Effects of Analgesia-Sedation on the Relationship Between NKG2D and Its Ligands MICA, MICB

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Abstract

Objective: This study aims to investigate the influence of analgesia-sedation on the expression of natural killer cells, and the NKG2D receptor and MICA/MICB ligand changes in patients who had cerebral digital subtraction angiography for either the diagnosis or treatment of intracranial vascular pathologies.

Methods: Forty-one male patients who were admitted for cerebral digital subtraction angiography were included in this study. The patients were divided into 2 groups: group I ($n = 7$) included patients who did not receive anesthesia, and group II ($n = 34$) comprised those who received anesthesia. For the molecules, the venous blood samples were collected from every patient before and after cerebral digital subtraction angiography.

Results: In group I, the natural killer cells, the NKG2D receptor, the MICA/MICB ligands, and the CD3 and CD8 cytokines were increased significantly after the DSA, but the CD16+56+ cells and the MHC-I molecules showed no statistically significant difference. In group II, the natural killer cells, CD16+56+ cells and the MICA/MICB levels did not show significant difference. On the other hand, the NKG2D, MHC-I, and the CD8+ and CD3+ cell levels increased significantly after the digital subtraction angiography. Comparing the group I and II after the digital subtraction angiography showed no significant difference regarding CD16+56+ and NKG2D. The natural killer (CD56+), and MICA/MICB levels decreased, and the MHC-I, CD8+, and CD3+ levels increased significantly in group II.

Conclusion: Analgesia-sedation combined with surgical stress in digital subtraction angiography causes some alterations in the immune status of patients. More data will enable us to administer the appropriate agents in order to strengthen their immune status during the preoperative period for decreasing the morbidity and/or mortality rate.

Keywords: Analgesia-sedation, angiography, NKG2D, immune system, natural killer cells

NKG2D ile Ligandları MICA, MICB Arasındaki İlişkiye Analjezi-Sedasyonun Etkileri

Öz

Amaç: Bu çalışma, intrakraniyal vasküler patolojilerin tanı veya tedavisi amacıyla serebral anjiyografi yapılan hastalarda analjezi-sedasyonun doğal öldürücü hücreler, NKG2D reseptörü ve MICA/MICB ligand değişikliklerinin ekspresyonu üzerindeki etkisini incelemeyi amaçlamaktadır.

Yöntemler: Çalışmaya serebral anjiyografi yapılması için başvuran 41 erkek hasta dahil edildi. Hastalar, anestezi almayan hastalar Grup I ($n = 7$) ve anestezi alan hastalar Grup II ($n = 34$) olacak şekilde sınıflandırıldı. Her hastadan inceleme amacıyla serebral anjiografi öncesi ve sonrası venöz kan örnekleri alındı.

Bulgular: Grup I' de, serebral anjiyografi sonrası doğal öldürücü hücreler, NKG2D, MICA/MICB, CD3+ ve CD8+ sitokinlerinin istatiksel olarak anlamlı bir şekilde arttığı; bunun yanında CD16+56+ ve MHC-I düzeylerinde istatiksel olarak anlamlı bir fark izlenmediği görüldü. Grup II' de ise doğal öldürücü hücreler, CD16+56 + ve MICA/MICB seviyelerinde anlamlı farklılık izlenmezken; serebral anjiyografi sonrası NKG2D, MHC-I, CD3 ve CD8 seviyeleri önemli ölçüde artmıştır. Serebral anjiyografi sonrası, bu iki grup karşılaştırıldığında CD16+56+ ve NKG2D değerlerinde anlamlı bir fark gözlenmedi. Doğal öldürücü (CD56+), MICA/MICB değerlerinde azalma saptanırken grup II' de MHC-I, CD3+, and CD8+ değerlerinde anlamlı artış meydana geldi.

Sonuç: Serebral anjiyografiye bağlı cerrahi stres ile birlikte analjezi-sedasyon, hastaların immün sistemlerinde bazı değişikliklere neden olmaktadır. Bu konu ile ilgili daha fazla veri, ameliyat öncesi dönemde uygun ajanların kullanımına yönlendirerek immün sistemin güçlenmesine; buna bağlı olarak morbidite ve / veya mortalite oranlarının azalmasına öncü olacaktır.

