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SAĞLIKLI PEDİYATRİK VE ERİŞKİN YAŞ GRUPLARINDA MİTOJENLERLE UYARILMIŞ LENFOSİT PROLİFERASYON DEĞERLERİNİN KARŞILAŞTIRILMASI

(COMPARISON OF MITOGEN-INDUCED LYMPHOCYTE PROLIFERATION VALUES IN HEALTHY PEDIATRIC AND ADULT AGE GROUPS)

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Bu tez çalışmasının kendi çalışmam olduğunu, tezin planlanmasından yazımına kadar bütün safhalarda etik dışı davranışımın olmadığını, bu tezdeki bütün bilgileri akademik ve etik kurallar içinde elde ettiğimi, bu tez çalışmasıyla elde edilmeyen bütün bilgi ve yorumlara kaynak gösterdiğimi ve bu kaynakları da kaynaklar listesine aldığımı, yine bu tezin çalışılması ve yazımı sırasında patent ve telif haklarını ihlal edici bir davranışımın olmadığı beyan ederim.

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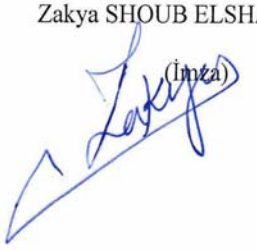

İTHAF (DEDICATION)

Every challenging work needs self-efforts as well as the guidance of elders especially those who were very close to our heart.

My humble effort I dedicate to my sweet and loving

My Husband, My sister, My mother

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KISALTMALAR LİSTESİ (LIST OF ABBREVIATIONS)

APC	Antigen-Presenting Cell
BCR	B Cell Receptor
CFSE	Carboxyfluorescein Succinimidyl Ester
CD	Cluster of Differentiation
FACS	Fluorescent-Activated Cell Sorting
IL	Interleukin
MHC	Major histocompatibility complex
NK	Natural killer Cell
PHA	Phytohaemagglutinin
PBS	Phosphate buffered saline
PBMCs	Peripheral blood mononuclear cells
RPMI	Roswell Park Memorial Institute
TCR	T cell receptor

ÖZET

SHOUB ELSHARI Z. (2018). Sağlıklı Pediyatrik ve Erişkin Yaş Gruplarında Mitojenlerle Uyarılmış Lenfosit Proliferasyon Değerlerinin Karşılaştırılması. İstanbul Üniversitesi, Sağlık Bilimleri Enstitüsü, İmmünoloji A.D. Yüksek Lisans Tezi, İstanbul.

Antijene yanıtlı lenfosit klonlarının prolifere olması, kazanılmış yanıtların ilk basamağı olup immün hücrelerin efektör fonksiyonlarını gerçekleştirebilmesi için önem taşımaktadır. İmmünolojik araştırmalar ve immün tanıda proliferasyon testlerinin kullanımı günden güne yaygınlaşmakta olup bu çalışmalarda sağlıklı kontrol olarak erişkin kanlarının kullanılabilirliği soru işaretidir. Bu çalışmanın amacı, pediyatrik ve erişkin sağlıklı kontrol gruplarında T lenfosit, B lenfosit ve total lenfositlerin mitojenlerle uyarılmış proliferasyon değerlerini karşılaştırmaktır. Çalışmaya 20 erişkin ve 19 pediyatrik birey katıldı. Gönüllülerden alınmış taze periferik kan örneklerinden periferik kan mononükleer hücreleri, fikol konsantrasyon gradyanı yöntemi ile saflaştırıldı, CFSE ile işaretlenen mononükleer hücreler, PHA ve CD2/CD3/CD28 monoklonal antikor kokteyli (CD-mix) ile 120 saat, 37C, %5 CO_2'li hücre kültürü ortamında uyarıldı. Kültür aşamasını takiben $CD4^+$ T ve $CD19^+$ B hücreleri, kendilerine özgül monoklonal antikorlarla işaretlenerek bu hücrelerin ve işaretlenmemiş total mononükleer hücrelerin proliferasyon değerleri akan hücre ölçer cihazı kullanımı ile belirlendi. Çalışmanın sonucları, pediyatrik ve erişkin yaş gruplarında, PBMC, $CD4^+$ T ve $CD19^+$ B hücrelerinin, mitojen uyarımı altında genelde benzer davranışlara sahip olduğunu, PHA uyarısı altında pediyatrik $CD4^+$ T hücrelerinin, erişkinlere göre anlamlı derece yüksek çoğaldıklarını ($p=0,012$) göstermiştir. Pediyatrik bireyler yaş gruplarına göre incelendiğinde, 0-2, 3-5 ve 6-18 yaş arası mitojen yanıtlarının benzerliği gözlenmiştir. Bu bulgular, pediyatrik hasta örnekleri için erişkin kontrol kan örneklerinin kullanılabilirliğini düşündürmektedir.

Anahtar Kelimeler: Akan hücre ölçer, proliferasyon, mitojen, lenfosit, hücre kültürü.

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ABSTRACT

SHOUB ELSHARI Z. (2018). Comparison of Mitogen-Induced Lymphocyte Proliferation Values in Healthy Pediatric and Adult Age Groups. İstanbul University, Institute of Health Science, Department of Immunology. M.Sc. Thesis. Istanbul.

The proliferation of antigen-specific lymphocyte clones, the initial step in acquired immunity, is important for effector functions of immune cells. Proliferation tests both in immunologic research and immune diagnosis are gaining attendance day by day, while utilization of adult healthy controls for comparison with pediatric patients is a question. This study aimed to investigate and compare mitogen-stimulated proliferation responses of peripheral blood mononuclear cells (PBMCs) and T- and B-lymphocytes in adult and pediatric healthy donors. 19 pediatric and 20 adult healthy donors were enrolled in this study. PBMCs purified from fresh peripheral blood samples of the donors by ficoll gradient centrifugation method were stained with CFSE and were cultured in a 37°C CO_2 incubator for 120 hours with the absence and existence of polyclonal activators; PHA and CD-Mix. Following cell culture, PBMCs were stained with antibodies against CD4 and CD19, and proliferation percentages of $CD4^+$ T cells and $CD19^+$ B cells together with total PBMCs were determined by flow cytometry. The results of this study revealed the similarity between pediatric and adult age groups, with respect to responses of PBMCs, T- and B-cells to mitogen stimulation. The only difference observed was a significantly high proliferation of pediatric $CD4^+$ T cells in response to PHA stimulation ($p=0.012$). Pediatric individuals were distributed into age groups of 0-2 years, 3-5 years and 6-18 years, and no difference of mitogen-triggered proliferative responses was observed among age groups. These findings propose the possibility of utilization of adult healthy controls as controls for pediatric patients.

Key Words: Flow cytometry, proliferation, mitogen, lymphocyte, cell culture

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1. INTRODUCTION AND PURPOSE

The proliferation or transformation of lymphocytes in response to an antigenic stimulation leads to lymphocyte expansion, which is important for immune cells to perform their effector functions. The utilization of proliferation assays to determine the history of cell division in the diagnosis of immune deficiencies is helpful for immunological studies [1]. Proliferation tests are needed to be performed by pediatric clinics for conditions such as immunodeficiencies, atopic diseases, lymphoproliferative disorders, and their importance for the diagnosis of the diseases is increasing day by day. The counts and proportions of lymphocyte subgroups in patients with inadequacy, in addition to the cellular functions analysis, is important and proliferation analyzes are important here.

In recent times, the proliferation assays are applied to evaluate the function of T and B lymphoblasts in response to stimulants as mitogens and are becoming a part of clinical practice. Furthermore, novel methodologies are being developed in order to provide safe and non-radioactive approaches; and also to render the laboratory work reliable and easier [2]. Mitogens like plant lectins are antigens to induce *in vitro* responsiveness which initiates the mitotic activity through intracellular signalling via binding to the surface of lymphocyte glycoproteins and transformed to the cell division. For example, Phytohemagglutinin (PHA) as mitogen works as a strong stimulant to activate and proliferate T cells [4]. Proliferation assays have been exploited in screening lots of studies with primary and secondary immunodeficiency conditions. Usually, the controls of the proliferation assays during laboratory clinical practice are adult healthy donors while this test is more applicable for neonates and elder children for investigating immune dysfunction [5]. However, antigen response *in vitro* might vary depending on the time period and the patient age with the history of the disease state and vaccination and also with lymphocytes absolute count [6]. Although the results of the proliferation tests are quantitatively expressed, the interpretation, in general, is qualitative [7]. In clinical practice, we need to utilize reference values to compare the outcomes of the patients. Unfortunately for pediatric age groups, utilizing the control group from a blood sample of healthy children is ethically difficult if there is definitely not a medical problem, though the little amount of blood sample required

(approximately 5-10 mL is collected). Because of this reason, many previous studies utilized peripheral blood mononuclear cells (PBMCs) from adult healthy volunteers for comparison of responses.

T cell functions are extremely important for the avoidance of viral and fungal infections, and also for the provision of help to B cells for isotype switch of antibodies against protein antigens. At present, besides laboratory screening tests, functional evaluation of T lymphocyte disturbance or combined T /B cell immunodeficiencies are essential, most of which are mostly related to the children. *In vitro* lymphocyte proliferation test analysis via dilution of a cytoplasmic dye called Carboxyfluorescein Succinimidyl ester (CFSE) is efficient for practical work. CFSE has the ability to enter the cell to integrate itself into the membrane and to emit fluorescence. In addition, flow cytometry is relevant to evaluate T cell functions by measuring responses to mitogens, Utilization of flow cytometry for T-cell proliferation analysis was reported to be appropriate for diagnosis and management of immune deficiency diseases [8]. The conventional method for estimation of lymphocyte proliferation is converted by the incorporation of tritiated (H^3) thymidine into newly divided cellular DNA [9]. However, this test is hard to utilize in a routine laboratory practice due to both some technical difficulties and also due to the lack of information on lymphocyte subsets [10].

The cells of adaptive immunity have the capacity to respond via antigen-specific receptors and clonally proliferate and rapidly differentiate into activated effector cells [11]. It is important to monitor lymphocyte proliferation while minimizing disruption of cell viability and function. Intracellular fluorescent dye CFSE has been found effective for investigation of lymphocyte division in many laboratories since its initial description by Lyons and Parish in 1994 [8, 12]. CFSE attached to intracellular proteins is equally distributed between each divided daughter cells during mitosis, which gives rise to a 50% drop of fluorescent signal in each cell division that can be recognized via flow cytometry [13].

During the evaluation of proliferative activity of pediatric patients, in order for the trial to be controlled, it is necessary to carry out the experiment together with the blood of a healthy control, which should normally be in similar same age group as the patient. However, it is difficult to take blood from a healthy pediatric under a certain

age group, due to both the limited amount of blood that can be taken, and the difficulty in taking blood from children without any medical reasons.

This study aims to investigate and compare total, $CD4^+$ T- and $CD19^+$ B-lymphocyte proliferation values of adult and pediatric healthy individuals in response to two major mitogens, by CFSE dilution method. No previous studies utilized CFSE staining to determine the proliferation levels of total lymphocytes, $CD4^+$ T- and $CD19^+$ B-lymphocytes in response to CD-mix and PHA mitogens and compare those levels in pediatric and adult healthy control groups. If the outcomes indicate a finding in this area then we could confirm that adult healthy controls can be utilized instead of the pediatric healthy controls and could exclude pediatric individuals as healthy controls, which will prevent paediatrics to give blood samples without a medical need. Also, reference values from adult and pediatric healthy controls are expected to be obtained as a result of this study.

