment only in positive control and there was no emerging band in peripheral blood samples of NPC patients (FIGURE 1A). The amplified of actin gene acted as a control (FIGURE 1B). It showed that none or very few EBVs was taken in the patients’ peripheral blood samples. Some of the samples were not good, for the example in sample 10-106, actin was slightly amplified, shown that the quality of this DNA sample was not good comparing to other samples.

FIGURE 1. (A) PCR Electrophoresis for LMP2A on peripheral blood samples NPC, agarose gel 2%. M is marker 100bp Vivantis, K+ is positive control, number on the upside panel is samples number (1-15). On the right panel is product basepair 320bp, showing by arrow. There is no DNA fragment amplification, (B) PCR of actin gene as a control gene, there is fragment amplification in all samples. Showing by headarrow is one of the sample which is too faint DNA amplification.
Amplification LMP2A gene from cytobrush sample of NPC patient

Thirty-two cytobrush samples of the NPC patients are amplified according to the prior PCR optimization. In this test, DNA band emerged only in positive control and most cytobrush samples with variable thickness of the band (FIGURE 2A). For sample number 11-01B, the gene is not amplified, while in PCR actin it is amplified. It is proof that there was not enough even none of EBVs in sample 11-01B so that there was no amplification of LMP2A gene. Likewise, in sample 11-11B, 11-13B, 11-14B and 11-15B, there were no amplified LMP2A gene whereas actin gene (control) were amplified (FIGURE 2B).

![FIGURE 2. (A) PCR Electrophoresis for LMP2A on cytobrush samples NPC, agarose gel 2%. M is marker 100bp Vivantis, K+ is positive control, number on the upside panel is samples number (1-12). On the right panel is product basepair 320bp, showing by arrow. DNA fragment amplification show in most of cytobrush samples. Showing by arrow is sample number 6 is no amplification; (B) PCR of actin gene as a control gene, there is fragment amplification in all samples. Showing by headarrow is sample number 6 (11-01).](image1)

Amplification LMP2A gene from peripheral blood sample of control

Thirty-two peripheral blood samples of control are amplified according to the previous PCR optimization. DNA band emerged only in positive control and it was not found any DNA band in sample groups (FIGURE 3A). Control gene actin was amplified in all samples (FIGURE 3B). It was proof that the quality of control DNAs were still good and there was no LMP2A gene in peripheral blood samples of the controls.

![FIGURE 3. (A) PCR Electrophoresis for LMP2A on peripheral blood control group, agarose gel 2%. M is marker 100bp Vivantis, K+ is positive control, number on the upside panel is samples number (1-15). On the right panel is product basepair 320bp, showing by arrow. There is no DNA fragment amplification; (B) PCR of actin gene as a control gene, there is fragment amplification in all samples.](image2)
Sequence variation of LMP2Agene from cytobrush samples

Twenty six of 32 cytobrush samples were successfully amplified. Only 16 of 26 successfully amplified samples that could be clearly analyzed using sequencing. Based on sequencing test, there was a change in gene sequence compared to wildtype (Gen Bank AJ 507799.2). It was also found another epitope besides CTL-HLA-A24, named CTL-HLA-A2, so that the results need to be analyzed thoroughly.

Distribution Sequence variation of LMP2Agene from cytobrush samples

Based on analysis, it was found five different variations in each sample which undergoing reanalysis to see the distribution between those variations in all samples (TABLE 2). The variations are: Type I: TCT>ACT (change in one nucleotide), Type II: GGC>GGA, CCA>CCC, TGC>TCC, GGT>GGC (change in four nucleotides) and Type III: GGC>GGA, CCA>CCC, TGC>TCC, GGT>GGC, TCT>ACT (change in five nucleotides). It was found, Type I with a change in one nucleotide as many as 37.5%, Type II with four changes of nucleotide as many as eight samples (50%) and Type III with five changes of nucleotide as many as 12.5% from all samples.
TABLE 2. Distribution Sequence variation of LMP2A gene from cytobrush samples

<table>
<thead>
<tr>
<th>Type</th>
<th>n (%)</th>
<th>Sequence variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>6 (37.5)</td>
<td>TCT&gt;ACT</td>
</tr>
<tr>
<td>Type II</td>
<td>8 (50)</td>
<td>GGC&gt;GGA, CCA&gt;CCC, TGC&gt;TCC, GGT&gt;GGC</td>
</tr>
<tr>
<td>Type III</td>
<td>2 (12.5)</td>
<td>GGC&gt;GGA, CCA&gt;CCC, TGC&gt;TCC, GGT&gt;GGC, TCT&gt;ACT</td>
</tr>
<tr>
<td>Total</td>
<td>16 (100)</td>
<td></td>
</tr>
</tbody>
</table>

The relationship between sequence variation of LMP2A gene and tumor stage

From the results above, it was showed the relationship between sequence variation of LMP2A gene and tumor stage (TABLE 3). In type I, it was found the same presentation between stage 4a and 4c which was 50%. In type II, the presentation differed between stage 3, 4a and 4b whereas the highest presentation was in stage 4b (62.5%). In Type III, it was also found the same presentation between stage 4a and 4b which was 50% each. Compared to Type II and Type III, only in Type I (the change of nucleotide TCT>ACT) stage 4c could be found.