Anahtar Kelimeler: Analjezi-sedasyon, anjiyografi, NKG2D, immün sistem, doğal öldürücü hücreler

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It has been well documented that anesthesia, any surgery, or any invasive intervention on the body can cause some modulatory changes in the immune response, and that cell-mediated immunity plays a

crucial role.[1] Any imbalance in the immunity may lead to an increased incidence of post-procedural infections. Furthermore, the co-existence of sepsis and multiple organ failure, which suggest immune functional imbalance, increases the incidence of post-procedural complications.[2] Post-operative infections can shadow the success of the surgical results in neurosurgery. Therefore, understanding immunity at the molecular level is of utmost importance for the prevention of catastrophic clinical sequelae.[3] Natural killer (NK) cells are indicators of a strong or competent immune system, and they are the main focus of recent studies regarding the immunity after surgery or digital subtraction angiography (DSA).[4,5]

Several in vivo and in vitro studies have tried to investigate immune function changes following surgery, but studies comparing the immune alterations before and after analgesia-sedation due to surgery or DSA are limited. In this study we have investigated the NKG2D receptor and the MICA/MICB ligand changes in patients with and without analgesia-sedation due to DSA for the diagnosis and treatment of some cerebrovascular diseases.

Methods

Study groups

This work was conducted at the Istanbul University, Cerrahpasa Medical Faculty, Departments of Physiology and Neurosurgery. Ethics committee approval was from İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine (Date: May 15, 2008, Number: 13214). Written informed consent was obtained from patients who participated in this study. A total of 41 male patients who needed cerebral DSA and were admitted to the Neuroradiology section were included in this study. All patients were categorized in ASA I (healthy (American Society of Anesthesiology Classification)), and ranged in age from 35 to 50 years.

The selection criteria for the subjects were as follows: patients who had (1) normal electrocardiography (ECG); (2) normal chest X-ray; (3) normal blood, urine, and coagulation tests; and (4) no history of prolonged used of any drug or of allergy. The group was divided into 2 subgroups. In group I, 7 patients who were not given analgesia-sedation were included; while 34 patients who were given analgesia-sedation before and after cerebral DSA were included in group II. Before and after DSA, a sample of blood from the venous line was col-lected from each patient from both groups, for the pur-pose of the study. All the procedures including cerebral DSA were performed after receiving written informed consent from either the patients or the next of kin.

Sample handling and anesthesia

Every patient had nothing per oral (NPO) for 6 hours and had 8 hours of sleep before the procedure. All patients were taken to the preparation room, where the room temperature was 20-22°C and all disturbances that might have resulted in stress were eliminated. The patients rested for 30-45 minutes before cerebral DSA. Then, 5 mL of blood sample was taken, twice in 2 separate tubes with ethylenediaminetetraacetic acid (EDTA), from both groups I and II. About 4-5 hours after the first blood sample was taken, the patients were monitored with ECG, pulse oximetry (SpO2), and non-invasive arterial pressure (NIAP). After the first arterial pressure was measured, patients from group II were pre-medicated with intravenous (IV) fentanyl (0.5 μg/kg) and IV midazolam (0.05 μg/kg). Five minutes later, the second NIAP measures were taken and an additional dose of fentanyl (0.5 μg/kg) was given. After DSA, 5 mL of blood sample was again taken twice, into 2 separate tubes with EDTA, from group II only.

Sample preparation

Whole blood is collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components are removed by washing the cells thrice in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x *g* for 5 minutes. Following that, 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Whole blood cells require lysis of RBCs following the staining procedure.

Immune fluorescein dyeing (NK counting)

For NK counting, 100 μL of cell suspension are taken, and 10 μL of fluorescein isothiocyanate (FITC) or phycoerythrin (PE) with specific antibody (CD3 FITC, CD16+56+ PE) is added. The samples are incubated in the dark at room temperature for 10 minutes, and then washed with PBS twice. The samples are fixed with 4% paraformaldehyde and kept in the refrigerator until flow cytometric analysis.

Cell count MICA/B-PE and monoclonal anti-human NKG2D-phycoerythrin cells to be used for staining with the antibody are first Fc-blocked by treatment with 1 μg of human or mouse IgG/105 cells for 15 minutes at room temperature. The excess blocking IgG is not washed from this reaction. Next, 25 μL of the Fc-blocked cells (1 × 105 cells) or 50 μL of packed whole blood is transferred to a 5 mL tube, followed by the addition of 10 μL of PE-conjugated anti-MICA/B reagent, and incubated for 30-45 minutes at 2-8°C. Following this incubation, unreacted anti-MICA/B reagent is removed by washing (described above) the cells twice in 4 mL of the same PBS buffer (note that whole blood requires an RBC lysis step at this point, using any commercially available lysing reagent, such as R&D Systems' Human Erythrocyte Lysing Kit, Catalog # WL1000). The cells are resuspended in 200-400 μL of PBS buffer for final

flow cytometric analysis. As a control for analysis, cells in a separate tube are treated with PE-labeled mouse IgG2A antibody.