2. GENERAL INFORMATION

The acquired immunity of the human immune framework plays an important role in combating invasive pathogens. T and B cells are important components of acquired immunity and respond to pathogens via soluble or cell-mediated effector mechanisms, specifically by recognition of pathogens with their receptors. The success of this mechanism is directly related to the proliferation capability of specific lymphocytes following the recognition phase. The evaluation of these responses under various stimuli is of great importance for basic and clinical investigations involving recurrent infectious diseases, autoimmune diseases, allergic diseases and immunodeficiencies [14]. The evaluation of lymphocyte proliferation in response to antigens by sensitive and useful methods like CFSE dilution is a great substantial for immunological investigations due to long-lived fluorescence and labeling properties by forming a covalent bond within the cells which give rise to higher intensity and low variance with minimum cell toxicity to follow at least 7 cell divisions [15]. The main method adopted as the gold standard for the measurement of the proliferative activity of lymphocytes is the utilization of radioactive (H^3) -thymidine. In this method, radioactive (H^3) -thymidine is added to the culture at the end of the culture period and is incorporated in the DNA of the cells in direct proportion with the rate of cell division. After the incubation, the measurement is performed with a scintillation counter. Because of the difficulties in evaluating the outcomes, the risks of utilizing radioisotopes, the necessity of special laboratory conditions, information about the proliferation of whole cells, and the impossibility of subgroup analysis, this method is problematic to be utilized in advanced immunological studies [10].

In humans, the utilization of CFSE dilution method by flow cytometry is widespread for evaluation of lymphocyte proliferation [16]. Non-radioactive stains with fluorescent capacities bind to intracellular proteins, and produce fluorescence signal that is distributed to the sister cells gradually, which dilutes fluorescent intensity into half in each cell division, and has serious methodological advantages due to its ability to be used together with surface markers, cost-effectiveness, and flow cytometry evaluation possibilities [10]. It is noted that CFSE is suitable to be used in a multicolour flow cytometer setting, because it can be utilized in combination with a large number of

different fluorochromes, with high fluorescence power, low variance and low cytotoxicity marking of cells [17]. When CFSE and (H^3) -thymidine methods are compared, CFSE has been reported to be a convenient method for a laboratory that performs flow cytometry studies [18], because of cost, infrastructure requirements, and the ability to demonstrate the proliferation of different cell subgroups. Furthermore, it has been shown that the outcomes of (H^3)–thymidine incorporation method is comparable with that of CFSE method, although with different display units [19].

Cell mitosis has to be triggered for the evaluation of lymphocyte proliferation. For this purpose, there are many stimulants for general reproduction or for special cell subgroups (such as T cells, B cells). Plant lectins bind to carbohydrate structures on the cell surface, activating intracellular signalling pathways and trigger cell division via upregulation of metabolic activity [6]. The lymphocyte proliferation triggering characteristic of Phytohemagglutinin, a herbal extract derived from the red kidney bean has been known for many years [4]. It is suggested that PHA, a lectin specific to N-acetyl galactosamine/galactose sugars, has many biologic effects along with its mitogenic properties [20]. Proliferative activity of 5 and 10 µg/mL PHA doses has been demonstrated previously [10]. PHA has been suggested to be a mitogen specific for T cells, as well as affecting B cell proliferation through T cells [21]. It is known that the monoclonal antibody cocktail (CD-mix; a combination of monoclonal antibodies against cell surface CD2, CD3 and CD28 molecules) on the T cell surface triggers T cell proliferation by binding to molecules of the immunological synapse, which are known to be required for initiation of specific immunity. Following activation of the T-cell receptor complex via CD-mix, cell activation, cytokine production and cell proliferation are triggered [22]. Both PHA and CD-mix are polyclonal activators, which means that they can activate all lymphocyte clones whereas allergens or vaccine antigens are monoclonal activators, meaning that they can only activate clones that recognize them specifically.

There are various studies in the field of immunology related to the evaluation of proliferative activity with CFSE [23]. In a study examining the immunomodulatory properties of natural killer (NK) cells, the ability of the IL-10 producing NK cell group to suppress allergen-stimulated T cell proliferation was demonstrated by utilization of this method [24]. In another study that investigated conditions that could break allergen-

specific tolerance, $CD4^+$ T cell proliferative responses to various allergens such as house dust mite, grass pollen was examined via CFSE method and it was shown that the innate inflammatory triggers and factors which can increase antigen-presenting cell capacity could lead to allergen-specific proliferation of T cells in healthy but allergen-sensitized individuals [25]. Tissue cell proliferation was assessed via the CFSE method in a study that examined the role of tonsils in the formation of tolerance to food allergens as well as airborne allergens [26] In another study examining the effects of ozone on human NK cells, the effect of ozone gas on human peripheral blood mononuclear cell proliferation was examined with CFSE [27]. A study investigated suppressive properties of T regulatory cells on both polyclonal and self-antigen specific T cell responses by CFSE dilution method [28].

The application of proliferation studies in the clinical field is still limited. In immune-compromised patients, the importance of evaluating proliferative activity together with immunophenotyping studies in which leukocyte rates are assessed has been addressed [29]. Examination of lymphocyte functions in patients with impaired immunity, of course, is gaining attention in terms of clinical conditions [2]. Unresponsiveness to mitogens is generally regarded as a marker for the presence of a severe immunodeficiency [6]. Although low proliferative values are generally thought to indicate increased side effects in live vaccine usage, such a finding has not yet been demonstrated [7].

It has been reported that the flow cytometer allows functional analyzes such as cellular phenotype, cell rates, cell signalling, and proliferative activity, which have considerable contributions to the diagnosis and follow-up of primary immunodeficiencies [30-32]. Also, it is notable that, with the ageing process the lymphoblasts appear in a certain way to be not fully efficient. but the transformation studies of lymphoblast cells confirmed the hypothesis that the function of the transformed cell is related to the decreased number of cell interactions, instead of a decrease in the activation of all cells. An elder individual may have low reactive cells but the activation impact is fully working [33]. Ageing was claimed to have effects on T-cells and the immune responses to infections as well as immunizations. Neoplasms observed in older individuals are quite different from the ones in young individuals, which is thought to arise from several factors, including the diminished proliferative

capacity, the variabilities in presence of NK cells, changes in TCR repertoire and differences in clonal expansion capacities. One possible and promising intervention that could be employed in an attempt to advance the immune response in the elderly is thymic reconstitution, which could avoid the loss of naïve T-cells and limit the build-up of exhausted and aged CD28null T-cells [34].

2.1. The impact of T cell mechanism

There are multiple causes of the major defects or changes in T cell function with ageing that may bring out diminished proliferative responses and also the inability to function by other means. Although it has been recognized as deficiency of the T cell quantities and Interleukin-2 (IL-2) secretion in the elder adults, there may be a lack of continuous supply of new partially differentiated cells that provide the basis for homeostasis of the human immune framework. In addition, the lack may be related to the loss of thymic activity and failure of the cellular interactions in the immune response and also an inability to respond to stimulatory signals, lack of actions for facilitating cell diversity may prevent age-related immune dysfunction [35]. In a study, $CD4^+$ T cells were raised with the presence of immobilized anti-CD3 with the presence of IL-4, the similar cytokine that tempted division-linked differentiation in B cells. CFSE dilution was utilized in this study to determine the quantitative analysis for differentiation and variable division numbers [36].

It has been declared that the T-cell-dependent path framework generates immune responses which need the presence of both B-cell and T-cell responses. the B-cells via proliferation would drive uptake and processing of antigens by the B-cell and presentation of peptides of that antigen to the specific T-cell. Furthermore, non-specific uptake and processing via professional APCs would lead to the presentation of the antigens on class II MHC to naïve T-cells. These activated T-cells, upon encountering the antigen-primed B-cell, deliver the cytokine signal required to cause the B-cells to convert to IgG-secreting plasma cells. The T-cell-independent response occurs via way of an outcome of cross-linking of B cell receptor (BCRs) via repetitive epitopes on the antigen/ mitogen. A cytokine signal required to enable the B-cells to mature into plasma cells [3] are presented in Figure 2.1. below.

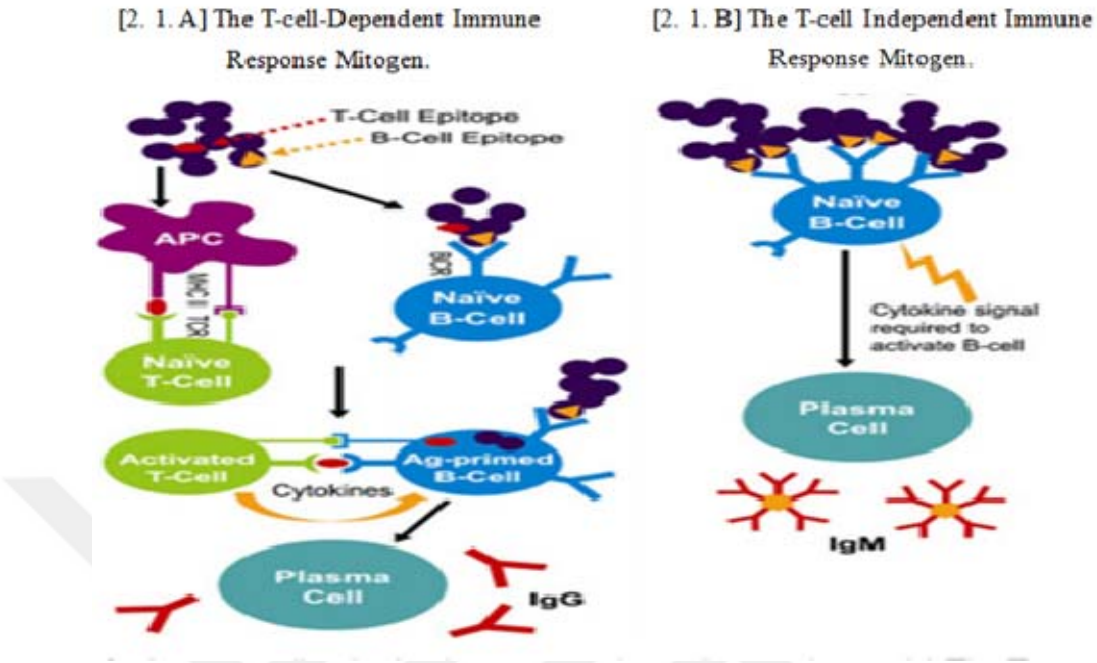

Figure 2-1: Basic theoretical path frameworks for immune cell activation by mitogens.

The T-cell-dependent path framework (2.1.A) and the T-cell-independent path framework (2.1.B) figures were adapted from [3].

2.2. $CD4^+$ T lymphocyte proliferation in age groups.

