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GENE TRANSFER INTO HEMATOPOIETIC CELLS: PROGRESS, PROBLEMS AND PROSPECTS*

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Gene therapy is the introduction of genetic material into somatic cells in order to correct a genetic defect or provide a new therapeutic function. Among the numerous potential cellular targets for gene therapy, hematopoietic stem cells (HSC) may be viewed as one of the best candidates for such genetic modification. More than 250 clinical gene transfer protocols have been reported from around the world, and almost one in three involves manipulation of hematopoietic cells. The introduction of a new gene into the DNA of HSC and expression of the gene product in their progeny are exciting approaches to treatment of congenital and acquired diseases. Although it has been shown that hematopoietic progenitor cells, or even LTC-IC, can be easily transduced with high efficiency by retroviral vectors in vitro, and that long-term expression and high transduction can be obtained in mice after transplantation of the gene manipulated cells, this has not been observed in primate models or in human clinical trials. The reason for this discrepancy is not properly known. The major problems of gene therapy for cancer have been transduction rate, selectivity, and effectiveness due to the heterogeneity of genetic alterations found in human tumors. Overcoming these limitations in gene therapy requires not only improvement in cell biology and immunology, but also development of better delivery systems. Nevertheless, gene therapy is still promising and offers encouragement for future applications in clinical practice. *Key words: hematopoietic stem cells, gene transfer, gene therapy, viral vectors, transduction.*

Developments in molecular biology have led to significant diagnostic methods and the characterization of disorders at the DNA level. In the last two decades, many molecular genetic techniques have been used to get more information about the DNA. The further characterization of the DNA helix, the discovery of restriction endonucleases, the development of vector systems, and the cloning of genes have been the milestones for gene therapy. Potential targets for gene therapy are hereditary diseases, especially single gene defects as well as acquired diseases such as cancer or certain infections.

Gene therapy is defined as the transfer of genetic material into the cells of an organism to treat disease. Molecular genetic techniques have been used to transfer genetic material into target cells for a diagnostic, preventive or therapeutic purpose. The material transferred may be one or a few genes in size. Gene

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therapy can be used for germ-line or somatic cells in humans, but for ethical reasons, only somatic gene therapy is currently being performed. The first genetic therapy to humans was actually attempted during the 1970s[1]. Shope rabbit papilloma virus which produces arginase was infected into children suffering from arginase deficiency. The clinical trial was based on animal experiments showing that the direct injection of a wild type virus could deliver the gene encoding arginase and decrease plasma arginine levels. Unfortunately, the results were not successful. During the 1980s, bone marrow cells of two children with thalassemia were transfected with the herpes simplex virus thymidine kinase (HSVtk) gene as the dominant selective marker by a calcium phosphate precipitation method[2]. The persistence of the transfected gene was observed for up to nine months, but then it became undetectable, probably because of the techniques used. Most current gene-therapeutic approaches are based on viral vectors used for transferring a foreign gene to the target cells. In 1989, a retroviral vector carrying a marker gene, the neomycin phosphotransferase II (neoR), was used to transduce autologous lymphocytes, and the cells were then infused into the patients in association with immunotherapy for malignant melanoma[3]. In 1990, the first gene therapy experiment with the possibility of producing clinical benefits was initiated[4,5]. Since then, great progress has been made on gene transfer strategies, but new developments are still required for substantial improvements in current gene transfer technologies[6-10].

Delivery Systems

Gene therapy requires not only the introduction of foreign genes into cells, but also their stable and appropriately regulated expression in the new environment. Normally, this can be obtained by replacement of the defective gene with a normal gene via homologous recombinatilon. This procedure alone is likely to ensure that the foreign gene will be regulated similar to the endogenous gene. Although this technique has been used successfully for mouse embryonal stem cells, it has not yet been applied in cells that are used for gene therapy. The currently available gene therapy approach is to insert additional functional genes into target cells. Many techniques have been developed for the introduction of foreign DNA into mammalian cells. These include chemical, physical, and fusion methods, receptor-mediated endocytosis, and recombinant viral vectors.

Nonviral Gene Delivery System

Gene transfer via these methods is called "transfection". The efficiency of gene transfer by these techniques is quite variable, depending on the cell type and cell cycle phase. Stable integration of a gene into DNA can be obtained, but it is very low, ranging from one transformation event in 10^2-10^5 cells. The integration

of a foreign gene occurs in random sites of the genome of target cells. The expression of a transgene depends on the site of integration. Variable expressions can be obtained from the same transgene due to the site of integration, and the corresponding gene products may also show differential stability in the target cells[11-13]. These methods, including $CaPO_4$ precipitation, microinjection of DNA and particle bombardment, electroporation. cationic lipids and liposomes, receptor-mediated gene delivery, and direct injection of naked DNA, can be used in some non-hematological diseases, but they are not ideal techniques for hematopoietic cells due to their low integration into DNA and transient expression.

Viral Gene Delivery Systems

Gene transfer via viral vectors is called "transduction". As a result of severe limitations of gene transfer efficiency and obvious difficulties for in vivo application by chemical or physical techniques, viral vectors have been widely used in gene therapy. Thus far, clinical protocols have used viral vectors for cellular gene transfer more frequently than nonviral methods. Commonly used viral vectors are retroviruses, adenoviruses, and adeno-associated viruses, but herpes viruses and vaccinia viruses are currently being developed for use in humans as well[14, 15].

Retroviral Vectors: Oncoviral vectors: Oncoviruses consist of a large class of enveloped viruses that contain single-stranded RNA as the viral genome[16]. They replicate via a chromosomally integrated DNA intermediate (provirus). During the normal viral life cycle, the viruses can bind to target cells via specific cell surface receptors the amphotropic receptor on primate and rodent cells[17] or the ecotropic receptor on rodent cells[18] and enter into the target cells via endocytosis[19]. Viral RNA is reverse transcribed to yield double-stranded DNA and is then transported into the nucleus where it is integrated in the host genome by a second virus-specific enzyme, the integrase. The double-stranded proviral DNA integrates stably and heritably into random sites of the host genome as a single copy colinear with the original viral genome. The viral genome is about 10 kb in size.

Most commonly used retroviral vectors are obtained from the Moloney murine leukemia virus (MoMLV). The MoMLV is a single-strand RNA virus consisting of three genomic parts. The long terminal repeats (LTRs) contain the viral promoter and enhancer regions which are responsible for the initiation or termination of transcription. LTRs also contain some sequences which are required for integration of retroviruses into the host genome. Psi (or encapsidation) is responsible for the packaging of the RNA viral genome into viral particles. The third region consists of the viral genes gag, pol, and env which encode viral structural proteins, enzymatic proteins (reverse transcriptase etc.) and the envelope protein, respectively.

Retroviral-mediated gene transfer is dependent on two important components: the viral vector (Fig. 1) and the packaging cell (Fig. 2). A packaging cell line (usually a murine fibroblast) is a cell that contains a retroviral genome from which the signals that are responsible for encapsidation (psi) of the viral genome into virus particles have been deleted[20, 21]. The viral genome in the packaging cells encodes all the components necessary for virus replication, but cannot create an infectious particle because of the lack of encapsidation. Packaging cells receiving a recombinant retroviral vector form a producer cell line. All important components are deleted in a retroviral vector except psi and replication signals. Up to seven kb genes of interest can be inserted into a retroviral vector by cloning, allowing incorporation of most cDNAs but very few full-length genomic genes. Vectors can contain a single therapeutic gene or several genes, including selectable markers such as the neoR. Several genes can be simultaneously expressed by the retroviral LTR or one gene may be expressed by the viral LTR and the second by an internal heterologous promotor. Using picarnovirus 5' non-translated sequences between the transgenes allows internal ribosomal attachment, resulting in translation of those genes from a single template. These vectors are called "polycistronic"[22-25]. Much effort has been made on the modification of the retroviral envelope to incorporate specific ligands such as erythropoietin, or the c-Kit receptor. Such genetically modified retroviral vectors should be able to target cells with specific ligand receptors, improving the specifity of vector attachment[26, 27]. The transduction of the gene into the target cells can be achieved either by cocultivation of the vector-producing cell line and the target cells, or by incubation of the target cells in the cell-free vector containing supernatant.