Flow cytometry

In this study, the data were analyzed using the FACSCalibur instrument (Becton Dickinson) using CellQuest software. Emission wavelengths of 530 nm for FITC, and 585 nm for PE/PI were used; and 488 nm wavelengths were used for the reflection. By using 2-colored fluorescein, forward-angled light scatter (FSC) and 90-degree side-scatter (SSC) diagrams, 10 000 cells were counted from every sample. CD56, CD16+56+, NKG2D, MICA/MICB, MHC-I, CD3, and CD8 were detected.

Monoclonal antibody labeling

For every monoclonal antibody, FITC and/or PE monoclonal antibody was added to the heparinized blood. After incubation, erythrocyte-lysing solution (FACS Lysing Solution) was added. Following the second incubation, CellQuest software was used for the analysis. Cytokine concentration was plotted on the horizontal column and mean absorbance values on the vertical column.

Statistical analysis

All data collected from each patient were organized in a database (Excel, Microsoft Corp.). The numerical variables were provided as the mean ± SD. For statistical analysis, we used the paired sample *t*-test to compare the values between before and after cerebral DSA for the same group. For the comparisons between groups I and II before and after cerebral DSA, the nonparametric independent samples *t*-test was used. A probability value less than .05 was considered statistically significant. All statistical calculations were performed using commercially available software (SPSS version 12.0, SPSS Inc., Chicago, IL, USA).

Results

In the group I (patients without anesthesia), comparing the levels before and after the DSA, the NK cells and NKG2D levels were increased very significantly after the DSA. The MICA/MICB levels also increased after the DSA. The CD3 and CD8 cytokines showed significant increase after the DSA, but the CD16+56+ and MHC-I showed no statistically significant difference (Figure 1).

In the group II (patients with anesthesia), the NK cells, CD16+56+, and MICA/MICB levels did not show significant difference before and after the DSA. On the other hand the NKG2D, MHC-I, and CD8+ levels increased very significantly, and the CD3+ levels also increased significantly after the DSA (Figure 2).

Comparing the groups I and II after the DSA showed no significant difference regarding CD16+56+ and NKG2D, but all other parameters showed some differences. The NK (CD56+) and MICA/MICB decreased significantly, the MHC-I increased significantly, and the CD8+ and CD3+ levels increased very significantly in the group II. Figures 1and 3 summarize the statistical results.

Discussion

Anesthesia accompanied by surgical stress is considered to suppress immunity, presumably by directly affecting the immune system. The primary purpose of immunity is to distinguish "self" from "non-self" and to clear "non-self" antigens from the body. The 2 major components of the immune response are the nonspecific innate immunity and the specific acquired immunity. Innate immunity is the first-line of defense against "non-self" invaders. The innate immunity response is rapid, nonspecific for the antigen, and requires no prior exposure to the antigen target to activate the nonspecific immune system components. Acquired immunity is more specialized than innate immunity. It supplements the protection provided by innate

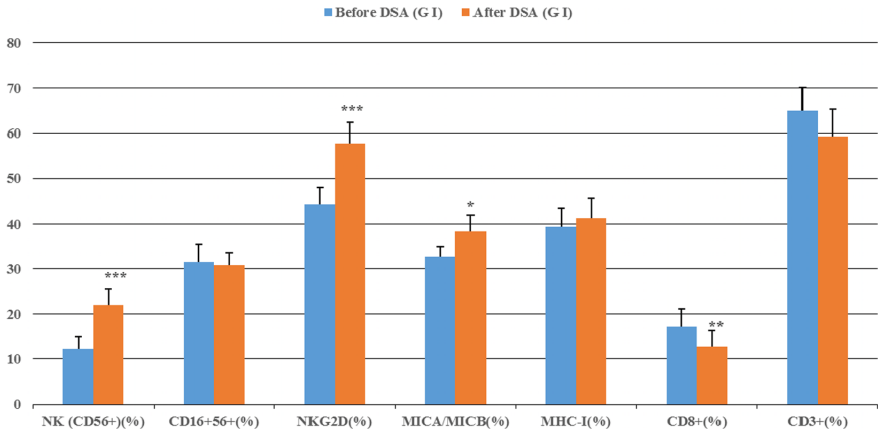

Figure 1. Levels of the parameters studied here in the Group I before and after the DSA. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