TABLE 3. The relationship between sequence variation of LMP2A gene and tumor stage

<table>
<thead>
<tr>
<th>Sequence variation</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4a</th>
<th>4b</th>
<th>4c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I (TCT&gt;ACT)</td>
<td></td>
<td></td>
<td></td>
<td>3(50)</td>
<td>3(50)</td>
<td></td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Type II (GGC&gt;GGA; CCA&gt;CCC; TGC&gt;TCC; GGT&gt;GGC)</td>
<td></td>
<td></td>
<td>1(12.5)</td>
<td>2(25)</td>
<td>5(62.5)</td>
<td></td>
</tr>
<tr>
<td>(n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type III (GGC&gt;GGA; CCA&gt;CCC; TGC&gt;TCC; GGT&gt;GGC; TCT&gt;ACT)</td>
<td></td>
<td></td>
<td></td>
<td>1(50)</td>
<td>1(50)</td>
<td></td>
</tr>
<tr>
<td>(n=2)</td>
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DISCUSSION

Based on subject characteristic, the number of male patients was more than female patients with the ratio of 2:1. This result was in line with Lo et al. research where male patients tends to be 2-3 times more than female patients. Sun D mentioned that NPC incidence in male patients tends to be higher by 2.5-2.6 times than female patients. In age characteristic, it found that the average of age was 50.9. It was shown that NPC occurrence mainly found in middle ages. Girri and Sarraf suggested that the peak of NPC occurrence was in the 50-59 of age. The unspecified symptoms of NPC (flu-like symptom, laryngitis or nasal inflammation in the early stage of cancer) is the main reason why most of NPC patients come to hospital at late stage. This study showed that most patients (81.3%) at the stage IV of NPC while the stage I was only 3.1%.

The most abundant LMP2A gene could only be amplified in cytobrush sample, whereas LMP2A was not expressed in peripheral blood sample.
of NPC patients or control gene. It might occur because cytobrush was taken from the site of cancer so the probability of captured EBV-infected B cell was much higher than in peripheral blood samples. For NPC peripheral blood samples, the volume of blood was only 100 µL and 900 µL lysis buffer. With only sucha small amount of blood volume, it contributed to the unamplified LMP2A gene because there are other types of cells in the blood sample. In addition, the possibilities were also caused by very low concentration of DNA in peripheral blood than in cytobrush samples. Junker\textsuperscript{16} said that so many years after acute infection of EBV, infected B cell and in latent phase in the peripheral blood accounts for more than 1/60 per 10\textsuperscript{6} B cells (± 10 mL blood). Kieff\textsuperscript{17} mentioned that EBV that infects B cell is found in only 1 of a million B cells.

According to the result of sequencing test from cytobrush samples, there was a change in gene sequence compared to wildtype (\textit{Gen Bank} AJ 507799.2). It was also found another epitope besides CTL-HLA-A24, named CTL-HLA-A2, so that the results need to be analyzed thoroughly.

From sequence analysis of LMP2A gene, it was found five nucleotide variations: the change of base sequence from GGC to GGA (C→A in position 1350, \textit{Gen Bank} AJ 507799.2), however, translated amino acid remained the same (Glycine, G). The second was the sequence change from CCA to CCC (A→C in position 1374, \textit{Gen Bank} AJ 507799.2), however, translated amino acid remained the same (Proline). The third was the sequence change from TGC to TCC (G→C in position 1375, \textit{Gen Bank} AJ 507799.2) and transformed cystein to serine. The fourth was the sequence change from GGT to GGC (T→C in position 1392, \textit{Gen Bank} AJ 507799.2), however, translated amino acid remained the same (Glycine). The fifth was the sequence change from TCT to ACT (T→A in position 1438, \textit{Gen Bank} AJ 507799.2) and transformed serine to threonine.