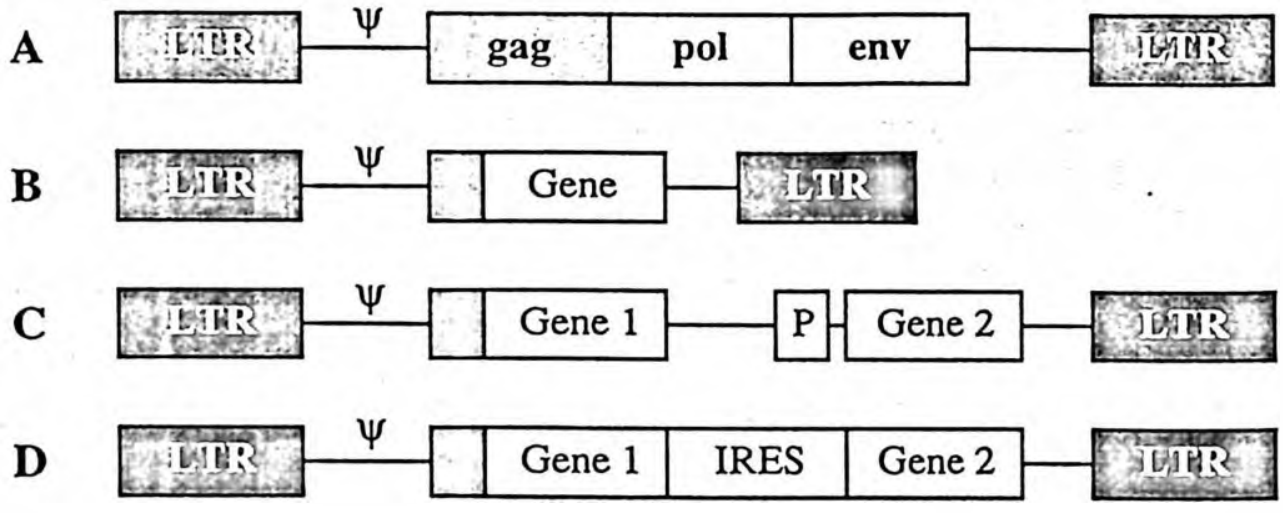

Fig. 1: Structure of retrovirus and retroviral vectors A) A retrovirus, with long terminal repeats (LTRs) at both 5' and 3' ends, the packaging signal ψ, and the viral replicate and the viral replicative and envelope protein genes (gag, pol, and env). B, C, and D) Replacement of the structural genes by gene or genes of interest. B) Single-gene vector. C) Two-genes vector with internal promoter. D) Bicistronic vector with internal ribosome entry site.

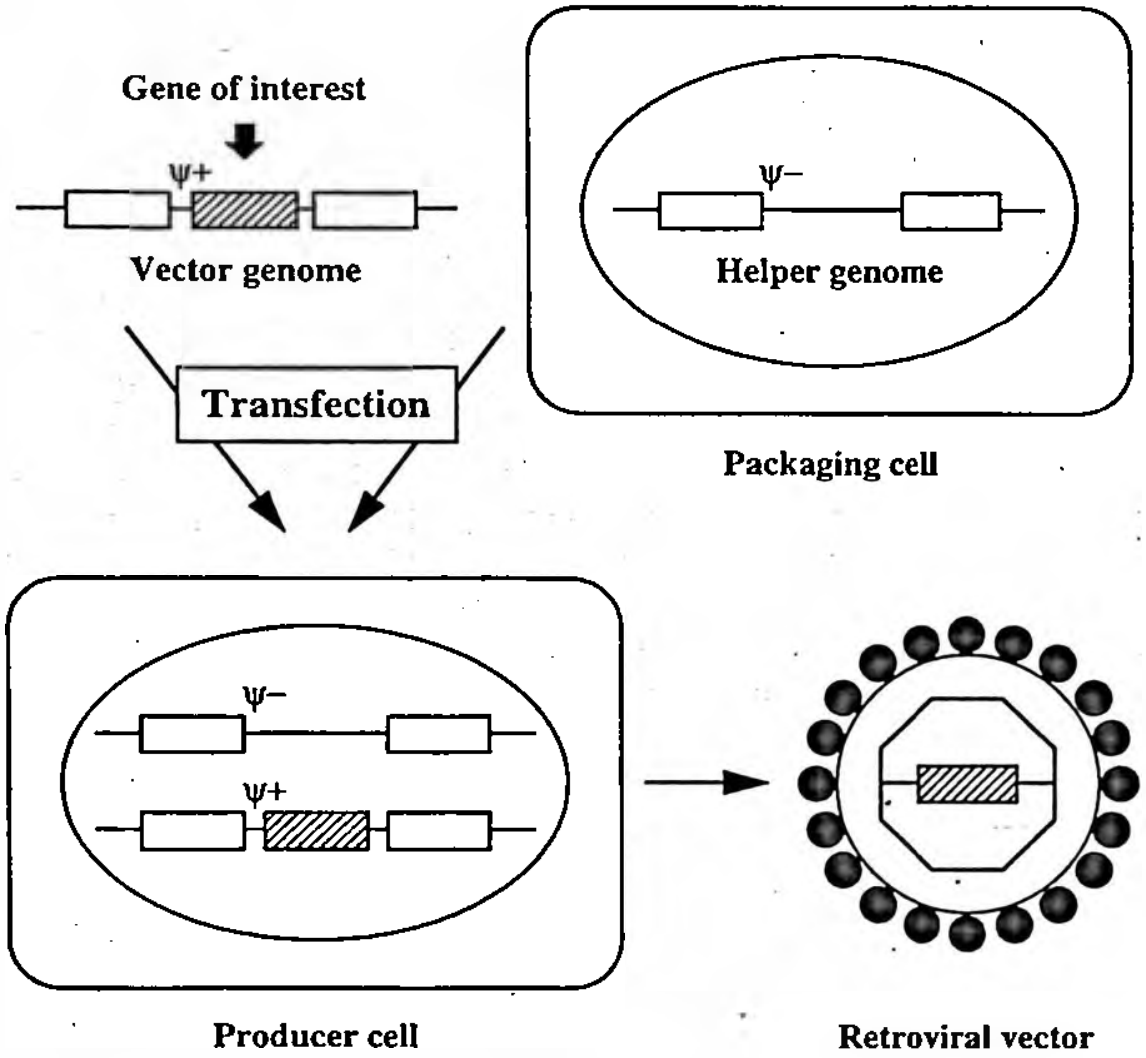

Fig. 2: Retroviral vector production using packaging cell lines. Packaging cell lines contain a retroviral genome which lacks the encapsidation sequence (ψ-) and therefore cannot incorporate the full-length viral genome into viral particles. Vector DNA introduced into the packaging cell line creates a producer cell line. Since the vector DNA contains the encapsidation sequence (ψ+), the vector genome is packaged into retroviral vector particles.

Retroviral vectors have some advantages over other viral vectors. They have been extensively investigated, therefore their features are well known. They are generally not harmful to target cells. The most important benefit is that retroviral vectors have the capacity to integrate into the genome of target cells. Another important feature of retroviral vectors is that the target cells need to be in the mitotic phase to allow gene transfer[28]. Depending on the target cells, this can be an advantage or a disadvantage. Amphotropic virus is rapidly inactivated in vivo in primates via a complement-mediated process.

Although retroviral-mediated gene transfer is an essential gene transfer method, it involves some potential risks[29-31]. Insertional mutagenesis, recombination with endogenous retroviral sequences, transfer of exogenous genetic material, and

production of replication-component murine retroviruses are major theoretical risks of this method. Insertional mutagenesis occurs when the integration of a retrovirus or retroviral vector causes disruption or abnormal regulation of a gene. This side effect has so far never been reported from using replication-incompetent retroviral vectors. The human genome contains many endogenous retroviral sequences. Because of mutations, they are replication-defective. It is theoretically possible that recombination can occur between these sequences and a retroviral vector, although the probability of this side effect is very low due to little homology. Another possible side effect is the contamination of the supernatant with replication-competent murine retrovirus (RCR). Packaging cell lines are designed to be free of replication-competent retrovirus but the generation of replication-competent virus via recombination in the producer cell line has been observed. The RCR could cause insertional mutagenesis via repetitive infections. Immunocompetent primates are able to clear and deactivate the RCR before it causes any damage. However, rhesus monkeys transplanted after total body irradiation with bone marrow cells transduced with a RCR-forming producer cell line did not develop antibodies against the RCR and developed T cell lymphoma nine months after transplantation[32]. Retroviral vectors in a clinical setting require extensive testing for the presence of RCR. So far, side effects related to RCR have not been reported in humans.

Lentiviral vectors: Lentiviruses also belong to the retrovirus family, but they can infect both dividing and non-dividing cells[33]. Therefore, much focus has been placed on the establishment of these vectors. The best-known lentivirus is the human immunodeficiency virus (HIV), which has been disabled as a vector for gene therapy. Up to 7.5 kb genes of interest can be inserted into the lentiviral vectors. HIV has the three gag, pol, and env genes, but it also carries genes for six accessory proteins called tat, rev, vpr, vpu, nef and vif. In HIV, the nuclear transport of preintegration complex is guided by nuclear localization signals present in the viral matrix.

Lentiviral vectors have been made with the transgene enclosed between the LTRs and a packaging sequence. Five genes (env, vif, vpr, vpu, and nef) can be deleted without affecting production of the vector or efficiency of infection[34]. The env gene of HIV is in dispensable for generating fully complement HIV-derived vector particles, as these can be pseudotyped with the surface glycoproteins of other viruses such as MoMLV or vesicular stomatitis virus (VSV). In spite of these advantages, a most legitimate concern about the clinical use of HIV-based vectors is linked to the possibility that the parental pathogenic virus might be reconstituted. Although such occurrence becomes unlikely, it nevertheless cannot be formally excluded. By contrast, a much high or degree of biosafety would be achieved if sequences coding critical virulence factors could be eliminated completely.