It has been known that lymphocyte functions significantly disturb and decline with increasing age within the ageing process. T lymphocytes appear more susceptible to age changes. In order to understand the changes of T cell proliferation with respect to advancing age, in response to T cell receptor (TCR) triggering by mitogens, activation of protein kinases, phosphorylation and decrease in interleukin 2 (IL 2) production were claimed to be adequate parameters [33]. Proliferation dynamics for $CD4^+$ T cells collected from two healthy donors correspondingly indicated that many of the cyton model parameters for describing cell proliferation can be reliably estimated utilizing our approach; however, they also show that substantial changes and experimental procedures may be required to ensure identifiability of the remaining cell proliferation parameters. They correspondingly considered with PHA-stimulated cells. Because being a non-specific T cell mitogen, PHA stimulates all T cells to begin dividing and therefore leads to cell proliferation [14].

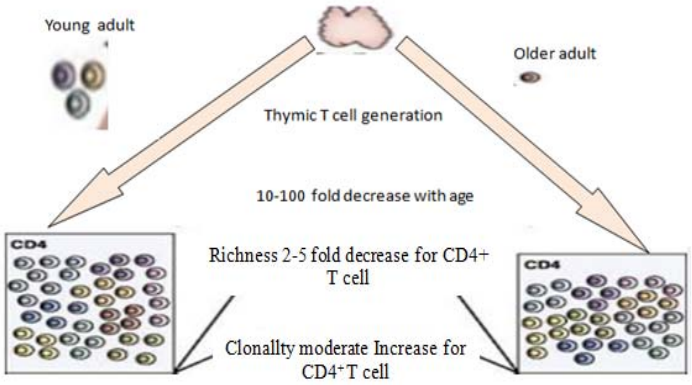

Figure 2-2: The impact of age on $CD4^+$ T cells.

The figure was adapted from [37].

T cell repertoire remains a daunting task; To maintain a large and at the same time diverse CD4 T cell repertoire via age. Population aspects, identification of potentially targetable faults is delivery renewed interest. Genomic and biochemical facts can be identified that are pertinent for ageing besides that is instructive to recognize naive T cell dysfunction. One hallmark sets naive T cell ageing apart from most additional tissues except stem cells; they initiate, however, do not complete differentiation stages toward memory cells. Maintaining quiescence besides avoiding differentiation may be the ultimate challenge to maintain the functions unique for naive T cells [37, 38].

2.3. $CD19^+$ B lymphocyte proliferation in age groups.

It has been determined that CD19 molecule inessential for B cell diversity and isotype switch but it plays an important role in a regulation response which influences with B-cell diversity Since humoral reactions to both T-cell-dependent and T-cell-independent antigens remained correspondingly affected via alterations in CD19 appearance, these variances are maximum expected to outcome from intrinsic variations in B-cell function rather than from the selective disruption of B-cell interactions thru T cells [39]. Lymphocytes essentially proliferate besides separate in reaction to the low attendance of an enormous array of antigens. Colligation of the membrane protein CD19 thru the antigen receptor of B lymphocytes reduced the threshold for antigen

receptor-dependent stimulation via 2 orders of magnitude. B lymphocytes proliferated when one-hundred antigen receptors per cell, 0.03 per cent of the total, were colligated with CD19. The B cell resolves the aforementioned dilemma via abstaining an accessory protein that allows activation once a lesser number of antigen receptors are occupied [40].

2.4. Flow cytometry

Flow cytometry is being progressed during the last 3 decades, from being a laboratory technique to a routinely used tool for both basic and clinical immunologists, in a wide area from routine diagnosis to highly advanced research [41]. It also has applications in molecular biology, cancer research, drug discovery, microbiology and virology [42-45]. This highly developed tool could be used to quantify various parameters as cell size and granularity, and surface as well as intracellular expressions of certain molecules. These parameters help for discrimination of certain cells types. In a typical flow cytometer, a laser beam is focused on the cells surrounded by a sheath fluid which are passing through a flow chamber in a single file manner. When the laser beam hits the particular cell, the beam is scattered in two different directions, which are recognised by two different detectors; forward scatter (FSC) which gives information about the size of the cell, and side scatter (SSC) which informs about the granular content of the cell. These two parameters are basic information one can obtain from a flow cytometer and are adequate for quantification of lymphocytes, granulocytes and monocytes in PBMC. If cells are labelled with certain monoclonal antibodies (MAbs) which are marked with certain fluorescent probes as fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), PE-cyanine 7 (PE-Cy7), laser beam induces excitation of this fluorescent molecule which causes emission of this fluorochrome, and this specific emission is captured by fluorescent detectors of the flow cytometer and are converted into electrical signals and are sent to a computer which enables evaluation of data gathered from a flow cytometer, by utilization of a specific software [46].

Flow cytometry can be used for immunophenotyping and/or sorting of various cell types with respect to their surface receptors termed as a cluster of differentiation (CD), investigation of the cell cycle, apoptosis, proliferation, cytokine production as well as ion exchange of cells [42, 47].

3. MATERIALS AND METHODS

3.1. Study samples

Blood samples obtained from 19 healthy paediatrics with age group (0 – 18) and 20 healthy adults with age group between (18 – 60). both participant groups were healthy with no health problems. The paediatric samples were provided from Istanbul University, Cerrahpasa Faculty of Medicine, Department of Pediatrics. Blood samples of the adult volunteers were provided by Istanbul University, Aziz Sancar Institute of Experimental Medicine. The warning signs of European Society for Immunodeficiencies (ESID) was followed as sample selection criteria. These are as follows: For pediatric controls; not having 4 or more ear infections per year, not having 2 or more sinus infections per year, not being under antibiotics treatment more than 2 months with observed limited effects, not suffering from 2 or more pneumonia per year, not having retarded growth or weight gain, not having deep tissue or organ abscesses, not having persistent oral candidiasis or skin infections with fungi, no requirement for intravenous antibiotic treatment against infections, not having 2 or more deep-localized infections including septicemia, and no familial history of primary immunodeficiencies. For adult controls; not having 2 or more ear infections per year, not having 2 or more new sinus infections per year with the absence of allergy, not having a pneumonia history per year for a period longer than one year, not having chronic diarrhea which ends up with weight loss, not having repetitive viral infections (including cold, warts, herpes, condyloma), no request for intravenous antibiotics for treatment of infections, not having repetitive deep abscesses in skin nor organs, not having persistent candidiasis nor fungal infections in skin or other locations, not having infection with otherwise harmless bacteria as mycobacteria, and not having familial history of primary immunodeficiencies. This study was approved by a local ethical committee of Istanbul University, Istanbul Faculty of Medicine. The signed consent from all donors was obtained.

3.2. Lymphocyte separation

Peripheral blood was collected in lithium heparin tubes (3 – 5 ml vacutainer; BD Vacutainer, UK) peripheral blood mononuclear cells were purified by ficoll concentration gradient centrifugation method. Briefly, the blood sample was diluted

with an equal volume of PBS solution with a ratio of (1: 1) and then layered over ficoll – hypaque (Capricorn, USA) with a ratio of (2: 1), and centrifuged at 20 ℃ at 2050 rpm for 20 minutes. The mononuclear cells layer (PBMC) was collected with a Pasteur pipette and transferred to a 15 ml Falcon tube (BD Biosciences, USA) and washed twice with PBS for 5 minutes at 20 ℃ at 1600 rpm. If erythrocyte contamination was observed in the cell pellet following 2 washing steps, erythrocytes were depleted with the addition of 2 ml of NH_4Cl solution and incubation for 10 minutes, and a washing step for 5 minutes at 20 ℃ at 1600 rpm.

3.3. CFSE staining and Cell culture

Peripheral blood mononuclear cells were counted under light microscopy by trypan exclusion method. Up to $2x10^7$ cells were suspended in 1 ml of RPMI1640 culture medium enriched with penicillin, streptomycin, L-glutamine, non-essential amino acids, sodium pyruvate and MEM vitamins (All from Gibco, Paisley, UK). 1 µl of 5 mM CFSE solution (Molecular Probes, Eugene, USA) was added to the PBMC suspension, incubated in the dark for 6 minutes at 4 ℃ then washed twice for 5 min at 2050 rpm at 4 ℃. Following CFSE staining, cells were counted under light microscopy with trypan exclusion method, and were cultured in round bottom, 48-well, flat bottom culture plates (NEST Biotechnology, China) for 5 days at 37 ℃ in 5 % CO_2 environment with 5 µl / ml phytohaemagglutinin (PHA, Thermo Fisher, USA) and 1/500 CD- Mix (Stem Cell, USA).

3.4. Determination of lymphocyte proliferation via flow cytometer

After 5 days of incubation with the absence and presence of mitogens, 100 µl cell suspension was taken from the respective well (us: unstimulated, PHA: Phytohemagglutinin-stimulated, CD-Mix: stimulated with CD-Mix) in 3 separate flow cell gauge tubes. 2.5 µl of anti-CD4 phycoerythrin-cyanin 7 (PE-Cy7) (BD Biosciences, USA) and 1 µl of anti-CD19 allophycocyanin (APC) (BD Biosciences, USA) fluorescent-labelled monoclonal antibodies were added to each tube and incubated at dark and at room temperature for 20 mins. After incubation, 5 ml of FACS buffer was added to each tube and washed at 20 ℃ for 5 minutes at 1600 RPMI. Following washing, the supernatant was removed, cells were resuspended in 500 µl of FACS buffer and analyzed in a FACs Calibur flow cytometry running CellQuest software (BD Biosciences, United States).

Analysis of data was performed with CellQuest software. The obtained data were evaluated as % proliferation. Total peripheral blood mononuclear cells, $CD4^+$ T cell and $CD19^+$ B cell proliferation percentages were evaluated separately for two different mitogens.

3.5. Statistical Analysis

The group's concordance to a normal distribution was evaluated by the Kolmogorov Smirnov Test. Differences between normally distributed groups were evaluated by Student's T-test. Differences between groups with no normal distribution were evaluated by the Mann Whitney U test. ($p<0.05$) was accepted as the statistical significance level. All data were expressed as (MEAN±SD).

4. RESULTS

Nineteen pediatric healthy children (11 female, 8 male, age: 7.16±6.07) and 20 healthy adult volunteers (8 female, 12 male, age: 35.95±5.56) were enrolled in this study. 6 of the children were aged between 0-2 years, 4 children were aged between 2-5 years and 9 children were aged between 5-18 years (Figure 4.1). Age groups were determined according to the expected changes in counts of neutrophils and lymphocytes in relation with advancing age [48]. Adult healthy volunteers were aged between 26-46 years. All of the controls enrolled in this study were healthy during their blood samples were taken.