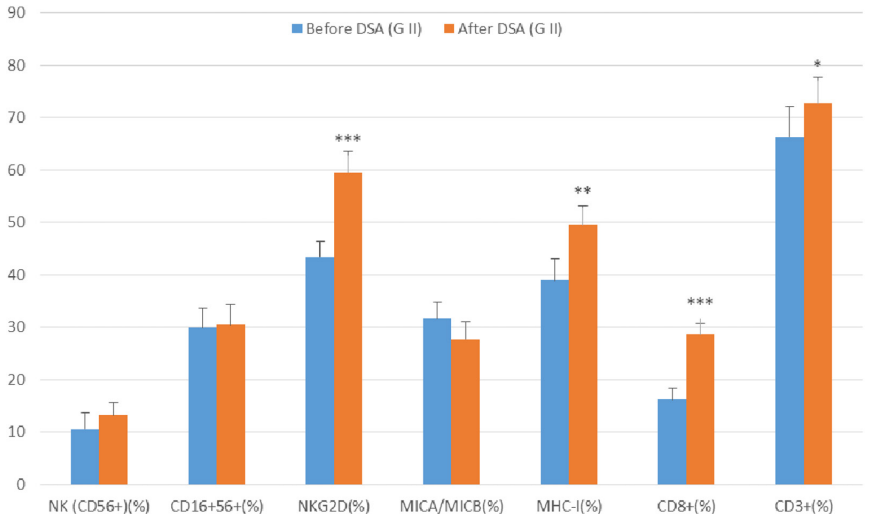

Figure 2. Levels of the parameters studied here in the Group II before and after the DSA. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

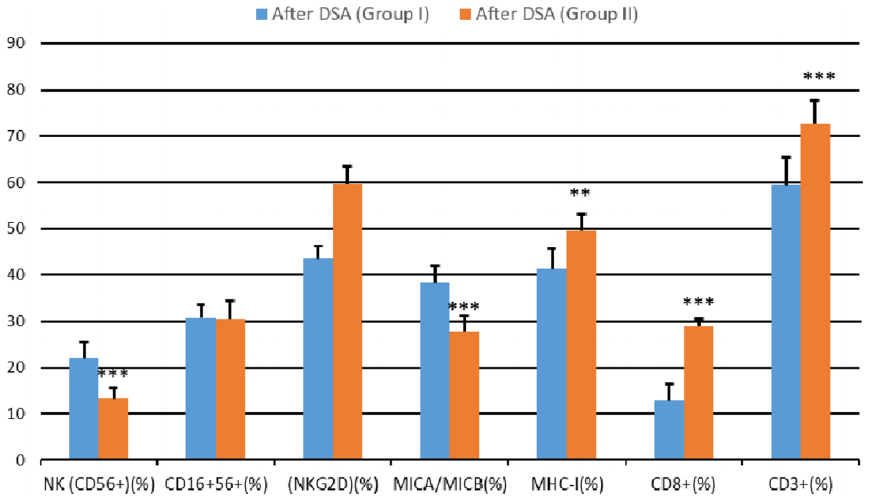

Figure 3. Levels of the parameters studied here in both groups after the DSA. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

immunity.[7] Innate immune responses are mediated by the NK cells and phagocytic cells such as monocytes, macrophages, and polymorphonuclear neutrophils, which use primitive nonspecific recognition systems to bind microorganisms, then neutralize and destroy them.[6,7]

The NK cells play an important role in particularly destroying the infected cells,[8] and, unlike the cytotoxic cells, they do not need to encounter the target antigens earlier and to have an interaction with cytokines for their action. They may act as the bridge between the natural and acquired immune systems by both secreting cytokines/chemokines and acting on cellular cytotoxicity. Especially useful in the early phases of host immune responses, NK cells are a distinct subpopulation of lymphoid lineage that can "naturally" kill certain tumor cells and infected cells without prior sensitization or MHC restriction.[9]