Adenoviral vectors: Adenoviral vectors are larger (35 kb) and more complex than retroviral vectors[35-37]. They are non-enveloped linear double-stranded DNA viruses that are taken up by the target cells via receptor-mediated endocytosis. After entering the target cells, they migrate to the nucleus and remain extrachromosomal. Adenovirus is a minor pathogen in humans and is not considered responsible for tumor formation. Adenoviruses they are easy to grow in large amounts and can infect nondividing cells. Many different cell types and tissues can be easily transduced by adenoviral vectors. Usually, the E1 coding region of the virus with the gene of interest is replaced. The human 293 cells are used as a source of the E1 proteins to produce vector particles. One major disadvantage of adenoviral vectors is that viral vectors remain extrachromosomal. Therefore, the expression of the foreign gene is particularly transient in dividing cells. The host immune response to the virus is also an important problem of the adenoviral vectors.

Adenoviral/Retroviral Chimeric Vectors

A new vector, combining the high titers and broad range infective capacity of the adenoviruses with the low immunogenicity and potentially stable, heritable expression of the retroviruses, has been developed[38, 39]. Adenoviral vectors have been used to deliver retroviral packaging plasmids directly to target cells, thereby converting them into retroviral packaging cells in situ, the retroviral particles subsequently being released at high local concentrations to infect surrounding cells. This chimeric system may allow delivery of high titers of retroviral particles coding a gene of interest into cell layers where the elusive stem cells may be found.

Adeno-Associated Virus: Adeno-associated virus (AAV) is a small, linear single-stranded DNA virus (4.68 kb)[36, 40, 41]. Although AAV is a simple virus, the expression and function of the AAV genes and gene products are less well characterized. Particularly, the AAV-2 has been investigated much more among AAVs. Heparan sulfate proteoglycan is considered a primary receptor for the AAV-2[42]. The virus requires coinfection with a helper virus such as adenovirus or some other virus in order to replicate, but AAVs can integrate into the host genome and remain as a provirus without a helper virus. The integration of wild-type AAV usually occurs in a specific site of chromosome 19. AAVs can infect almost all cultured cells that have been tested. Although more than 80 percent of humans are seropositive for antibodies against the AAV, infection has not been associated with any disease.

AAV-based vectors are simple vectors having two coding sequences: rep, which produces four proteins that mediate replication and integration, and cap, which produces the three capsid proteins. They contain the viral terminal repeat (TR) sequences flanking the transcription unit of interest. In most commonly used recombinant AAVs, the rep gene has been deleted because of a negative effect

of its protein to the transduction rate. In the absence of rep, AAV vectors may not always integrate into the host genome, but even if they do, the integration occurs at random. Also, AAVs tend to integrate in multiple copies in the genome (average 2-3 copies), but they have a high frequency of rearrangement. It is not known whether primary cells must undergo a round of cell division for integration to occur. A gene of about 4.5 kb in size can be inserted into the AAV vectors. Long-term gene expression can be obtained in non-dividing cells by these vectors. Production of recombinant AAV requires coinfection of a permissive cell line such as Detroit-6 with a plasmid containing TR sequences flanking the gene of interest as well as plasmids containing the cap and rep genes. In addition, transfected cells require superinfection with adenovirus to provide additional helper functions. Even though the vast majority of the helper adenovirus can be removed from AAV vectors by isopyknic centrifugation and heat treatment, denatured adenoviral protein can still be a potential source of contamination. Also, cell lysis is necessary to release recombinant AAV vectors. Therefore, vectors are quite difficult to grow in large amounts. High titer viral stocks can be obtained, although the percentage of infectious particles could be quite low. In a recent study, a mini adenoviral genome incapable of expressing adenoviral structural proteins but still able to supply the essential helper function required for AAV was generated[43]. These constructs have increased AAV production 40-fold over the conventional packaging strategy and appear to have resolved the high-titer AAV production problem without any contamination of adenovirus.

Herpes Simplex Virus Vectors: Herpes simplex virus (HSV) vectors may provide a unique strategy for the persistence of foreign DNA in cells of the central nervous system because of their tropism to these cells[44]. HSV vectors are non-integrating and therefore their use in dividing cells will probably not provide long-term expression. Although they have a complex regulation system, a replication deficient viral stock has been achieved, but it seems that even replication-incompetent virus is toxic to the target cells. Studies are being performed to make these vectors safer. Recently, a high rate of gene transfer into hematopoietic cells has been achieved with a HSV type-2 vector[45].

Gene Transfer into Hematopoietic Progenitors

The Biology of Hematopoietic Progenitor and Stem Cells

The turnover of cells in the hematopoietic system in human beings is estimated to be close to 10^{15} cells per day, including 200 billion erythrocytes and 70 billion neutrophilic leukocytes. All these cells are produced by a small population of bone marrow cells called hematopoietic stem cells (HSC)[46]. A lymphohematopoietic stem cell is defined as a cell with great self-renewal and proliferative capacity as well as the capacity to differentiate into the progenitors

of all the blood cell lineages, including erythrocytes; neutrophilic, eosinophilic, and basophilic granulocytes; mast cells; monocytes and macrophages; platelets; T lymphocytes; B lymphocytes; and natural killer (NK) cells[46-48].

Very recently, it has been shown in a murine model that myogenic progenitors are recruited from the bone marrow and access a site of muscle regeneration via peripheral circulation[49]. Genetically marked bone marrow, obtained from a transgenic line in which the lacZ gene is under the control of the muscle-specific promotor, has been transplanted into SCID-beige mice. When muscle regeneration is induced in the transplanted animals, lacZ-positive nuclei are found incorporated into the regenerated fibers. These data indicate that bone marrow-derived cells can migrate into areas of muscle degeneration, undergo myogenic differentiation, and participate in regeneration of the damaged fibers[49].

Self-renewal refers to the capacity to produce daughter cell(s) with identical characteristics. Another common definition of HSC is that the cells are capable of long-term reconstitution of the hematopoietic system of recipient animals. HSC represent up to 0.05 percent of the cells in the bone marrow. Many functional or indirect techniques have been developed to get more information about HSC, including spleen colony assay, clonal and suspension culture assays, and reconstitution of lethally irradiated or genetically deficient mice. These primitive cells can be obtained from bone marrow, spleen, or bood with the support of some cytokines. One important type of cell is the long-term culture initiating cell (LTC-IC). These cells require the presence of an adherent marrow stromal cell layer. The cells are grown on irradiated stromal layers and assayed with semi-solid clonal assays five weeks after culturing. Xenogeneic reconstitution systems have proven to be useful in vivo models for human hematopoiesis. Several groups have shown that the long-term engraftment of human hematopoietic primitive cells, which are called the long-term repopulating cells (LTRCs), can be obtained in different animal models such as immunodeficient mice[50, 51] or pre-immune fetal sheep[52]. The majority of HSC are in G_0 phase of the cell cycle in the steady state; just a small population of HSC go to mitosis randomly. It has been claimed that the G_0 state corresponds to time during which sten cell repairs DNA damage, thus allowing maintenance of the genetic integrity of the stem cell populations[46]. The product of HSC is progenitor cells. A progenitor cell is the differentiated product of a stem cell that has restricted its lineage potential and that has little, if any, renewal capability. Progenitor cells normally become restricted to a single blood cell lineage such as colony forming unit-granulocyte-macrophage (CFU-GM), burst-forming unit-erythroid (BFU-E), or colony-forming unit-megakaryocyte (CFU-MK). These progenitor cells are responsible for short-term engraftment in vivo. The expression of cell-surface antigens by HSC is being investigated and allows us to obtain more information about HSC. The demonstration that progenitor

cells in human bone marrow cells express the CD34 antigen has enabled many investigators to explore the hematopoietic stem-cell compartment in humans[53]. CD34 is a highly glycosylated membrane glycoprotein of unknown function[54, 55]. Expression of CD34 is only found in hematopoietic precursors and vascular endothelial cells. The CD34+ cell population is heterogeneous. CD34 expression is highest in the earliest progenitors, and progressively reduced with maturation. Recently, CD34+ progenitors have been extensively used in a clinical setting[56]. The average of CD34+ cells in the bone marrow is 0.5-2 percent. High enrichments of human hematopoietic progenitor cells can also be obtained from CD34+ cell populations. These cells are CD34+CD38-HLADR-. They are also Thy+, c-Kit+, and have low retention of the mitochondrial dye, rhodamine 123, but are negative for lineage markers. Survival and proliferation of the HSC is regulated by cytokines which prevent cells from apoptotic death[57]. A number of direct-acting and indirect-acting cytokines have been identified and their genes cloned. IL-1, IL-3, IL-6, IL-11, stem cell factor (SCF), leukemia inhibitory factor (LIF), and GM-CSF are early-acting cytokines that regulate the proliferation of the HSC. It has been claimed that IL-3 and GM-CSF maintain survival in the G_0 of the HSC[46].