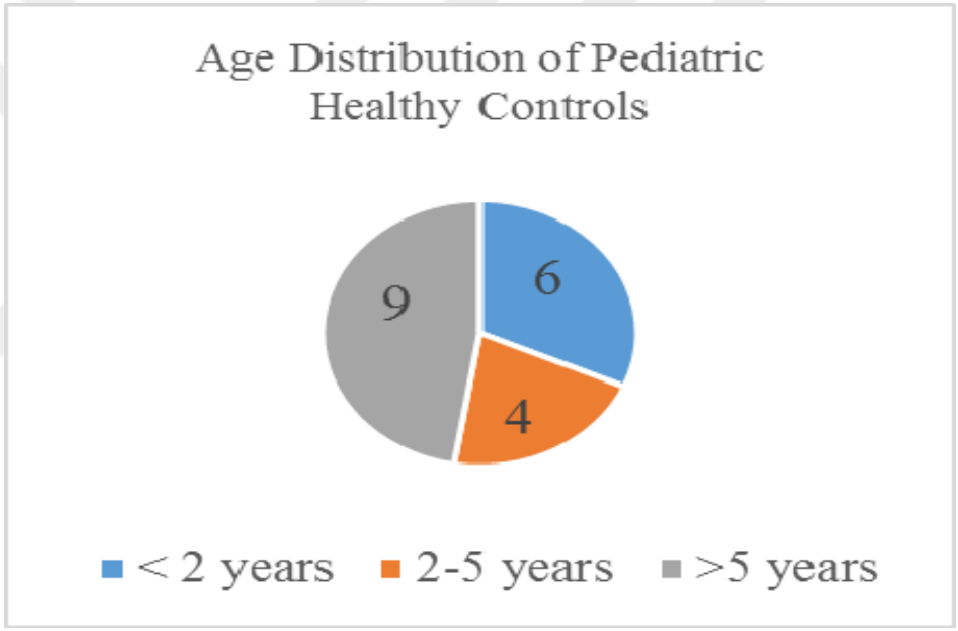

Figure 4-1: Age distribution of pediatric healthy volunteers enrolled in this study.

Proliferation levels of PBMCs were expressed as % proliferation. Spontaneous proliferation was marked on the unstimulated cells and gate was used for both mitogens. (Figure 4.2).

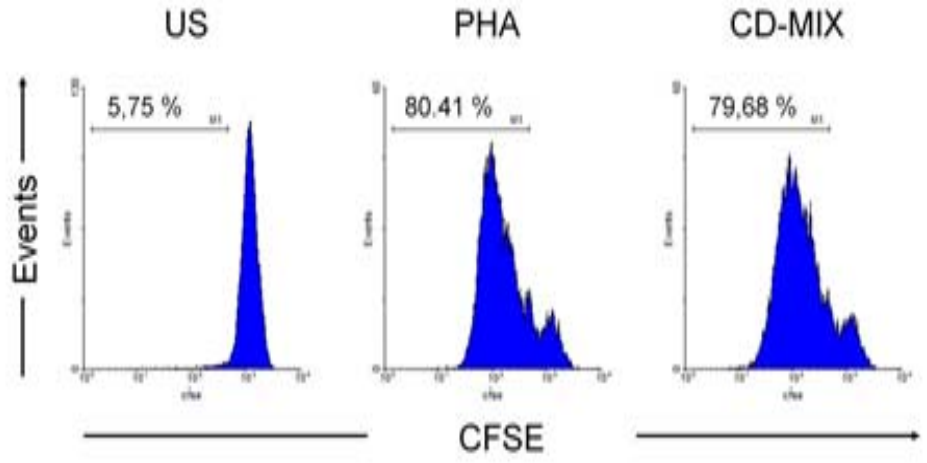

Figure 4-2: A demonstrative histogram graphic of the proliferation of PBMCs in response to mitogens.

When proliferation levels of total PBMCs were investigated, both pediatric and adult healthy control groups revealed similar proliferative capabilities in response to both mitogens. No significant differences were observed among adult and pediatric healthy control groups (Figure 4.3).

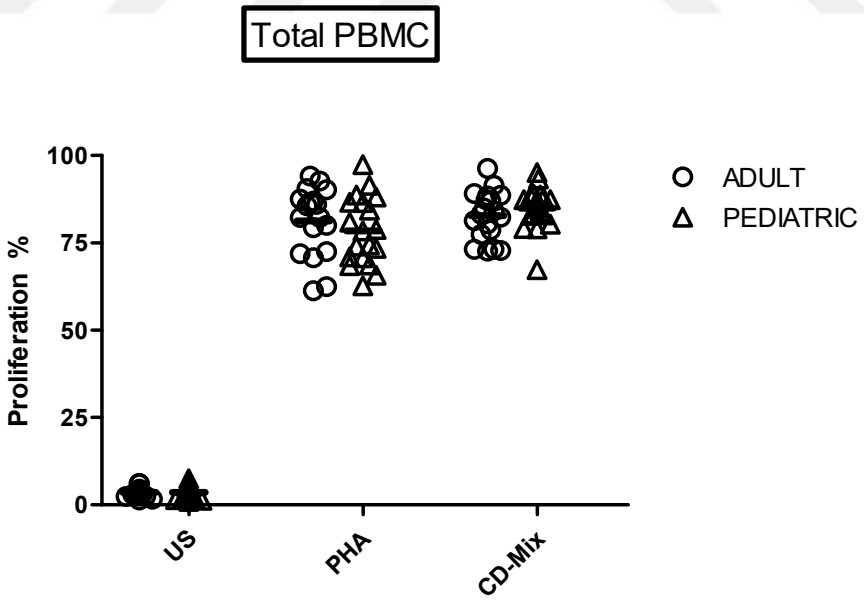

Figure 4-3: Total PBMC proliferation levels of adult and pediatric healthy controls, with the absence and existence of mitogens.

Lines indicate mean values of the groups.

With the aim of investigation of $CD4^+$ T lymphocyte proliferation, PBMCs were stained with anti-CD4-PeCy7 monoclonal antibody after the termination of cell culture. Density plot was used for the presentation of CD4+ T cell proliferation. Proliferating cell percentages were used as data. The quadrant was set according to the unstimulated condition and was used for PHA- and CD-Mix-stimulated conditions (Fig. 4.4). $CD4^+$ T cells of pediatric controls were observed to respond to PHA significantly stronger than that of adult controls, (40.35±11.23 % vs 29.66 ±10.85 %, respectively, p=0.012). CD-mix induced pro-liferative values of pediatric and adult control groups were observed to be similar to each other (43.66±10.15 % vs 49.32±14.26 %, respectively) (Figure 4.5).

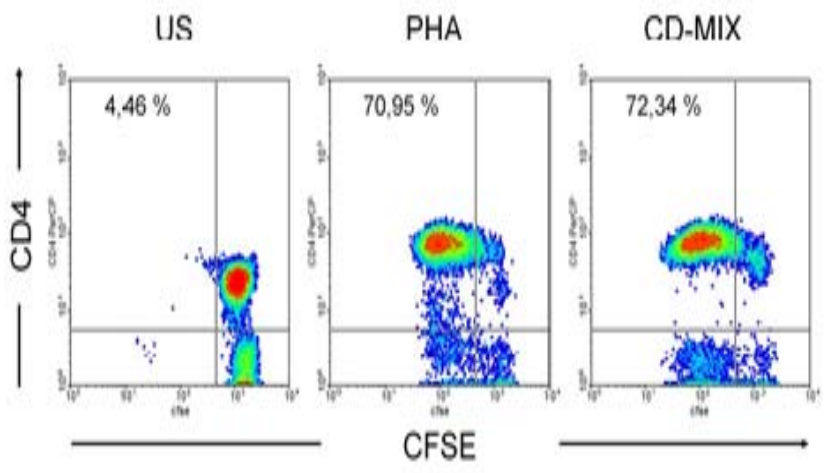

Figure 4-4: A demonstrative density plot graphic of $CD4^+$ T cell proliferation in response to PHA and CD-Mix mitogens.

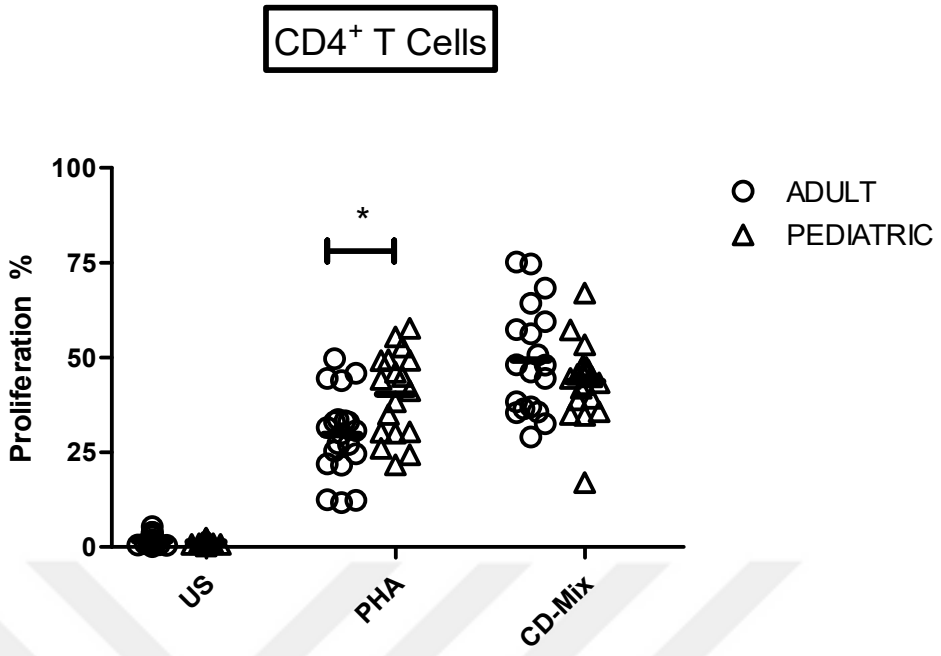

Figure 4-5: Proliferation levels of $CD4^+$ T cells of pediatric and adult healthy controls in response to mitogens; PHA and CD-Mix.

Lines indicate mean values of relevant groups. (* p=0.012)

With the aim of investigation of $CD19^+$ B lymphocyte proliferation, PBMCs were stained with anti-CD19-APC monoclonal antibody following the termination of cell culture. Density plot was used for the presentation of CD19+ B lymphocyte proliferation. Proliferating cell percentages were used for data evaluation. The quadrant was set according to the unstimulated condition and was used for PHA- and CD-Mix-stimulated conditions (Fig. 4.6). When proliferation levels of $CD19^+$ B cells were investigated, no significant differences were observed among the adult and pediatric control groups in response to both PHA and CD-Mix mitogen stimulations. In other words, B cell responses following cell culture with PHA- and CD-Mix mitogens were concordant (Figure 4.7).

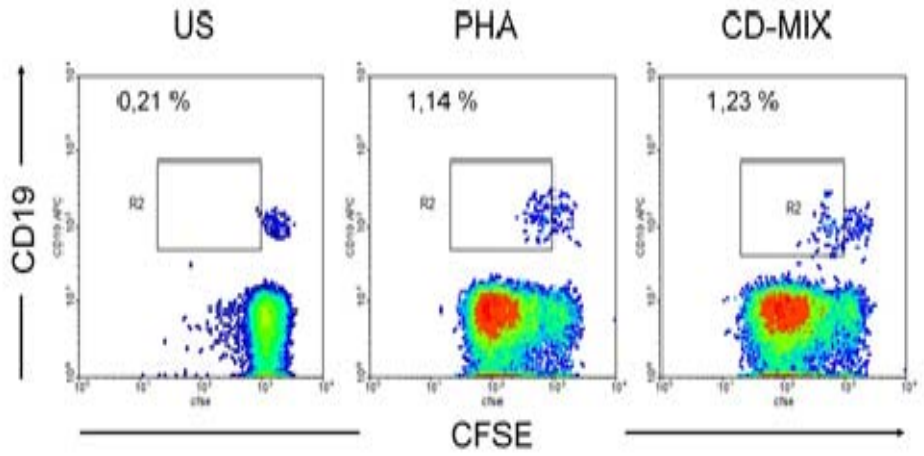

Figure 4-6: A demonstrative density plot graphic of CD19$^+$ B cell proliferation with the absence or existence of mitogens PHA and CD-Mix.