Cystein and serine are non-charged amino acid, so that the change from cystein to serin will not affect the whole polarity and charge of amino acid sequence. But another possibility emerges: it may cause the difference of binding action with HLA amino acids. The change of amino acid sequence may cause non optimal recognition of the CTL. Research conducted by Wiqoyah\textsuperscript{18} towards three epitopes from lymphoblastoid cell line from NPC patients found that there were change of amino acid at epitope 8 from epitope TYGPVFMC, epitope 1 from epitope CLGGLLLTMV and epitope 6 from epitope SSCPLSKILL. The difference between our research and Wiqoyah’s is in type and samples amount.

Khanna et al.\textsuperscript{13} mentioned that the change of amino acid sequence in the anchor epitope CTL will cause nonbinding epitope with its HLA, so that CTL will not recognize HLA-epitope complex. In the other hand, the change of amino acid sequence in the part that binds T cell receptor (TCR) will cause CTL to recognize the epitope but not optimal. Ming Lung et al.\textsuperscript{19} suggested that the expression of LMP2A in NPC is highly related to the evading mechanism of EBV against immune surveillance represented by CTL.

The 119 amino-terminal cytoplasmic domain of LMP2A coded from the first exon hold an important part in LMP2A function. In that part there is phosphorylation spot for tyrosine, serine and threonine as well as binding spot for certain protein kinases such as asyrosine kinase from src family coded by Lyn and Fyn, which will later function in inhibiting B cell’s signal transduction by interacting with Lyn protein kinase. Tanaka et al.\textsuperscript{20} stated that LMP2A is the specific target of CTL, so that the mutation of this target will cause disturbance in CTL response towards LMP2A causing rapid growth of cells in the host body.
Serin amino acid change is reported to be the most common in NPC in China. This change is advantageous for the EBV to be persistent in B cells. This also showed that LMP2A has an important role that causes EBV to be latent in the host.

We found five different variations in each sample which underwent reanalysis to see the distribution between those variations in all samples. The variations are: Type I: TCT>ACT (change in one nucleotide), Type II: GGC>GGA, CCA>CCC, TGC>TCC, GGT>GGC (change in four nucleotides) and Type III: GGC>GGA, CCA>CCC, TGC>TCC, GGT>GGC, TCT>ACT (change in five nucleotides). It can be suggested that Type I with a change in one nucleotide as many as 37.5%, Type II with four changes of nucleotide as many as eight samples (50%) and Type III with five changes of nucleotide as many as 12.5% from all samples.

From the results above, it was obtained the relationship between sequence variation of LMP2A gene and tumor stage which are: In type I showed the same presentation between stage 4a and 4c which was 50% each. In Type II, the presentation differed between stage 3, 4a and 4b whereas the highest presentation was in stage 4b (62.5%). In Type III, it was also found the same presentation between stage 4a and 4b which was 50% each. Compared to Type II and Type III, only in Type I (the change of nucleotide TCT>ACT) stage 4c could be found. From these results, there was a association between sequence variation and tumor stage. Previous study showed that detected EBV in NPC case had a connection with its stage, whereas there was more positive EBVs in late cancer stage. LMP2A are important for the migration of the cell, its increase the metastasis progress. LMP2A mimicry BCR and activated Akt/Pkb from PI3K pathway, the function of pro apoptosis protein Bad and Glycogensintese kinase 3β was inhibited. And its caused β catenin are not degradation, as we know β catenin play important role in cell differentiation and oncogenic transformation. It was also a E cadherin component that have function in adhesion of the cell.

In our research, we found another variation compared the previous research conducted by Wiqoyah. The variation is in the upstream sequence of LMP2A from GGC>GGA which is silent mutation and the other variation is in downstream sequence of LMP2A from TCT>ACT which is missense mutation. It is assumed that those sequence variations might be affecting structure change of LMP2A amino acids, so that interaction between LMP2A and CTL epitope is not immunogenic enough to activated CTL, results in escape from immune surveillance. To prove this evidence, further study is needed.

CONCLUSION

It is concluded that cytobrush is still the best choice for screening or diagnosis of NPC. Moreover, the finding of five variations of LMP2A gene which are: the change of base sequence from GGC to GGA, from CCA to CCC, from TGC to TCC, from GGT to GGC, and from TCT to ACT. CCA>CCC and TGC>TCC variation are found on epitope TYGPVMCL which is recognized by HLA-A2 and TYGPVFMSL caused by missense mutation. Variation of CCA>CCC does not change the amino acid sequence thus does not alter epitope’s shape. Moreover, on epitope associated with HLA-A2 (CLGLLLTVMV), there is a change of base sequence from GGT to GGC, however, this change does not transform translated amino acid, it is still glycine (silent mutation). In addition, the new variations found in the upstream sequence of LMP2A from GGC>GGA which is silent mutation and the other variation is in downstream sequence of LMP2A from TCT>ACT which is missense mutation.
ACKNOWLEDGMENTS

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