Under normal circumstances, the majority of HSC are present within the bone marrow, but it has been shown that a detectable number of HSC circulate in the peripheral blood in normal individuals[58-61]. The reason for peripheral blood progenitor cells (PBPC) being in circulation is unclear. Steady state peripheral blood contains approximately 0.1 to 0.2 percent CD34+ cells, about one-tenth of the level in normal bone marrow. Therefore, it is difficult to collect enough PBPC from normal individuals for therapeutic purposes, but PBPC levels can be markedly increased by mobilizing strategies based on cytokine injections and/or on the hematopoietic rebound effect that occurs following chemotherapy. The advantages of PBPC over bone marrow autologous transplantations are that 1) PBPC can be harvested without the prerequisite of a healthy pelvic bone marrow and without a general anesthesia, 2) rapid hematopoietic reconstitution is seen in those patients transplanted with PBPC collected after mobilization, and 3) tumor contamination of the harvest is less in PBPC than in bone marrow. For these reasons, PBPC transplantation is commonly used world-wide. Moreover, allogeneic PBPC mobilized by cytokines has become a new source for stem cell expansion and transplantation[62]. Ex vivo stem cell expansion has been developed by using cytokines. Incubation of selected CD34+ cells in combination with high-dose cytokines has been the most commonly studied technique of ex vivo hematopoietic culture[63-65].

Another source of HSC is cord blood. The presence of hematopoietic progenitor cells in umbilical cord blood was first reported in 1974[66]. The main sources of cells for transplantation and engraftment of the hematopoietic system are adult

bone marrow and PBPC. Recently. cells from the umbilical cord and placental blood of children at birth have been used for transplantation purposes. Approximately one percent of the nucleated cells present in unfractionated cord blood express the CD34 antigen.

Gene Transfer Studies

Since methods were developed allowing the transfer of foreign genes into eukaryotic cells, the HSC have been an obvious and desirable target for gene therapy[67-71]. There are some advantages of HSC for transferring a new gene: 1) the HSC can be easily removed from the patient, 2) they can be manipulated ex vivo, 3) they can be cryopreserved for later use, 4) they can be returned to the patient, 5) transduced cells can be expanded without losing the new gene, and 6) successful gene transfer into a few pluripotent HSC may be sufficient to repopulate the recipient with enough modified cells to treat some diseases. Therefore, these cells have been extensively used in gene transfer experiments.

Animal Models: The successful gene transfer into hematopoietic progenitor cells by recombinant retroviral vectors was first reported in 1983[72]. Murine bone marrow cells were transduced with a retroviral vector carrying the dominant selectable marker gene neoR. As a result, 0.3 percent of G418 (analog of neomycin) resistant CFU-GM was obtained. After this result, several other groups also showed that more primitive progenitors cells could be transduced by means of retroviral vectors, although the transduction rate was quite low[73-75]. After the discovery and cloning of many of the hematopoietic growth factors, they have been used to stimulate and maintain the HC, thus these cells are made susceptible to retroviral transduction in culture. Particularly, the combination of IL-3 and IL-6 with or without SCF was found to be one of the best cocktails[76, 77]. One other way to stimulate HSC was to inject 5-fluorouracil (5-FU) into donor mice before the harvest of bone marrow in an attempt to induce mitosis of primitive cells[78]. This method was later modified to optimize for a better yield of cycling HSC[79, 80]. A high transduction rate of a foreign gene into long-term repopulating cells has been obtained in recipient mice with engraftment of multiple hematopoietic lineages by using these methods. The expression of the recombinant gene in the murine HSC was observed for up to 12 months in some animal experiments indicating the possibility of gene transfer into pluripotent stem cells[81-84]. In spite of successful gene transfer into murine HSC, long-term engraftment of the cells containing a foreign gene in large animals was not too effective. Both in rhesus monkeys and in dogs, the long-term engraftment of cells carrying the recombinant gene was obtained, but the transduction rate was quite low[85-89].

Gene Transfer into Human Hematopoietic Cells: Committed human progenitor cells as well as LTC-IC have been transduced by retroviral vectors in the

presence of cytokines, or in the presence of marrow stromal cells with or without cytokines[90-93]. It has also been shown that specific extracellular matrix component, such as fibronectin, increase retroviral-mediated gene transfer into HSC[94]. Apart from in vitro experiments, SCID, NOD-SCID, or SCID-beige mice have been used for evaluation of normal HSC as well as HSC carrying a new gene[50, 95, 96]. The human HSC are capable of generating human hematopoiesis in these xenograftic mouse models. As previously discussed, since PBPC have some advantages over bone marrow, they are very attractive target cells for retroviral-mediated gene transfer[97-100]. PBPC have been chosen as target cells for many clinical protocols dealing with gene therapy[101]. Cord blood cells are also other important target cells for retroviral-mediated gene transfer[102].

Applications of Gene Transfer into Hematopoietic Cells

Gene Marking

Gene marking can be used to determine whether harvested progenitor cells in marrow or blood are contaminated with tumorigenic cells. The technology can also provide some information about the contribution of marrow or blood HSC to long-term engraftment. Autologous bone marrow or peripheral blood stem cell transplantation has been extensively used for malignant diseases such as leukemia and lymphoma, but relapse remains the major cause of treatment failure. For many malignancies, it is not known whether tumor relapse following autologous stem cell transplantation results from residual malignant cells in the patient, from the tumor cells in the harvest or from both sources. Because the source of relapse is unknown, it was difficult to evaluate whether marrow purging, which has some side effects to the normal HSC, was necessary. Studies to address these issues have been performed by gene marking experiments.

The first gene marking study was to transduce tumor infiltrating lymphocytes to determine whether they homed selectively to tumors[3]. In 1991, nucleated bone marrow cells of patients with acute myelocytic leukemia (AML) and neuroblastoma were transduced with a retroviral vector carrying the neoR gene[103, 104]. In the AML study, four out of 12 patients relapsed, and in two, the marker gene was present in the tumor cells. In the neuroblastoma study, five patients relapsed, and gene-marked tumor cells were found in four patients. Similar results have been obtained in patients receiving autologous bone marrow transplantation for chronic myelocytic leukemia (CML)[105]. These results clearly show that harvested bone marrow in remission could ontain residual tumor cells that can contribute to the relapse of diseases. Moreover, in the studies of AML and neuroblastoma, the recombinant gene was found in about 2-15 percent of hematopoietic progenitor cells after transplantation. The marker gene continued to be detected and expressed for up to five years in the mature progeny of marrow precursor

cells, including peripheral blood T and B cells and neutrophils, suggesting successful stem cell gene transfer[106]. A similar attempt was made for patients with multiple myeloma and breast cancer[107]. In this study, the levels of gene transfer into HSC were quite low and the marker gene (neoR) was not detected in the tumor cells. After showing that myeloma cells can be tranduced by a retroviral vector carrying a marker gene[108], some clinical protocols for gene marking of tumor cells in patients with multiple myelomas are in progress[109-112].

Correction of a Single Gene Defect

One of the major targets of gene therapy is the replacement of defective or missing genes in congenital diseases affecting the hematopoietic cells.

Adenosine Deaminase Deficiency: The first application of modern gene therapy was initiated in September 1990[4,5]. Two children with adenosine deaminase deficiency (ADA) were treated by autologous lymphocytes which had been transduced with a retroviral vector carrying the ADA gene following stimulation with IL-2 and anti-CD3 monoclonal antibodies. After ex vivo expansion, the cells were repeatedly infused intravenously. Over two years following the lasat infusion of transduced cells, T lymphocyte clones were found to contain the foreign gene. Both children showed improved immune function, increased T cells and ADA levels. In spite of these encouraging results, lymphocyte gene therapy has some problems. Particularly, the lifespan of most circulating T cells is probably short. Therefore, repeated collections and infusions of T cells will probably be necessary. The attempt has been made to transduce CD34+ progenitor cells. One of these patients was treated by transduction of G-CSF mobilized peripheral blood CD34+ cells, using another retroviral vector carrying the ADA gene with an internal promotor. In this way, the vector could be distinguished from the first vector by polymerase chain reaction (PCR), but peripheral blood leukocytes containing the second vector were not detected by PCR after therapy[113]. A number of patients with ADA deficiency have also been treated by other groups. In one study, T lymphocytes and bone marrow CD34+ cells of two children were transduced with two different vectors carrying an ADA gene[114]. The patients currently have about one percent transduced CFU-GM in their marrow and about one percent of their circulating T lymphocytes express the ADA. In a second study, the bone marrow of three patients was transduced with a retroviral vector carrying the ADA gene[115]. They cocultured the patients' CD34+ cells on the fibroblasts producing the ADA vector. Vector-containing cells from the bone marrow of one patient were detected six months after treatment. In a third study, three newborn children were treated with CD34+ cord blood cells transduced with a retroviral vector carrying the ADA cDNA[116]. Four years later, they continued to produce leukocytes containing the ADA cDNA, with aproximately 100-fold

higher frequencies of gene-containing T lymphocytes (1-10%) than other lineage (0.01-0.1%). In the summer of 1997, PEG-ADA replacement was discontinued in one patient; multiple laboratory and clinical parameters of immune function declined during the two-month period and the enzyme treatment was restarted[117].