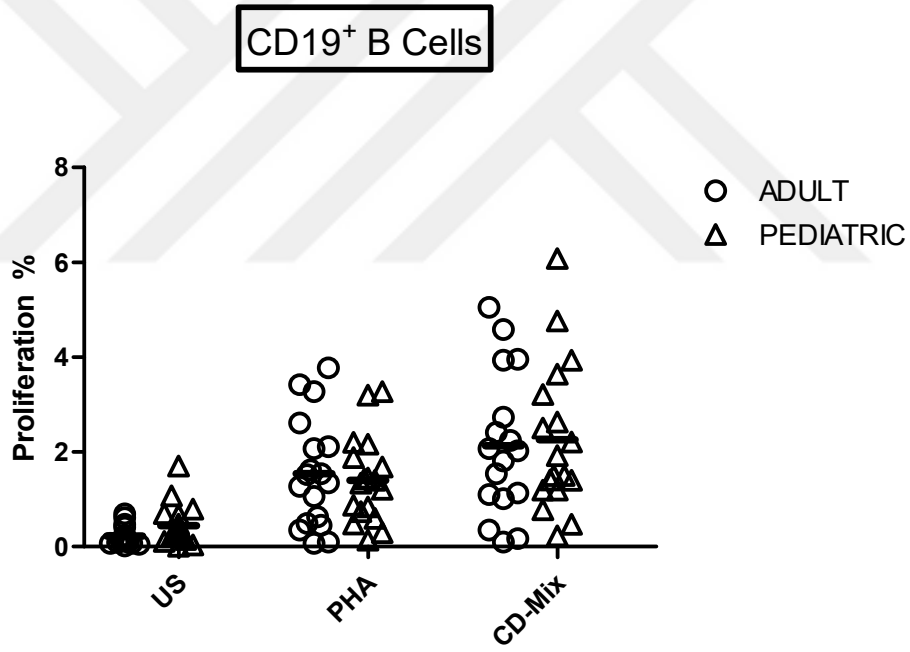

Figure 4-7: Proliferation levels of CD19$^+$ B cells of pediatric and adult age groups, in response to mitogens; PHA and CD-Mix.

Lines indicate mean values of the relevant groups.

Responses of PBMCs, CD4$^+$ T cells and CD19$^+$ B cells to two polyclonal activators; PHA and CD-Mix, in different age groups of pediatric controls were investigated in this study. No significant differences in proliferative values of PBMCs

(Figure 4.8), $CD4^+$ T cells (Figure 4.9), as well as $CD19^+$ B cells (Figure 4.10) among different age groups, were observed.

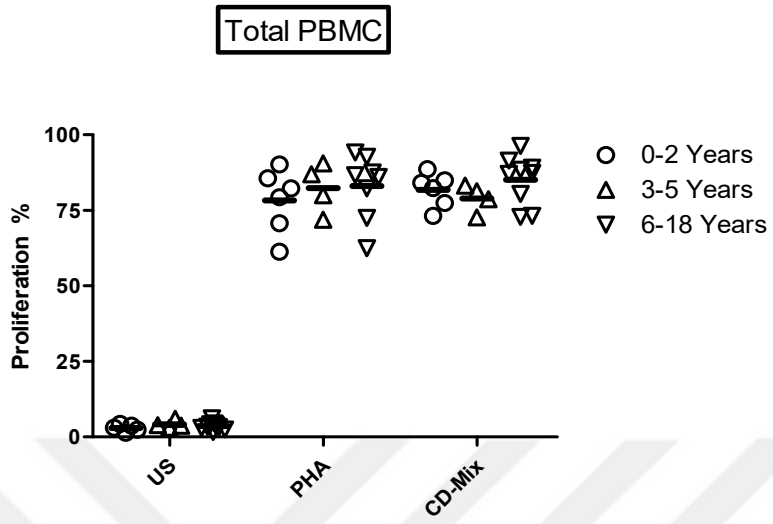

Figure 4-8: Proliferation values of total PBMCs in different age groups of pediatric healthy controls.

Lines indicate mean values of the groups.

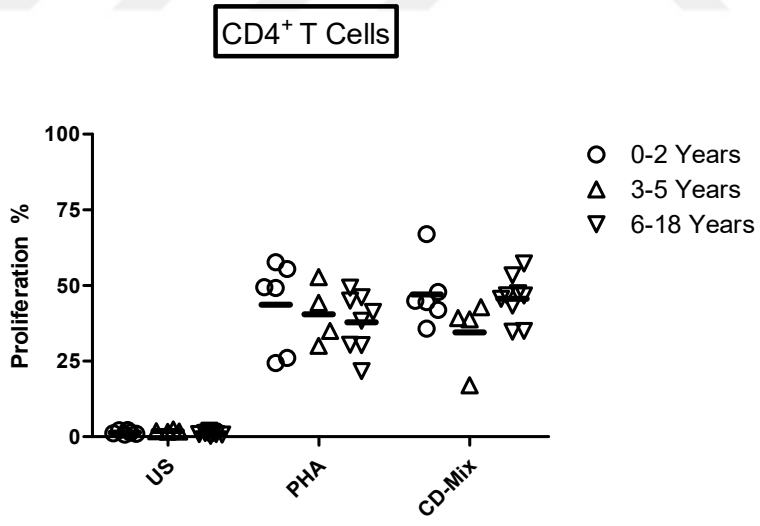

Figure 4-9: Proliferation values of $CD4^+$ T cells in different age groups of pediatric healthy controls.

Lines indicate mean values of the groups.

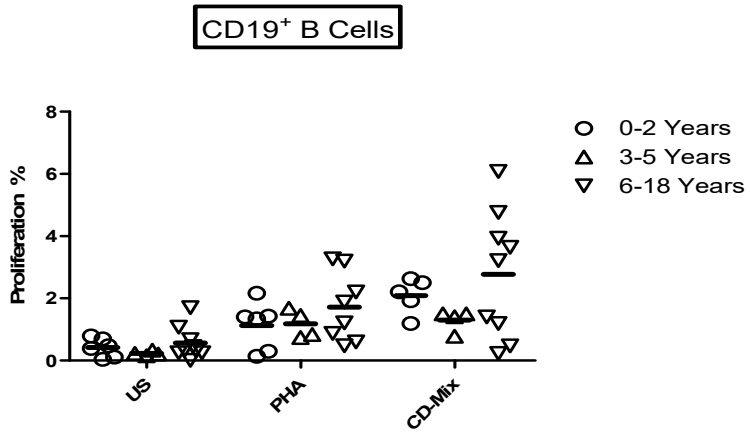

Figure 4-10: Proliferation values of CD19+ B cells in different age groups of pediatric healthy controls.

Lines indicate mean values of the groups.

When cumulative data of this study was evaluated, preliminary reference values for proliferative capacities of total PBMCs, $CD4^+$ T cells and $CD19^+$ B cells were obtained for different pediatric age groups and adults. These preliminary values are summarized in Table 4.1.

Table 4-1: Reference values for the mitogen-induced proliferation of PBMCs, $CD4^+$ T cells and $CD19^+$ B cells in adult and pediatric healthy controls

			PHA-Stimulated			CD-Mix Stimulated		
	n		**PBMC**	**$CD4^+$**	**$CD19^+$**	**PBMC**	**$CD4^+$**	**$CD19^+$**
Adult	**20**	Mean±SD	78.42±9.66	29.66±10.85	1.54±1.15	84.91±6.02	49.32±14.26	2.13±1.51
		Cut-off	59.09	7.96	-	72.87	20.80	-
Pediatric								
Total	**19**	Mean±SD	81.27±9.82	40.35±11.23	1.40±0.90	82.74±6.88	43.66±10.15	2.25±1.56
		Cut-off	61.63	17.89	-	68.99	23.36	-
1-2 y	**6**	Mean±SD	78.24±10.56	43.68±14.72	1.13±0.77	81.75±5.60	46.95±10.63	2.09±0.57
		Cut-off	57.12	14.25	-	70.56	25.70	0.94
3-5 y	**4**	Mean±SD	82.33±8.22	40.48±10.07	1.18±0.46	78.94±4.60	34.47±11.77	1.30±0.35
		Cut-off	65.90	20.34	0.26	69.75	10.93	0.60
6-18 y	**9**	Mean±SD	83.00±10.66	37.78±9.53	1.71±1.11	85.10±8.05	45.54±7.40	2.77±2.04
		Cut-off	61.68	18.72	-	69.01	30.73	-

Reference values summarized in the table are derived from % proliferation values of healthy donors of the relevant age groups. Cut-off values were evaluated with the formula: (mean-(2xSD)) of the relevant group. (All values indicate proliferation percentages of relevant cell groups, n: number of individuals in each group).

5. DISCUSSION

The proliferation of the antigen-specific lymphocyte clones is the initial step in acquired immunity, which is important for the effector functions of the immune cells. Proliferation tests both in immunologic research topic as tolerance studies, antigen responsiveness and determination of efficacy of inhibitory molecules, and immune diagnosis of especially immune deficiency diseases is gaining attendance day by day, while utilization of adult healthy controls for comparison with pediatric patients is a question.In this study, the rates of mitogen induced proliferation of total lymphocytes and CD4 and CD19 sub-groups were evaluated in adult healthy individuals and different age groups of pediatric individuals. Our results revealed similar proliferative capacities of lymphocytes in all pediatric age groups. All but one parameter were concordant in adult and pediatric control groups, while PHA-responsiveness of $CD4^+$ T cells was observed to be stronger in pediatric controls, in comparison with adult healthy controls. This variability may be attributed to some facts. A previous study reported diminished proliferative responses of $CD4^+$ T cells in elder adults with ages over 65 years old to PHA and anti-CD3 stimulations, in comparison with young adults aged between 20-30 years old [49]. While, another study reported a slightly decreased growth of $CD4^+$ T cells in elder adults following PHA stimulation, which was observed to be completely similar among elder and younger adults under anti-CD3 stimulation. The counts of activated cells in both age groups that continue to live and proliferate in the culture were observed to be unnoticeable [35]. The reduced proliferation is related to the decreased efficiency of signal transduction via comparing this to the mobilization of intracellular free calcium activity after stimulation with PHA. Calcium was measured in $CD4^+$ T cells of young and old adults utilizing a flow cytometry assay with the dye indo-l Compared to cells from young adults, $CD4^+$ cells from old adults showed a calcium response which was up to ten percent lower following PHA stimulation. This appeared to be related to decreased calcium channel activity in old adults, rather than the mobilization of intracellular calcium stores. The reduced proliferation of T cells from old adults was not found to be related to the decreased efficiency of transmembrane signal transduction [49]. Also, in a study, no functional difference was observed between healthy young and elder persons when expression of co-stimulatory

molecules; CD27 and CD28 on $CD4^+$ T cells were investigated [50] . In our study, adult population (35.95±5.56 years, range: 26-46 years) possibly fits young adult group, therefore the observed variability of $CD4^+$ T cell responses could possibly be a property of pediatric age group. More pediatric healthy controls are needed to increase the size of the age-related subgroups, which therefore may help for more detailed investigations to be converted.