In the present study, we found that the NK (CD56+) cells increased significantly in patients who did not receive anesthesia, while no change was detected in patients with anesthesia. Suppression of the NK cells causes repeated complications and frequent metastases. It is underlined that a decrease in NK cells in the peroperative period is associated with increased mortality.[10-12] Woods and Griffi[13] found that the volatile anesthetics, halothane and enflurane, reversibly inhibited NK cell activity dose-dependently in vitro. In addition, a study using an animal model indicated that the halothane-induced suppression of NK cell activity increased tumor metastases in vivo.[14]

NK cells are recognized by their surface antigens CD16 and CD56. The CD16 receptor is an Fc receptor for IgG, which enables NK cells to lyse IgG-opsonized target cells (antibody-dependent cellular cytotoxicity). In the present study, there was no significant change after the DSA in the expressions of CD16+56+ in

patients with and without anesthesia. This situation suggests that NK cell's ability to lyse the target cells has not been affected in both circumstances. The MHC-I molecules showed significant increase only in patients with anesthesia. The MHC cells are the molecules that protect the normal cells from being lysed by the NK cells. Thus, action of NK cells may be controlled by a precise balance among the cell surface receptors.[15] The NK cells are controlled by both activator and inhibitor surface receptors, and the stimulation of these receptors at different levels directly affects the cells' response.

In humans, the MHC-I molecules are found on the telomeric end and code the gene loci of classic transplantation antigens (HLA-A, -B, and -C), non-classical Class-I antigens, pseudogenes (HLA-E, -F, and -G), and MICA/MICB.[16] Although several activator receptors have been identified in humans and rats, the relationship between these receptors and their effect on the different NK cells, unfortunately, has not yet been exposed. Some experimental research showed that some activator receptors act as primary receptors for the recognition of the target cells.[16-19] In our study, after the DSA, the MICA/MICB levels increased, and the CD8+ decreased in the patients without anesthesia but CD8+ significantly increased in patients without anesthesia. Furthermore, in the same group MICA/MICB did not show any difference.

The question of how anesthetics inhibit the NK cells still awaits a response. Depending on our results, we can speculate that anesthetics suppress the NK cells by induction of the CD8+ T cells.[9] This suppression effect is also seen on the CD15+56+ cells and the MICA/MICB ligands. Additionally, increase in MHC-I strengthens the restrictive effect on NK cells. In patients with anesthesia, despite the increase in NKG2D, the signal transduction is normally interrupted, by MICA/MICB ligands that decrease after anesthesia, and may result in a decrease in the number of NK cells or a suppression of the NK activities.[20]

The NKG2D ligand and the MHC-I-related chains (MICA and MICB) depend on MHC molecules, and after stress like DSA, their expressions increase.[20,21] Data related to cytotoxicity and cytokine production by these molecules are scarce. We believe that the control of the receptors such as NKG2D is held under the strict supervision. Long-term surgical stress may have harmful effects on NKG2D-mediated signal mechanisms.[8] NKG2D ligands are not expressed under normal conditions, but they may be induced by cellular stress, originating from the "induced" or "stressed self" hypothesis.[22] Physiological and psychological stress in the peroperative period may result in a decrease in the number of activities of CD8 lymphocytes and NK cells, and also suppress cell-mediated immunity. The NK cells are important immune cells that kill target cells very rapidly because of their antigenic properties. Stress due to DSA and anesthesia can also cause peroperative immune suppression which affects rate of post-procedural or post-operative infection rate. Nevertheless, although increase in the levels of the receptors and ligands in patients who had DSA without anesthesia may seem to prevent post-procedural complications due to anesthesia if given, excessive immune response may cause a response to the body's own autoantibodies which result in destruction of the immune system. In order to prevent such destruction for the autoimmunity, for example, for MHC-I, stimulation of the inhibitor receptors on the surface of NK cells may be needed; however, this needs further extensive studies. Thus, anesthesia does not have only a negative effect on the immunity, it may also have a beneficial effect. Anesthesiologists should decide optimal anesthetic techniques in consideration of long-term morbidity, mortality, and prognosis of the patients.

Conclusion

Surgical stress, like digital cerebral angiography, causes some alterations in the immune status of the patients, especially when combined with anesthesia.

According to our study, when NKG2D levels are increased by analgesia and stress, a transformation in effector cell activation and killing of the ligand-expressing target cells can result. Since general anesthesia aggravates immune suppression, the option of local or regional anesthesia should primarily be used.