Gaucher's Disease: Gaucher's disease is a lipid storage disorder and results from deficiency of the lysosomal enzyme glucocerebrosidase (GC). Accumulation of large amounts of glycolipids in reticuloendothelial cells causes hepatosplenomegaly, pancytopenia, lytic bony lesions, and neurologic symptoms with a wide range of severity. The disease is the most common disease among the lysosomal storage disorders. There are three variants of Gaucher's disease. In the type I variant, generalized clinical manifestations may be treated largely by the genetic correction of HSC. The GC gene has been succcessfully transduced into murine and human hematopoietic progenitor cells[25, 81, 82, 93, 118].

A number o patients with Gaucher's disease have been treated by different institutions. In one study, CD34 separated bone marrow cells or G-CSF mobilized PBPC from three adult patients with moderately-severe Type I disease were transduced with a retroviral vector expressing the human GC cDNA. Gene-corrected cells were engrafted and persisted for at least three months post-infusion, even without myeloablation[119]. However, the level of corrected cells was too low to result in any clinical benefit. In the second study, a G-CSF mobilized and enriched population of CD34 cells from PBPC was also transduced with a similar vector[120]. The results to date reveal the presence of the GC transgene and an increase in GC enzyme activity in peripheral blood leukocytes in the patients studied. In one case, the levels increased to above 50 percent of normal and this effect has persisted for 12 months following the last transplantation[120].

Fanconi's Anemia: Fanconi's anemia is a rare genetic disorder characterized by progressive bone marrow failure and a predisposition to leukemia[121]. The Fanconi's anemia group A (FAA) and group C (FAC) genes have been cloned and encode DNA repair proteins[121]. Transduction of hematopoietic progenitor cells from Fanconi patients with retroviral vectors expressing FAC and FAA cDNA corrected the hypersensitivity of DNA cross-linking agents, such as mitomycin, to hematopoietic cells and improved their viability in vitro[122, 123].

Three patients with Fanconi's anemia group C have been treated with gene therapy[124]. Each patient received three or four cycles of gene transfer, each consisting of one or two infusions of autologous PBPC that had been transduced ex vivo with a retroviral vector carrying the normal FAC cDNA. Although there was an in vivo selective advantage resulting from the FAC gene transfer, long-term hematopoietic reconstitution with gene-corrected clones was not observed.

Chronic Granulomatous Disease: Chronic granulomatous disease (CGD) is characterized by granuloma formation and recurrent life threatening infections. Neutrophils, eosinophils, monocytes and macrophages are unable to inactivate bacteria, fungi, and parasites. The main defect is in the enzyme NADPH oxidase which consists of at least four subunits. The most common autosomal form results from a failure to produce the $p47^{phox}$. Transfer of the $p47^{phox}$ gene into PBPC derived from $p47^{phox}$-deficient patients has shown that gene expression and metabolic correction can be obtained[99].

A phase I clinical trial of gene therapy for $p47^{phox}$-deficient CGD was conducted by treating five adult patients with a single cycle of therapy with ex vivo transduced PBPC[125]. The ex vivo correction of oxidase activity in neutrophils differentiated in culture from the transduced PBPC as measured in chemiluminescence assay ranged from 23 to 65 percent, but very small number of oxidase-positive neutrophils appeared in the peripheral blood of all five patients and could be detected for three to six months.

Myeloablation was not used in these clinical protocols dealing with Gaucher's disease, Fanconi's anemia, and chronic granulomatous disease. After transduction, the cells were infused into the recipients in the form of as blood transfusion. This could be one important reason for low engraftment of gene-corrected cells into bone marrow. Although several studies have currently reported that a high level of engraftment of bone marrow in the mouse may be obtained without myeloablatilon, a very high number of marrow cells were used in these experiments[126, 127].

Hemoglobinopathies: Particularly, β-thalassemia is the one of the main target diseases for gene therapy[128-131]. The genetic correction of thalassemia major requires insertion of a normal β-globin gene. The new β-globin gene must produce an amount of β-globin equal to the amount of α-globin produced within the cell. Otherwise, the patient will remain anemic. This is very difficult with currently available vectors. The β-globin genes are coordinately regulated at the level of gene transcription. It has been shown that both gene proximal promoters and enhancers as well as a far upstream 15kb dominant regulatory region, termed the locus control region (LCR), are required for high level expression. LCR appears to be responsible for regulating the β-globin production to match the α-globin quantity in the cell. Addition of LCR to the constructs containing genomic fragments of the β- or γ- genes resulted in high level expression in tissue culture cells[132]. However, the expression level of these constructs in primary cells was quite low. Recently, constructs containing LCR elements and a human β-globin gene altered to reduce recombination were reported to induce high levels of expression in MEL cells and relatively high expression in mice[133], but these results require confirmation in non-human primate models. Further work on the role of the cis- and trans-acting elements in globin

gene regulation, vector development, and improved stem cell transduction is required before gene therapy becomes a treatment modality for hemoglobinopathies. An alternative approach, gene conversion utilizing an RNA-DNA oligonucleotide, was used for the correction of the mutation in lymphoblastoid cell lines from a patient with sickle cell disease[134]. Further studies are required to assess the feasibility of this approach in primary cells.

Hemophilia: Coagulation factor deficiencies such as hemophilia A and B do not require cell-specific gene expression. The production of coagulation factors can take place in hematopoietic cells[135]. Another important factor is that only 10 percent of normal factor concentration will probably be enough for effective therapy[136, 137]. Hematopoietic cells, bone marrow stroma cells, skin fibroblasts, hepatocytes, myoblasts, and vascular endothelial cells have been used as target cells in gene therapy studies. Some encouraging results have been obtained from mice and dog experiments[138, 139]. A phase A clinical trial of gene therapy for hemophilia B was conducted by treating four patients with autologous fibroblasts which had been transduced ex vivo with a retroviral vector carrying hFIX cDNA[140]. The genetically modified cells were amplified and implanted into the patients subcutaneously[140]. It was reported that the level of hFIX protein was elevated (5% of normal level), blood clotting activity increased, and hemorrhage tendencies were alleviated. Treatment-related side effects have not been observed so far (4 years).

Modifying the Drug Sensitivity of Progenitor Cells

Bone marrow suppression is one of the most common toxicities of chemotherapy regimens. A number of drug resistance genes have been identified that might be useful in gene therapy strategies to prevent chemotherapy toxicity. The ability to render HSC resistant to one or more chemotherapeutic drugs can be implicated to prevent some side effects of these drugs on marrow cells, or can be used as a dominant selectable marker, allowing the in vivo selection of transduced over-naive cells. Murine hematopoietic progenitor cells have been successfully transduced with a retroviral vector carrying the multiple drug resistance (MDR1) gene in vitro[141]. Mice with MDR1-gene modified bone marrow have been shown to tolerate much higher doses of chemotherapy[142-144]

Clinical trials using this approach are currently in progress and more than 40 patients have been treated so far[101, 112]

Gene Therapy for Cancer

There are several potential applications for the use of gene therapy in the treatment of cancer, including solid and hematological malignancies[106, 145, 146]

Suicide Gene Therapy

Another potential principle for use of gene technology in the treatment of cancer is the insertion of a suicide gene into tumor cells. A suicide gene is a gene encoding a protein that is toxic to the genetically modified cell. Some suicide genes may express products that are directly toxic for the cell, such as diphtheria toxin or pseudomonas exotoxin, both of which inhibit protein synthesis. Other suicide genes are encoding enzymes that selectively convert nontoxic prodrugs into highly toxic metabolites.

One of the most commonly used and investigated suicide gene is the HSVtk[147]. The transfer of the HSVtk gene into tumor cells using retroviral vectors or adenovirus vectors followed by the administration of GCV or acyclovir provides a potential strategy for the treatment of some malignancies[148-151]. GCV is converted by the HSVtk enzyme into a monophosphate form and subsequently to GCV-di-and triphosphate by endogenous mammalian kinases. GCV-triphosphate competes with normal nucleotides for DNA replicatilon in mammalian cells and can cause cell growth inhibiton and cell death[152]. Therefore, cells that express HSVtk become sensitive to the toxic effect of GCV and can be eradicated in vivo by the administration of GCV.

In a series of animal experiments, tumor cells were transduced with a retroviral vector coding the HSVtk. In spite of a rather low gene transfer into tumor cells, a complete tumor eradication was obtained after GCV treatment in some animals. This unexpected finding is called the "bystander effect", a phenomenon where HSVtk-transduced tumor cells are toxic to nearby untransduced cells when GCV is injected[148, 153]. After the discovery of the bystander effect, such a strategy has been used to treat various experimental hematological[154] and non-hematological tumors. It has been shown that gap junctions may play an important role in the mechanism of the bystander effect in vitro[155-158] and in vivo[159]. Apart from a local bystander effect, a distant bystander effect has also been observed in vivo[154, 160].

Gene Transfer to Donor Lymphocytes

Infusion of donor lymphocytes has become increasingly common as treatment for relapse of hematological malignancies following allogenic bone marrow transplantation (BMT). Although such adoptive immunotherapy approaches have produced clinical benefits, they are complicated by the frequent development of severe graft versus host disease (GVHD). Several gene transfer studies in allogenic BMT are providing a means of monitoring such approaches, ablating donor lymphocytes in the event of severe GVHD[161]. Recently, patients who relapsed after T cell-depleted BMT have been treated with donor lymphocytes

transduced with a suicide gene (HSVtk). Some patients have developed severe GVHD, which has been effectively controlled by GCV-induced elimination of the transduced cells[162].