Taken together, our data support the hypothesis that healthy blood samples of relatively young adults could be used as control blood for pediatric patients, in lymphocyte proliferation studies which utilize PHA and CD-Mix as polyclonal activators. This may avoid unnecessary blood sampling from healthy pediatric to be used as a control for a diseased counterpart. Diminished responses of adult $CD4^+$ T cells in response to PHA should be kept in mind and should further be investigated. The reference values achieved from this study may be used for evaluation of pediatric patients.

As conclusion, the results of this study has presented that, there big similarities between healthy adults and healthy pediatric individuals in terms of mitogen-stimulated lympho-cyte proliferation and in this case the utilization of healthy adult blood samples for com-parison with pediatric patient values will be possible. A conclusion that comes out alter-natively is that this study revealed the necessity and importance of forfeiting attention to the age group of healthy controls in terms of proliferation. More and more studies will utilize proliferation tests in the near future, and an answer to the healthy controls issue may be arised by this study.

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FORMLAR

BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU

Araştırma Katılımcıları: 0-12 Yaşlar

Sağlıklı Pediyatrik ve Erişkin Yaş Gruplarında Mitojenlerle Uyarılmış Lenfosit Proliferasyon Değerlerinin Karşılaştırılması

Sevgili Anne – Baba,

Sizleri, yakın zamanda kullanıma girmiş olup bazı hastalıkların tanısı ve takibinde yardımcı olan yeni bir yöntemin kullanımına katkı sağlayacak bir araştırmaya katılmaya davet ediyoruz. Bu çalışmaya katılım kararından önce, yapılacak çalışma hakkında bilgi vermek istiyor, eğer katılmaya karar verirseniz olur formunu imzalamanızı rica ediyoruz. Katılmak istememeniz halinde çocuğunuzun takip veya tedavisiyle ilgili hiçbir değişiklik olmayacak, herhangi bir dezavantajınız olmayacaktır. Ayrıca, herhangi bir nedenle bu çalışmadan ayrılmak isterseniz inceleme sonuçlarınız isteğe bağlı silinecektir.

Bu çalışmaya katılmanız halinde, zaten rutin kontrol için çocuğunuzdan kan alınması esnasında bize, bir defaya mahsus olmak üzere, 3-4 mililitre daha kan vermenizi rica ediyoruz. Böylece, çocuğunuz için ilave bir iğne deliği ve ilave sıkıntıya neden olmayacağız. Bu kan miktarı, çocuğunuzun sağlığı üzerinde hiçbir olumsuz etkiye yol açmayacak kadar düşüktür. Doktorunuzun kontrolünde çalışmaya katılım yapılacağı için kendinizi güvende hissedebilirsiniz. Bu çalışmada, çocuğunuza doğrudan herhangi bir tedavi veya işlem yapılmayacak, herhangi bir madde verilmeyecektir. Katılım, sadece kan vererek gerçekleşecektir. Alınan kan örnekleri laboratuvarda incelenecektir, çalışmanın genetik araştırmalarla hiçbir ilgisi olmayıp, sadece hücre fonksiyonları incelenmektedir.

Bu çalışmaya katılmak normal kan verme işleminin sıradan ve önemsiz yan etkileri olan çok az bir acı ve kan alma yerinde morluk oluşumuna neden olabilir. Ancak, bu işlemi gerçekleştiren kişilerin deneyimleri sayesinde çoğunlukla bu yan etkiler de gözlenmeden kan alma işlemi tamamlanmaktadır. Zaten rutin kontroller için kan veriyor olduğunuzdan çalışmaya katılmak ilave bir sıkıntıya neden olmayacaktır.

Bu çalışmanın amacı, sağlıklı çocukların akyuvar hücrelerinin çalışma kapasitesini ölçüp normal değerlerini saptamak ve erişkinlerdeki değerlerle karşılaştırmaktır. Çocuğunuzun çalışmaya katılması için sadece kan vermesi yeterlidir, çalışmayla ilgili başka bir test, tetkik, ölçüm istenmeyecektir. Bu araştırmaya katılmanızın çocuğunuzun sağlığı ve bakımına doğrudan yararı olmayacaktır, ancak çalışma sonucu elde edilecek veriler ile ciddi sağlık problemleri yaşayan pek çok çocuğun sonuçlarının doğru yorumlanması ve tanı almasına yardımcı olabilirsiniz. Hasta çocuklardan istenen lenfosit proliferasyon (çoğalma) testi çalışmasında sağlıklı bir kontrolün kanı da gereklidir. Bizim çalışmamız, erişkin hücrelerinin çoğalmasıyla çocukların hücrelerinin çoğalmasını karşılaştıracaktır. Sizin katılımınızla yapılacak bu karşılaştırma, erişkin kanlarının kontrol olarak kullanılabileceği sonucunu ortaya koyarsa, hasta çocukların karşılaştırmaları için sağlıklı çocukların kan vermesinin önüne geçilebilir.

Bu çalışmaya katılmanız halinde, tüm bilgilerinizin gizli tutulacağını, katılan gönüllü isimlerinin kesinlikle saklanacağını, verilerin tamamen bilimsel çalışmalar için kullanılacağını garanti ederiz. Buradan elde edilecek verilerin ticari amaçlarla kullanımı söz konusu değildir.

Araştırmaya katılım kararını vermeden önce, araştırma süreci, beklenen sonuçlar hakkında ilgili kişilere dilediğiniz soruyu sorabilirsiniz.

Sizlerden, çalışmaya katılmanız halinde hiçbir ücret alınmayacak ve ödeme yapılmayacaktır. Çalışmaya katılmanız veya çalışmadan ayrılmanız, olağan tıbbi bakımlarınızı hiçbir şekilde etkilemeyecektir.

Çalışmayla ilgili tüm sorularınız için Prof. Dr. Yıldız Camcıoğlu'na, veya Doç. Dr. Umut Küçüksezer'e (0212) 4142000 / 33342 no'lu telefondan ulaşabilirsiniz.

Yukarıdaki tüm bilgiler, tarafıma …… tarafından anlatıldı.

☐ Bu bilgi formunu okudum, araştırmanın konusunu anladım, açık olmayan herşey konusunda doktorumla görüştüm.

☐ Bu araştırmaya isteyerek katılıyorum, herhangi bir neden göstermeden herhangi bir zamanda, herhangi bir endişe duymadan çalışmadan ayrılabileceğimi biliyorum.

☐ Kan incelemelerinin değerleri saklanabilir ve ismim kullanılmaksızın bilimsel olarak kullanılabilir.

GÖNÜLLÜ ONAY FORMU

Yukarıda gönüllüye araştırmadan önce verilmesi gereken bilgileri gösteren metni okudum. Bunlar hakkında bana yazılı ve sözlü açıklamalar yapıldı. Bu koşullarla söz konusu klinik araştırmaya kendi rızamla hiçbir baskı ve zorlama olmaksızın katılmayı kabul ediyorum.

Veli veya vasinin

Adı-Soyadı: Tarih:

Adresi: Telefon no:

İmzası:

Açıklama Yapanın

Adı Soyadı: İmza:

Birimi: Tarih:

BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU

Araştırma Katılımcıları: 12-18 Yaşlar

Sağlıklı Pediyatrik ve Erişkin Yaş Gruplarında Mitojenlerle Uyarılmış Lenfosit Proliferasyon Değerlerinin Karşılaştırılması

Değerli Genç Kardeşim,

Seni, yakın zamanda kullanıma girmiş olup, özellikle çocuklarda görülen bazı hastalıkların tanısı ve takibinde yardımcı olan yeni bir yöntemin kullanımına katkı sağlayacak bir araştırmaya katılmaya davet ediyoruz. Bu çalışmaya katılım kararından önce, yapılacak çalışma hakkında bilgi vermek istiyor, eğer katılmaya karar verirsen olur formunu imzalamanı rica ediyoruz. Katılmak istememen halinde takip veya tedavinle ilgili hiçbir değişiklik olmayacak, herhangi bir dezavantajın olmayacaktır. Ayrıca, herhangi bir nedenle bu çalışmadan ayrılmak istersen inceleme sonuçların isteğe bağlı olarak silinecektir.

Bu çalışmaya katılman halinde, zaten rutin kontrol için senden kan alınırken, bize, bir defaya mahsus olmak üzere, 3-4 mililitre daha kan vermeni rica ediyoruz. Böylece senin için ilave sıkıntıya neden olmayacağız. Bu kan miktarı, sağlığın üzerinde hiçbir olumsuz etkiye yol açmayacak kadar düşüktür. Doktorun kontrolünde çalışmaya katılım yapacağın için kendini güvende hissedebilirsin. Bu çalışmada, sana doğrudan herhangi bir tedavi veya işlem yapılmayacak, herhangi bir madde verilmeyecektir. Katılımın, sadece kan vererek gerçekleşecektir. Alınan kan örnekleri laboratuvarda incelenecektir, çalışmanın genetik araştırmalarla hiçbir ilgisi olmayıp, sadece hücre fonksiyonları incelenmektedir.

Bu çalışmaya katılmak normal kan verme işleminin sıradan ve önemsiz yan etkileri olan çok az bir acı ve kan alma yerinde morluk oluşumuna neden olabilir. Ancak, bu işlemi gerçekleştiren kişilerin deneyimleri sayesinde çoğunlukla bu yan etkiler de gözlenmeden kan alma işlemi tamamlanıyor. Zaten rutin kontroller için kan veriyor olduğun için, bir kez daha belirtelim ki çalışmaya katılmak ilave bir sıkıntıya neden olmayacaktır.

Bu çalışmanın amacı, sağlıklı çocukların akyuvar hücrelerinin çalışma kapasitesini ölçüp normal değerlerini saptamak ve erişkinlerdeki değerlerle karşılaştırmaktır. Çalışmaya katılman için kan vermen dışında başka bir test, tetkik, ölçüm istenmeyecektir. Bu araştırmaya katılmanın, sağlığın ve bakımına doğrudan yararı olmayacaktır, ancak çalışma sonucu elde edilecek veriler ile ciddi sağlık problemleri yaşayan pek çok çocuğun sonuçlarının doğru yorumlanması ve tanı almasına yardımcı olabilir, bu genç yaşında hasta çocuklara önemli katkıda bulunabilirsin. Hasta çocuklardan istenen lenfosit proliferasyon (çoğalma) testi çalışmasında sağlıklı bir kontrolün kanı da gereklidir. Bizim çalışmamız, erişkin hücrelerinin çoğalmasıyla çocukların hücrelerinin çoğalmasını karşılaştıracaktır. Katılımın sayesinde yapılabilecek bu karşılaştırma, erişkin kanlarının kontrol olarak kullanılabileceği sonucunu ortaya koyarsa, hasta çocukların karşılaştırmaları için sağlıklı çocukların kan vermesinin önüne geçilebilir.