More data related to the immune mechanisms will lead us to administer appropriate agents to the patients in order to strengthen the immune status during the pre-operative period, thus decreasing the morbidity and/or mortality rate.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine (Date: May 15, 2008, Number: 13214).

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References

1. Von Dossow V, Sander M, MacGill M, Spies C. Perioperative cell-mediated immune response. *Front Biosci*. 2008;13:3676-3684. [CrossRef]
2. Wakefield CH, Carey PD, Foulds S, Monson JR, Guillou PJ. Changes in major histocompatibility complex class II expression in monocytes and T cells of patients developing infection after surgery. *Br J Surg*. 1993;80(2):205-209. [CrossRef]
3. Herzer G, Trimmel H. Neuroanesthesia, principles of optimized perioperative management. *Anaesthesist*. 2010;59(4):371-384. [CrossRef]
4. Page GG. Surgery-induced immunosuppression and postoperative pain management. *AACN Clin Issues*. 2005;16(3):302-9; quiz 416. [CrossRef]
5. Türeci E, Kızılkılıç O, Akyol S, et al. The effects angiography. *Turk Neurosurg*. 2011;21(3):340-346. [CrossRef]
6. Souza-Fonseca-Guimaraes F, Adib-Conquy M, Cavaillon JM. Natural killer (NK) cells in antibacterial innate immunity: angels or devils? *Mol Med*. 2012;18:270-285. [CrossRef]
7. Hogan BV, Peter MB, Shenoy HG, Horgan K, Hughes TA. Surgery induced immunosuppression. *Surgeon*. 2011;9(1):38-43. [CrossRef]
8. Champsaur M, Lanier LL. Effect of NKG2D ligand expression host immune responses. *Immunol Rev*. 2010;235(1):267-285. [CrossRef]
9. Markovic SN, Murasko DM. Anesthesia inhibits interferon induced natural killer cell cytotoxicity via induction of CD8+ suppressor cells. *Cell Immunol*. 1993;151(2):474-480. [CrossRef]
10. Bahram S, Inoko H, Shiina T, Radosavljevic M. MIC and other NKG2D ligands: from none to too many. *Curr Opin Immunol*. 2005;17(5):505-509. [CrossRef]
11. Stephens HA. MICA and MICB genes: can the enigma of their polymorphism be resolved? *Trends Immunol*. 2001;22(7):378-385. [CrossRef]
12. Zwirner NW, Fuertes MB, Girart MV, Domaica CI, Rossi LE. Cytokine-driven regulation of NK cell functions in tumor immunity: role of the MICA-NKG2D system. *Cytokine Growth Factor Rev*. 2007;18(1-2):159-170. [CrossRef]
13. Woods GM, Griffiths DM. Reversible inhibition of natural killer cell activity by volatile anaesthetic agents in vitro. *Br J Anaesth*. 1986;58(5):535-539. [CrossRef]
14. Coudert JD, Held W. The role of the NKG2D receptor for tumor immunity. *Semin Cancer Biol*. 2006;16(5):333-343. [CrossRef]
15. Shankarkumar U. The human leukocyte antigen (HLA) system. *Int J Hum Genet*. 2004;4:91-103.
16. Jamieson AM, Diefenbach A, McMahon CW, et al. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. *Immunity*. 2002;17(1):19-29. [CrossRef]
17. Bacon L, Eagle RA, Meyer M, et al. Two human ULBP/RAET1 molecules with transmembrane regions are ligands for NKG2D. *J Immunol*. 2004;173(2):1078-1084. [CrossRef]
18. Kumar S. Natural killer cell cytotoxicity and its regulation by inhibitory receptors. *Immunology*. 2018;154(3):383-393. [CrossRef]
19. Pende D, Cantoni C, Rivera P, et al. Role of NKG2D in tumor cell lysis mediated by human NK cells: cooperation with natural cytotoxicity receptors and capability of recognizing tumors of nonepithelial origin. *Eur J Immunol*. 2001;31(4):1076-1086. [CrossRef]
20. Fernández-Messina L., Reyburn H. T., Valés-Gómez M., Human NKG. 2D-ligands: cell biology strategies to ensure immune recognition. *Front Immunol*. 2012;3:299. [CrossRef]
21. González S, Groh V, Spies T. Immunobiology of human NKG2D and its ligands. *Curr Top Microbiol Immunol*. 2006;298:121-138. [CrossRef]
22. Cosman D, Müllberg J, Sutherland CL, et al. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity*. 2001;14(2):123-133. [CrossRef]