Immunotherapy

Immunotherapy is basically a tumor cell vaccination. Murine tumors were genetically altered ex vivo with one or more cytokines including IL-1β, IL-2, IL-4, IL-6, IL-7, TNF-α, IFN-gamma, and GM-CSF, and then reimplanted into mice[163, 164]. With a few exceptions, most tumor cells engineered to express cytokines are promptly rejected in the syngeneic animals. Most treated mice were systemically immune to reimplantation of the non-modified cells as well. Depending on the cytokine and tumor type, any of several mechanisms, including macrophages, neutrophils, eosinophil, and T cells, were shown to be responsible for the rejection of the tumor, but the primary target for obtaining an immune response to tumors is the T lymphocytes, specifically the major histocompatibility complex (MHC) class I-restricted, cytotoxic (CD8) T cell[165, 166]. In spite of encouraging results from cytokine gene alteration of tumors in the mice, it is difficult to translate this method to a clinical setting. First, the tumors used in these studies were quite immunogenic and therefore were not good models for human cancer. Second, live tumor cells were used as vaccine as it is known that irradiated tumor cells are less immunogenic than live tumor cells. Third, with few exceptions[167, 168], when animals with established large tumors were treated, the results were much less satisfactory. Nonetheless, many clinical protocols have been approved and initiated worldwide[112]. These studies are an attempt to immunize tumor-bearing patients against autologous tumors by modifying their tumors with a cytokine gene that is expected to raise the host immune response to the tumor. A biopsy of tumor tissue is obtained from a patient and the tumor cells are grown in culture. Once the cells are growing, a vector is used to transfer a cytokine gene into the cultured cells. After expansion of the cells with or without selection, the cells are irradiated and then injected into the patient. Injections of the cells have to be repeated several times. Melanoma, neuroblastoma, breast, colorectal, renal cell carcinoma, and small cell lung cancer are the primary targets of the tumor vaccine studies. This technique has been used for some hematological malignancies such as multiple myeloma.

The in vitro growth of primary tumor cells is not always an easy task. Therefore, in some studies, fibroblasts which are much easier to cultivate in vitro have been transduced with a vector carrying a cytokine gene[169, 170]. These cells were mixed with tumor cells and then injected into animals. The local productilon of cytokines by the fibroblasts could induce a strong immune response to the neighboring tumor cells resulting in a systemic anti-tumor immunizing response. A variation of this approach is to transfer a cytokine gene into tumor infiltrating

lymphocytes (TIL) in an attempt to make these cells more effective in killing tumor cells[3, 106]. TIL are lymphocytes that tend to localize in tumor cells. In the first gene transfer protocol in humans, these cells were used as target cells[3]. TIL were obtained from biopsies of melanoma tissues, expanded in vitro in the presence of IL-2, transduced with a retroviral vector carrying the neoR gene, and then returned to the patients by intravenous infection. The aim was to analyze the trafficking, homing, and survival of TIL. The results demonstrated that the TIL may home to metastatic melanoma tumor cell deposits. In the subsequent experiments, the neoR gene was replaced with TNF-α cytokine gene to enhance their anti-tumor efficacy[171].

Another approach of immunotherapy is the transfer of co-stimulatory molecules such as B7 into tumor cells. B7 is a membrane bound molecule which is the ligand for the CD28 receptor of lymphocytes[172]. The B7-CD28 interaction is critical for activating T lymphocytes. Some tumor cells engineered to express B7 are rejected in the syngeneic animals, and there are systemic immune responses capable of offering protection against challenges from the non-modified cells[173].

Also of current interest is the transfer of HLA genes into tumor cells. The aim is to elicit an immune response to the foreign HLA antigen in the hope that other surface antigens are also recognized and an imune response is simultaneously developed against them. Dendritic cells (DC) are the natural antigen-presenting cells which express high levels of co-stimulatory molecules such as B7.1, B7.2, CD40[174]. They have a unique function and ability to capture, process, and present antigens in peptide-HLA complexes to unsensitized T lymphocytes and to deliver the co-stimulatory signals necessary for T cell activation. DC originate from hematopoietic progenitor cells in the bone marrow. Large numbers of DC can be generated from the bone marrow or peripheral mononuclear cells in the presence of GM-CSF+TNF-α or CM-CSF+IL-4[175]. It has been demonstrated that DC pulsed with synthetic tumor peptides could induce protective and therapeutic anti-tumor immunity[176]. Another approach may be to transduce these cells with a tumor-specific antigen or tumor-associated antigen, and/or a cytokine gene. Since the antigen is produced in the transduced cells, tumor-specific CD8+ cytotoxic T lymphocytes may recognize tumor-antigen peptides presented by class 1 MHC molecules. .

Modification of Tumor Suppressor Genes

It is known that several tumor suppressor genes encode vital cell cycle regulatory or other growth-related functions. The protein of the p53 tumor suppressor gene can arrest the replication of a cell with damaged DNA until the DNA is repaired. If it is not repaired, cells go into apoptosis. Losing of both alleles of p53 may transform normal cells into malignant cells. Defects in the function of this gene represent the most common alteration in human cancer. Transfer of the wild-type p53 gene into tumors with a mutated p53 gene resulted in the elimination

of the malignant clone[177]. Recently, a retroviral vector carrying the wild-type p53 gene was directly injected into tumor deposits in nine patients with non-small cell lung cancers. Although the efficiency of transduction of retroviruses into tumor cells was low, tumor regression was noted in three patients. It has been suggested that a bystander effect may have contributed to the tumor regressions observed[178].

Antisense Strategies

Ribozymes: Ribozymes are small, catalytic RNA molecules annealing to complementary sequences of mRNA, cleaving the mRNA and thereby suppressing the translation of mRNA. They have been demonstrated to be efficacious and specific in eliminating some transcripts in cellular and in vitro systems. The hammerhead ribozymes are well known ribozymes for purposes of gene therapy[179, 180]. These ribozymes consist of three basepaired stems, I, II and III, and a core region in which 13 residues are very specific for cleaving 3' to any GUX sequence (X can be C, A, or U). In the ribozymes, specific sequences can be introduced into stem I and III that arrange complementary recognition between the ribozyme and target RNA. It has been shown that designed ribozymes can inhibit the activities of some oncogenes[181]. Viral vectors are being constructed to carry a ribozyme sequence in the development of these strategies for gene therapy.

Oligodeoxynucleotides: Oligodeoxynucleotides are short synthetic nucleotide sequences of DNA that are synthesized as exact reverse complements of the desired mRNA target nucleotide sequence[182, 183]. Oligos of more than 15 to 17 nucleotides in length would have a unique sequence relative to the entire genome and should normally be able to bind to the RNA species and interrupt the translation of mRNA into protein by the destruction of molecules with RNase H. Oligos complementary to genomic DNA can also interact with DNA and form a triple helix which would inhibit transcription. Antisense oligonucleotides can be administered systemically. The main problems of the technique are degradation of the oligonucleotides in plasma and low cellular uptake. This method could potentially be used for example in CML to block the gene product of the pathological bcr/abl gene sequence, and subsequently induce disease control. In CML, both the bcr/abl and myb genes can be targeted. Growth inhibition has been obtained in vitro with synthetic antisense oligonucleotides against bcr/abl in CML[184], myc in lymphoma[185], and myb in leukemia cell lines[186]. A K-ras gene in the antisense orientation was successfully used to transduce human lung cancer cell lines using a retroviral vector[187]. A protocol for ex vivo purging of CD34+ enriched CML marrow cells with c-myb antisense DNA followed by autologous transplantation is in progress[183].

These approaches also have limitations. An important issue is the targeted delivery of the vectors that carry antisense oncogenes or tumor suppressor genes. Another issue is whether it is necessary to deliver the genes into each malignant cell, in which case reaching them through multiple cell layers of solid tumor masses poses a major difficulty.

Conclusions and Future Perspectives

Although it has been shown by several groups that hematopoietic progenitor cells or even LTC-IC can be easily transduced with high efficiency by retroviral vectors in vitro, and that long-term expression and high transduction can be obtained in mice after transplantation of the gene manipulated cells, this has not been observed in primate models or in human clinical trials. The reason for this discrepancy is not properly known. Since there is no well defined method to evaluate the pluripotent stem cells in vitro, it is not clear whether true repopulating stem cells are transduced by retroviral vectors. A low transduction rate into true stem cells may be due to lack of cell cycling and integration, but could also be due to insufficient viral receptor density. The presence and density of viral receptors on pluripotent stem cells are unknown. New in vitro methods and animal models for evaluation of the true stem cells need to be developed.

Although more than 250 clinical gene transfer trials have been initiated[101], and more than 2.000 patients have been treated[112], very few patients have directly benefitted from them. However, they have been very important in establishing proof of principle: that it is possible to transfer genes into human cells and achieve long-term expression in vivo with very few side effects to the patients. For therapy of most single gene defects to be effective, better delivery systems are required, as well as more precise knowledge of the optimal conditions for transduction of hematopoietic cells. Low dose myeloablative conditioning could increase engraftment of gene-corrected cells in the patients. New vectors for in vivo gene transfer and targeting will possibly reduce the need for ex vivo manipulation and reinfusion of hematopoietic cells in the future.