Bu çalışmaya katılman halinde, tüm bilgilerinin gizli tutulacağını, katılan gönüllü isimlerinin kesinlikle saklanacağını, verilerin tamamen bilimsel çalışmalar için kullanılacağını garanti ederiz. Buradan elde edilecek verilerin ticari amaçlarla kullanımı söz konusu olmayacaktır.

Araştırmaya katılım kararını vermeden önce, araştırma süreci, beklenen sonuçlar hakkında ilgili kişilere dilediğin soruyu sorabilirsin.

Sizlerden, çalışmaya katılmanız halinde hiçbir ücret alınmayacak ve ödeme yapılmayacaktır. Çalışmaya katılman veya çalışmadan ayrılman, olağan tıbbi bakımlarını hiçbir şekilde etkilemeyecektir.

Çalışmayla ilgili tüm soruların için Prof. Dr. Yıldız Camcıoğlu, veya Doç. Dr. Umut Küçüksezer'e (0212) 41 42000 / 33342 no'lu telefondan ulaşabilirsin.

Yukarıdaki tüm bilgiler, tarafıma …….. tarafından anlatıldı.

☐ Bu bilgi formunu okudum, araştırmanın konusunu anladım, açık olmayan herşey konusunda doktorumla görüştüm.

☐ Bu araştırmaya isteyerek katılıyorum, herhangi bir neden göstermeden herhangi bir zamanda, herhangi bir endişe duymadan çalışmadan ayrılabileceğimi biliyorum.

☐ Kan incelemelerinin değerleri saklanabilir ve ismim kullanılmaksızın bilimsel olarak kullanılabilir.

GÖNÜLLÜ ONAY FORMU

Yukarıda gönüllüye araştırmadan önce verilmesi gereken bilgileri gösteren metni okudum. Bunlar hakkında bana yazılı ve sözlü açıklamalar yapıldı. Bu koşullarla söz konusu klinik araştırmaya kendi rızamla hiçbir baskı ve zorlama olmaksızın katılmayı kabul ediyorum.

Gönüllünün

Adı-Soyadı: Tarih:

Adresi: Telefon no:

İmzası:

Açıklama Yapanın

Adı Soyadı: İmza:

Birimi: Tarih:

BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU

Araştırma Katılımcıları: Erişkin

Sağlıklı Pediyatrik ve Erişkin Yaş Gruplarında Mitojenlerle Uyarılmış Lenfosit Proliferasyon Değerlerinin Karşılaştırılması

Değerli Katılımcı,

Sizleri, yakın zamanda kullanıma girmiş olup bazı hastalıkların tanısı ve takibinde yardımcı olan yeni bir yöntemin kullanımına katkı sağlayacak bir araştırmaya katılmaya davet ediyoruz. Bu çalışmaya katılım kararından önce, yapılacak çalışma hakkında bilgi vermek istiyor, eğer katılmaya karar verirseniz olur formunu imzalamanızı rica ediyoruz. Çalışmaya katılıp katılmamakta serbestsiniz. Ayrıca, herhangi bir nedenle bu çalışmadan ayrılmak isterseniz inceleme sonuçlarınız isteğe bağlı silinecektir.

Bu çalışmaya katılmanız halinde, sizden bir defaya mahsus olmak üzere, 3-4 mililitre kan vermenizi rica ediyoruz. Bu kan miktarı, sağlığınız üzerinde hiçbir olumsuz etkiye yol açmayacak kadar düşüktür. Bu çalışmada, size herhangi bir tedavi veya işlem yapılmayacak, herhangi bir madde verilmeyecektir. Katılım, sadece kan vererek gerçekleşecektir. Alınan kan örnekleri laboratuvarda incelenecektir, çalışmanın genetik araştırmalarla hiçbir ilgisi olmayıp, sadece hücre fonksiyonları incelenmektedir.

Bu çalışmaya katılmak normal kan verme işleminin sıradan ve önemsiz yan etkileri olan çok az bir acı ve kan alma yerinde morluk oluşumuna neden olabilir. Ancak, bu işlemi gerçekleştiren kişilerin deneyimleri sayesinde çoğunlukla bu yan etkiler de gözlenmeden kan alma işlemi tamamlanmaktadır.

Bu çalışmanın amacı, sağlıklı çocukların akyuvar hücrelerinin çalışma kapasitesini ölçüp normal değerlerini saptamak ve erişkinlerdeki değerlerle karşılaştırmaktır. Sizin katılımınızla erişkin lenfositlerinde hücre çoğalması (proliferasyonu) değerleri belirlenecektir. Çalışmayla ilgili başka bir test, tetkik, ölçüm istenmeyecektir. Hasta çocuklardan istenen lenfosit proliferasyon (çoğalma) testi çalışmasında sağlıklı bir kontrolün kanı da gereklidir. Bizim çalışmamız, erişkin hücrelerinin çoğalmasıyla çocukların hücrelerinin çoğalmasını karşılaştıracaktır. Sizin katılımınızla yapılacak bu karşılaştırma, erişkin kanlarının kontrol olarak kullanılabileceği sonucunu ortaya koyarsa, hasta çocukların karşılaştırmaları için sağlıklı çocukların kan vermesinin önüne geçilebilir.

Bu çalışmaya katılmanız halinde, tüm bilgilerinizin gizli tutulacağını, katılan gönüllü isimlerinin kesinlikle saklanacağını, verilerin tamamen bilimsel çalışmalar için kullanılacağını garanti ederiz. Buradan elde edilecek verilerin ticari amaçlarla kullanımı söz konusu değildir.

Araştırmaya katılım kararını vermeden önce, araştırma süreci, beklenen sonuçlar hakkında ilgili kişilere dilediğiniz soruyu sorabilirsiniz.

Sizlerden, çalışmaya katılmanız halinde hiçbir ücret alınmayacak ve ödeme yapılmayacaktır. Çalışmaya katılmanız veya çalışmadan ayrılmanız, olağan tıbbi bakımlarınızı hiçbir şekilde etkilemeyecektir.

Çalışmayla ilgili tüm sorularınız için Doç. Dr. Umut Küçüksezer'e (0212) 4142000 / 33342 no'lu telefondan ulaşabilirsiniz.

Yukarıdaki tüm bilgiler, tarafıma …….. tarafından anlatıldı.

☐ Bu bilgi formunu okudum, araştırmanın konusunu anladım, açık olmayan herşey konusunda ilgili kişilerle görüştüm.

☐ Bu araştırmaya isteyerek katılıyorum, herhangi bir neden göstermeden herhangi bir zamanda, herhangi bir endişe duymadan çalışmadan ayrılabileceğimi biliyorum.

☐ Kan incelemelerinin değerleri saklanabilir ve ismim kullanılmaksızın bilimsel olarak kullanılabilir.

GÖNÜLLÜ ONAY FORMU

Yukarıda gönüllüye araştırmadan önce verilmesi gereken bilgileri gösteren metni okudum. Bunlar hakkında bana yazılı ve sözlü açıklamalar yapıldı. Bu koşullarla söz konusu klinik araştırmaya kendi rızamla hiçbir baskı ve zorlama olmaksızın katılmayı kabul ediyorum.

Gönüllünün

Adı-Soyadı: Tarih:

Adresi: Telefon no:

İmzası:

Açıklama Yapanın

Adı Soyadı: İmza:

Birimi: Tarih:

ETİK KURUL KARARI

İSTANBUL TIP FAKÜLTESİ KLİNİK ARAŞTIRMALARI ETİK KURULU KARAR FORMU

ARAŞTIRMANIN AÇIK ADI	**"Sağlıklı Pediyatrik ve Erişkin Yaş Gruplarında Mitojenlerle Uyarılmış Lenfosit Proliferasyon Değerlerinin Karşılaştırılması"**

	Belge Adı	Tarihi	Versiyon Numarası	Dili
DEĞERLENDİRİLEN BELGELER	ARAŞTIRMA PROTOKOLÜ	07.06.2017		Türkçe ■ İngilizce ☐ Diğer ☐
	BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU	■		Türkçe ■ İngilizce ☐ Diğer ☐
	OLGU RAPOR FORMU	☐		Türkçe ☐ İngilizce ☐ Diğer ☐
	ARAŞTIRMA BROŞÜRÜ	☐		Türkçe ☐ İngilizce ☐ Diğer ☐

	Belge Adı		Açıklama
DEĞERLENDİRİLEN DİĞER BELGELER	TÜRKÇE ETİKET ÖRNEĞİ	☐	
	SİGORTA	☐	
	ARAŞTIRMA BÜTÇESİ	■	
	BİYOLOJİK MATERYEL TRANSFER FORMU	☐	
	HASTA KARTI/GÜNLÜKLERİ	☐	
	İLAN	☐	
	YILLIK BİLDİRİM	☐	
	SONUÇ RAPORU	☐	
	GÜVENLİLİK BİLDİRİMLERİ	☐	
	DİĞER:	■	Anabilim Dalı Başkanlığından Üst Yazı ve Akademik Kurul Kararı, Literatür Kaynağı, Sorumluluk Paylaşım Belgesi, Olgu Rapor Formu, İlgili Elemanların Bilgilendirildiğine Dair Belge, CV, CD

KARAR BİLGİLERİ	**Karar No:12**	**Tarih: 23/06/2017**
	İstanbul Üniversitesi Aziz Sancar Deneysel Tıp Araştırma Enstitüsü, İmmünoloji Anabilim Dalında görevli **Doç. Dr. Umut Can KÜÇÜKSEZER' in** sorumluluğunda ve Yüksek Lisans Öğrencisi Zakia SI IOUD' un yürüteceği yukarıda bilgileri verilen araştırma başvuru dosyası ile ilgili belgeler araştırmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş, gerçekleştirilmesinde etik ve bilimsel sakınca bulunmadığına toplantıya katılan Etik Kurul üye tam sayısının salt çoğunluğu ile karar verilmiştir.	