We have far to go before the extraordinary potential of gene transfer for therapy of hematological malignancies can be fully realized. However, the promises are still great, and the problems have almost been identified. Therefore, new developments are excepted to establish gene therapy as an effective revolutionary approach.

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ECHOCARDIOGRAPHIC ASSESSMENT OF LEFT AND RIGHT VENTRICULAR DIASTOLIC FUNCTIONS IN CHILDREN WITH DILATED CARDIOMYOPATHY*

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SUMMARY: Alehan F, Özkutlu S, Alehan D, Saraçlar M. (Cardiology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Echocardiographic assessment of left and right ventricular diastolic functions in children with dilated cardiomyopathy. Turk J Pediatr 1998; 40: 337-346.

We evaluated left and right ventricular diastolic functions by pulsed Doppler echocardiography in 16 children with dilated cardiomyopathy and in 20 healthy age-matched control subjects. The cardiomyopathy group demonstrated an abnormal relaxation pattern of the left ventricle. In the cardiomyopathy group compared to normal subjects, peak early filling velocities (43.3 ± 11 cm/s versus 60.4 ± 11 cms/s, $p < 0.01$), the corresponding velocity-time integrals (3.3 ± 1.4 cm versus 4.6 ± 1.2 cm, $p < 0.01$) and the ratio of peak early filling velocity to late filling velocity (1.22 ± 0.47 versus 1.49 ± 0.23, $p < 0.05$) were significantly lower whereas isovolumic relaxation time was significantly longer (58.9 ± 19.8 ms versus 49.7 ± 8.9 ms, $p < 0.05$). In addition, right ventricular diastolic filling was also impaired in children with dilated cardiomyopathy. Peak early filling velocities (41 ± 7.9 cm/s versus 47.5 ± 8.8 cm/s, $p < 0.05$) and the corresponding velocity time integrals (3.0 ± 1.0 cm versus 3.87 ± 1.1 cm, $p < 0.05$) were significantly decreased, while isovolumic relaxation time was significantly increased (60.6 ± 16.3 ms versus 52.2 ± 12.8 ms, $p < 0.05$) in the cardiomyopathy group. The study suggests that abnormalities of both right and left ventricular diastolic function may occur, and should be searched for, in pediatric patients with dilated cardiomyopathy.
Key words: dilated cardiomyopathy, diastolic function, Doppler echocardiography, children.

Impaired systolic function and reduced cardiac output are the hallmarks of dilated cardiomyopathy. However, an increasing number of patients with signs and symptoms of congestive heart failure, in the absence of overtly abnormal systolic performance, are now being recognized[1, 2]. This has emphasized the importance of diastolic filling and has shifted the interest from systolic functions to diastolic functions. Consequently, the possible role of impaired diastolic function in dilated cardiomyopathy was only recently appreciated.

Several studies have shown the utility of Doppler echocardiography in assessing left ventricular filling and have demonstrated diastolic abnormalities in adult patients with dilated cardiomyopathy[3-5]. However, there are only a few reports assessing diastolic function in pediatric patients with dilated cardiomyopathy, particularly in terms of right ventricular function.

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The purpose of this study was to evaluate left and right ventricular diastolic functions in children with dilated cardiomyopathy using Doppler echocardiographic indices.

Material and Methods

Patient group consisted of 16 patients with clinical and echocardiographic diagnosis of dilated cardiomyopathy in the absence of clinical evidence of myocarditis and primary valvular disorder (mean age 6.55 years, range 2 months-15.5 years). All patients had cardiomegaly as determined from chest x-ray (cardiothoracic ratio > 0.5). M-mode echocardiographic study demonstrated left venticular enlargement (end-diastolic dimension greater than 25% above the normal value as predicted from the age and body surface area)[6], decreased fractional shortening (< 25%) and a marked increase of E point-septal separation[7]. Mean ejection fraction measured in the study group was also reduced (41.6 ± 11.2%). Two dimensional echocardiography revealed a dilated nonhypertrophic left ventricle with diffuse hypokinesia or akinesia of left ventricular wall motion in all patients. None of the cases had significant arrhythmia, atrioventricular block or pacemaker implants at the time of the study: Presumed etiology of dilated cardiomyopathy was idiopathic in 15 patients and adriamycin-induced in one. Each patient was clinically stable during the time of examination. None of the patients was receiving parenteral inotropic or ventilatory support at the time of the study. Digoxin was being administered to all but one patient. Three patients were receiving a diuretic, five captopril, and three both, in addition to digoxin. One patient was treated with captopril alone.

Control group consisted of 20 healthy children with a similar age distribution (mean age. 6.6 ± 2.5 years).

Echocardiographic Examination

Complete two-dimensional and Doppler echocardiographic examinations were performed on each subject by one investigatior using a Toshiba Sonolayer SSH 160 A echocardiographer with 2.5, 3.75, and 5 MHz transducers. Examinations were recorded on a standard VHS videotape and measurements were made from the replay. Continuous wave and color Doppler echocardiography were applied to each patient with standard methods to rule out valvular abnormalities that are not infrequent in patients with dilated cardiomyopathy[8].

Each subject underwent pulsed Doppler echocardiographic examination of mitral and tricuspid valves from an apical four-chamber view. The Doppler cursor was placed at the tips of the valve leaflets, in the left and right ventricular inflows, almost parallel to the direction of flow. We simultaneously recorded the subjects' electrocardiogram, phonocardiogram and respiration (using a pressure transducer placed over the subphrenic region) (Fig. 1). Care was taken to keep the positions of the body and the transducer unchanged during the recording.

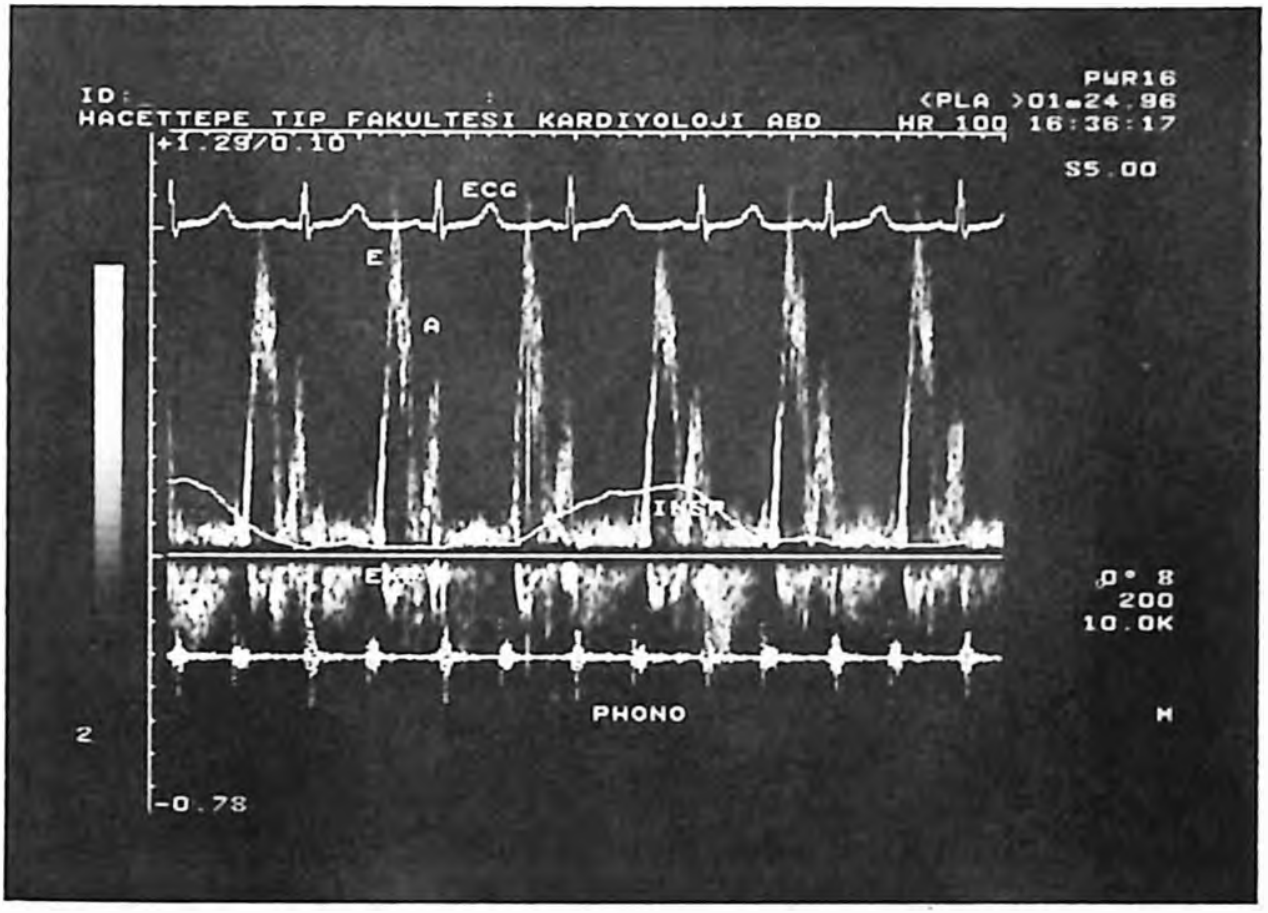

Fig. 1: Pulsed Doppler transmitral spectral tracing with simultaneous respiratory recordings. The electrocardiogram (ECG), phonocardiogram (PHONO), inspiration (INSP), and expiration (EXP) are labeled. E: peak early filling velocity (peak E); A: peak late filling velocity (peak A).