İSTANBUL TIP FAKÜLTESİ KLİNİK ARAŞTIRMALARI ETİK KURULU

ÇALIŞMA ESASI	19.08.2011 tarihli, 28030 sayılı Resmi Gazetede yayınlanan Klinik Araştırmalar Hakkındaki Yönetmelik
BAŞKANIN UNVANI / ADI / SOYADI:	Prof. Dr. A. Yağız ÜRESİN

Unvanı/Adı/Soyadı	Uzmanlık Alanı	Kurumu	Cinsiyet		Araştırma ile ilişki *		Katılım **		İmza
Prof. Dr. A. Yağız ÜRESİN	Farmakoloji ve Klinik Farmakoloji	İstanbul Tıp Fakültesi (Etik Kurul Başkanı	E ■	K ☐	E ☐	H ■	E ☐	H ■	İZİNLİ
Prof. Dr. Berrin UMMAN	Kardiyoloji	İstanbul Tıp Fakültesi (Etik Kurul Başkan Yardımcısı)	E ☐	K ■	E ☐	H ■	E ■	H ☐	
Prof. Dr. Ahmet GÜL	Romatoloji	İstanbul Tıp Fakültesi	E ■	K ☐	E ☐	H ■	E ■	H ☐	
Prof. Dr. Oğuzhan ÇOBAN	Nöroloji	İstanbul Tıp Fakültesi	E ■	K ☐	E ☐	H ■	E ■	H ☐	
Dr. Sevda ÖZEL YILDIZ	Biyoistatistik	İ.Ü. İstanbul Tıp Fakültesi	E ☐	K ■	E ☐	H ■	E ■	H ☐	

* :Araştırma ile İlişki
** :Toplantıda Bulunma

İ.Ü. İstanbul Tıp Fakültesi Klinik araştırmalar Etik kurulu 13.04.2013 tarih, 28617 sayılı Resmi Gazetede yayınlanan Klinik Araştırmalar Hakkında Yönetmelik çerçevesinde kurulmuş ve T.C. Sağlık Bakanlığı Türkiye İlaç ve Tıbbi Cihaz Kurumu tarafından onaylanmıştır. İlgili yönetmelik kapsamında kalan araştırmalar Sağlık Bakanlığından izin almak zorundadır. Yönetmelik kapsamı dışında kalan araştırmalar ise Etik Kurul bünyesinde oluşturulmuş 5 kişilik alt komisyon tarafından değerlendirilmekte olup Sağlık Bakanlığı iznine tabi değildir.

T.C.
İSTANBUL ÜNİVERSİTESİ
İSTANBUL TIP FAKÜLTESİ
KLİNİK ARAŞTIRMALAR ETİK KURULU

Sayı : 817 Tarih : 30.06.2017
Konu: Doç. Dr. Umut Can KÜÇÜKSEZER hk.

Sayın Doç. Dr. Umut Can KÜÇÜKSEZER
İmmünoloji Anabilim Dalı

İlgi : İmmünoloji Anabilim Dalının 07/06/2017 gün ve 215487 sayılı yazısı

Sorumlu araştırıcılığını üstlendiğiniz ve Yüksek Lisans Öğrencisi Zakia SHOUB' un yürüteceği 2017/739 dosya numaralı "Sağlıklı Pediyatrik ve Erişkin Yaş Gruplarında Mitojenlerle Uyarılmış Lenfosit Proliferasyon Değerlerinin Karşılaştırılması" başlıklı çalışma kurulumuzun 23/06/2017 gün ve 12 sayılı toplantısında görüşülerek etik yönden uygun bulunmuş olup, tutanaklar ekte sunulmuştur.

Bilgilerinizi rica ederim.

Prof. Dr. A.Yağız ÜRESİN
İstanbul Tıp Fakültesi Klinik Araştırmalar
Etik Kurul Başkanı

Eki: İstanbul Tıp Fakültesi Klinik Araştırmaları Etik Kurulu Karar Formu

İSTANBUL TIP FAKÜLTESİ KLİNİK ARAŞTIRMALARI ETİK KURULU KARAR FORMU

ETİK KURUL BİLGİLERİ	ETİK KURULUN ADI	**İSTANBUL TIP FAKÜLTESİ KLİNİK ARAŞTIRMALARI ETİK KURULU**
	AÇIK ADRESİ:	**İ.Ü.İSTANBUL TIP FAKÜLTESİ HULUSİ BEHÇET KÜTÜPHANESİ KAT:3 FATİH/İSTANBUL**
	TELEFON	**0 (212) 414 21 53**
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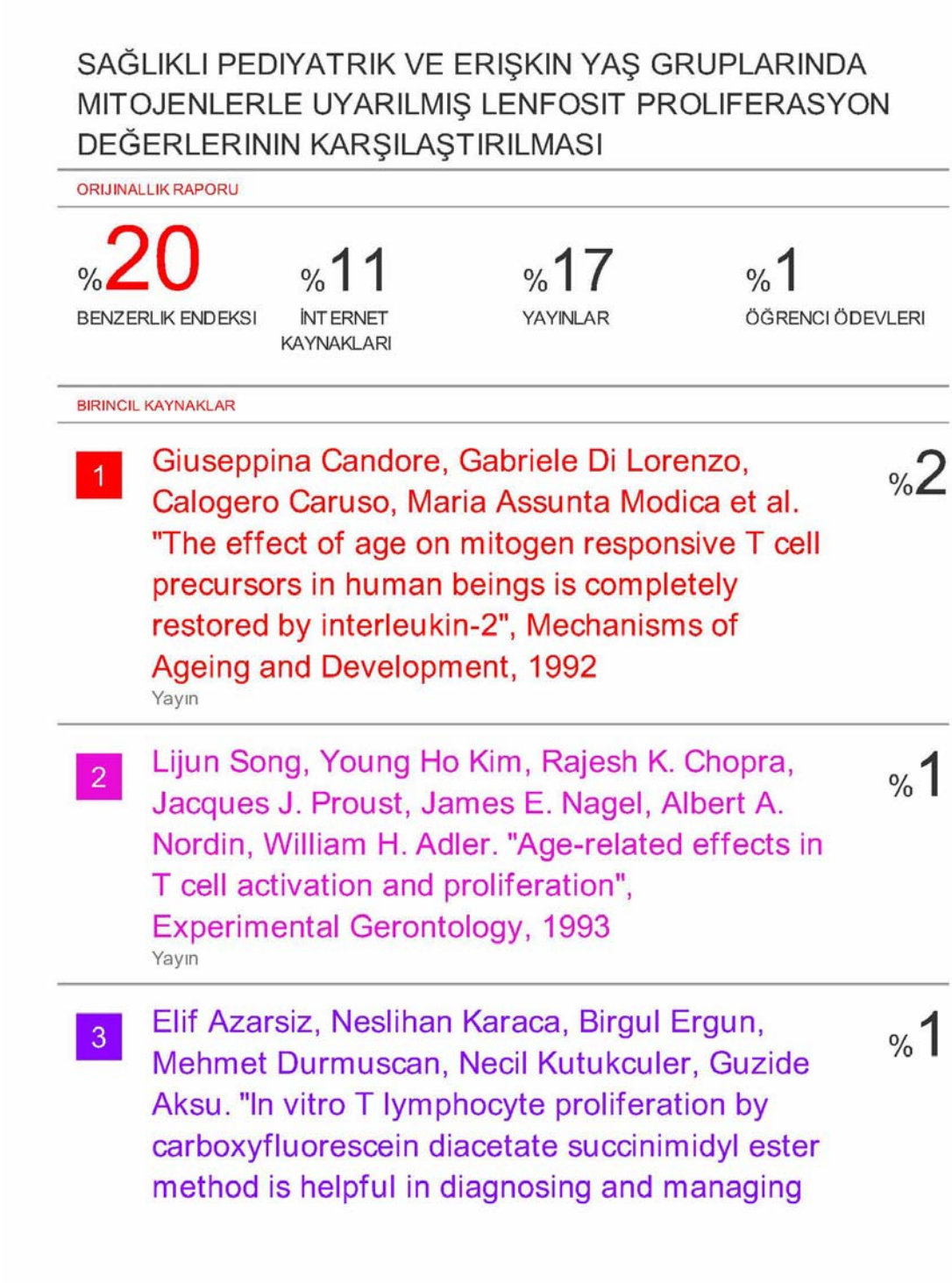
	E-POSTA	**itfetikkurul@istanbul.edu.tr.**

BAŞVURU BİLGİLERİ	ARAŞTIRMANIN AÇIK ADI	**"Sağlıklı Pediyatrik ve Erişkin Yaş Gruplarında Mitojenlerle Uyarılmış Lenfosit Proliferasyon Değerlerinin Karşılaştırılması"**			
	ARAŞTIRMA PROTOKOL KODU	**---**			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACI UNVANI/ADI/SOYADI	**Doç. Dr. Umut Can KÜÇÜKSEZER**			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ UZMANLIK ALANI	**İmmünoloji**			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ BULUNDUĞU MERKEZ	**İstanbul Üniversitesi Aziz Sancar Deneysel Tıp Araştırma Enstitüsü, İmmünoloji Anabilim Dalı**			
	DESTEKLEYİCİ	**İstanbul Üniversitesi Bilimsel Araştırma Projeleri Birimi**			
	DESTEKLEYİCİNİN YASAL TEMSİLCİSİ	**---**			
	ARAŞTIRMANIN FAZI	FAZ 1	☐		
		FAZ 2	☐		
		FAZ 3	☐		
		FAZ 4	☐		
	ARAŞTIRMANIN TÜRÜ	Yeni Bir Endikasyon	☐		
		Yüksek Doz Araştırması	☐		
		Diğer ise belirtiniz :			
	ARAŞTIRMAYA KATILAN MERKEZLER	TEK MERKEZ ■	ÇOK MERKEZLİ ☐	ULUSAL ■	ULUSLAR ARASI ☐

İNTİHAL RAPORU İLK SAYFASI



SAĞLIKLI PEDIYATRIK VE ERIŞKIN YAŞ GRUPLARINDA MITOJENLERLE UYARILMIŞ LENFOSIT PROLIFERASYON DEĞERLERININ KARŞILAŞTIRILMASI

ORIJINALLIK RAPORU

%20	%11	%17	%1
BENZERLIK ENDEKSI	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCI ÖDEVLERI

BIRINCIL KAYNAKLAR

1	Giuseppina Candore, Gabriele Di Lorenzo, Calogero Caruso, Maria Assunta Modica et al. "The effect of age on mitogen responsive T cell precursors in human beings is completely restored by interleukin-2", Mechanisms of Ageing and Development, 1992 Yayın	%2
2	Lijun Song, Young Ho Kim, Rajesh K. Chopra, Jacques J. Proust, James E. Nagel, Albert A. Nordin, William H. Adler. "Age-related effects in T cell activation and proliferation", Experimental Gerontology, 1993 Yayın	%1
3	Elif Azarsiz, Neslihan Karaca, Birgul Ergun, Mehmet Durmuscan, Necil Kutukculer, Guzide Aksu. "In vitro T lymphocyte proliferation by carboxyfluorescein diacetate succinimidyl ester method is helpful in diagnosing and managing	%1

ÖZGEÇMİŞ

Kişisel Bilgiler

Adı	ZAKYA SHOUB	**Soyadı**	ELSHARİ
Doğ.Yeri	DERNA	**Doğ.Tar.**	29 September 1987
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Yük.Lis.		
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Lise		

İş Deneyimi (Sondan geçmişe doğru sıralayın)

	Görevi	**Kurum**	**Süre (Yıl - Yıl)**
1.	Teacher assistant	Faculty of medical technology	2011-2015
2.			-
3.			-

Yabancı Dilleri	**Okuduğunu Anlama***	**Konuşma***	**Yazma***	**KPDS/ÜDS Puanı**	**(Diğer) Puanı**
English	Good	Good	Good	70	70

*Çok iyi, iyi, orta, zayıf olarak değerlendirin

	Sayısal	**Eşit Ağırlık**	**Sözel**
LES Puanı			
(Diğer) Puanı			

Bilgisayar Bilgisi

Program	**Kullanma becerisi**

Yayınları/Tebligleri Sertifikaları/Ödülleri

Özel İlgi Alanları (Hobileri):