Doppler Analysis

The pulsed Doppler recordings were analyzed using an off-line computer system. For the determination of ventricular filling dynamics, the cardiac cycles with the most clearly defined flow velocity waveform and with the maximum early diastolic filling velocity were chosen and measurements were made from the end expiratory cardiac cycles. The following parameters were obtained from the spectral display: peak early filling velocity (peak E) (in centimeters per second) and peak late (atrial) filling velocity (peak A) (in centimeters per second), from which the ratio of early to late peak filling velocity (peak E/A ratio) was derived; early diastolic velocity-time integral (E integral) (in cm) and late diastolic velocity-time integral (A integral) (in cm), from which E/A integral was derived; total diastolic filling period (TDF), defined as the interval between the onset and termination of diastolic filling velocity; the acceleration time (AT), defined as the interval between the onset of diastolic flow and peak E; deceleration time (DT), defined as the interval between peak E and the termination of early diastolic filling; early acceleration slope (OE slope); early deceleration slope (EF slope);

and isovolumic relaxation time (IVRT), defined as the interval between closure of the aortic or pulmonary valve (in respect to the ventricle being studied), determined from phonocardiogram, and onset of diastolic flow.

Data Analysis

All Doppler measurements reported are the average of three cardiac cycles at end-expiration. All values are reported as mean ± 1 SD. A two-tailed unpaired t-test was used for statistical analysis; $p < 0.05$ indicated a significant difference between the groups.

Intraobserver Variability

All echocardiographic studies and measurements were performed by the same cardiologist (S.Ö). Intraobserver variability was calculated as described previously[9] and was low for most of the Doppler indices for Doppler velocity measurements $r = 0.99$, for all time interval measurements $r = 0.99$, for velocity-time integral measurements $r = 0.98$, and for acceleration and deceleration slope $r = 0.97$.

Results

The cardiomyopathy group consisted of 11 female and five male patients. There were no significant differences in age, heart rate (104 ± 23 beats/min versus 99 ± 13 beats/min) or systolic blood pressure (95 ± 16 mmHg versus 104 ± 18 mmHg) between the cardiomyopathy group and the control group, respectively. Clinical data of the patients are illustrated in Table I.

Comparison of left ventricular filling in patients with cardiomyopathy and normal children showed significant differences in various parameters. Peak early filling velocities (Peak E) and the corresponding velocity-time integrals (E integral) were significantly lower (43.3 ± 11.4 cm/s versus 60.4 ± 11.4 cm/s, $p < 0.01$; and 3.3 ± 1.4 cm versus 4.6 ± 1.2 cm, $p < 0.01$) in the cardiomyopathy group (Fig. 2). In addition, the ratio of peak early filling velocity to late filling velocity was significantly lower, whereas isovolumic relaxation time was significantly longer, in patients with cardiomyopathy (Table II).

Significant differences were also obtained in some indices of right ventricular filling. Peak early filling velocities (41 ± 7.9 cm/s versus 47.5 ± 8.8 cm/s, $p < 0.05$) and the corresponding velocity-time integrals (3.0 ± 1.0 cm versus 3.87 ± 1.1 cm, $p < 0.05$) were significantly decreased, while isovolumic relaxation time was significantly increased ($p < 0.05$), in the cardiomyopathy group compared to normal children (Fig. 3). Right ventricular filling parameters are given in Table III.

The demonstrated abnormalities of left and right ventricular diastolic filling were compatible with the impaired relaxation pattern of both ventricles.

Table I: Clinical Data of the Patients

Patient	Sex	Age (mo)	Weight (kg)	Heart Rate (bpm)	Drug Therapy
1	F	70	17	83	Dig
2	M	135	29	75	Dig+cap
3	F	48	12	115	Dig+cap
4	F	188	49.5	100	Dig
5	F	43	15	125	Dig+diur+cap
6	F	48	14.5	78	Dig+diur
7	M	90	17	113	Dig+cap
8	F	12	10.5	132	Dig+diur
9	F	152	40	99	Dig
10	M	156	34	114	Dig+diur
11	F	149	45	63	Dig
12	M	96	23	84	Cap
13	F	12	9.5	96	Dig+diur+cap
14	F	39	12.5	115	Dig+diur+cap
15	M	2	4.4	148	Dig+cap
16	F	18	7.9	130	Dig+cap

bpm : beats per minute cap : captopril dig : digoxin
diur : diuretic F : female M : male mo : month

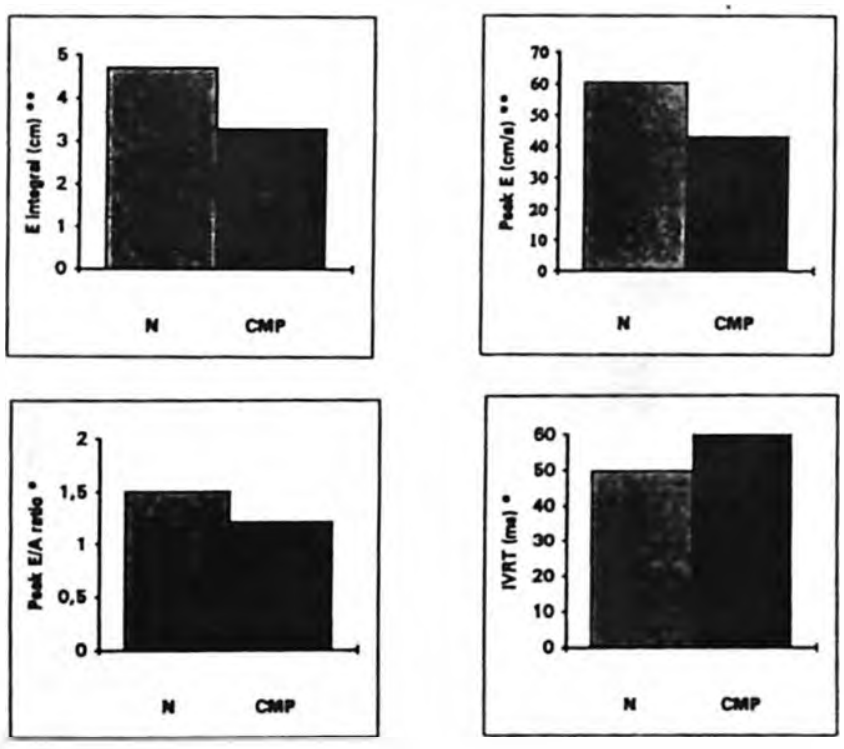

Fig. 2: Left ventricular echocardiographic parameters of normal subjects (N) and patients with cardiomyopathy (CMP). In the cardiomyopathy group, peak early filling velocity (Peak E), ratio of early to late filling velocity (Peak E/A ratio), and early diastolic velocity-time integral (E integral) were significantly decreased, while isovolumic relaxation time (IVRT) was significantly increased when compared with normal subjects ($^{*}p < 0.05$; $^{**}p < 0.01$).

Table II: Left Ventricular Diastolic Parameters in Normal Subjects and in Patients with Cardiomyopathy

	Cardiomyopathy	Normal	p Value
Peak E (cm/s)	43.3 ± 11.4	60.4 ± 11.4	< 0.01
Peak A (cm/s)	37.6 ± 10	40.5 ± 6.8	NS
Peak E/A ratio	1.22 ± 0.47	1.49 ± 0.23	< 0.05
E integral (cm)	3.31 ± 1.4	4.69 ± 1.2	< 0.01
A integral (cm)	1.85 ± 0.6	2.19 ± 0.6	NS
E/A integral	1.85 ± 0.7	2.14 ± 0.5	NS
AT (ms)	66.3 ± 15	70.3 ± 12.2	NS
DT (ms)	82.1 ± 25.7	89.2 ± 16.4	NS
TDF (ms)	281 ± 67	294.5 ± 40	NS
EF Slope (cm/s^2)	5.7 ± 1.9	5.44 ± 1.5	NS
OE Slope (cm/s^2)	8.83 ± 3.7	9.48 ± 2.1	NS
IVRT (ms)	58.9 ± 19.8	49.7 ± 8.9	< 0.05

AT: acceleration time; A area: late (atrial diastolic velocity-time integral. DT: deceleration time; EF slope: early deceleration slope. E area: early diastolic velocity-time integral. IVRT: isovolumic relaxation time. NS: non-significant. OE slope: early acceleration slope. Peak A: peak late filling velocity. Peak E: peak early filling velocity. TDF: total diastolic filling period. For definitions see the methods section.

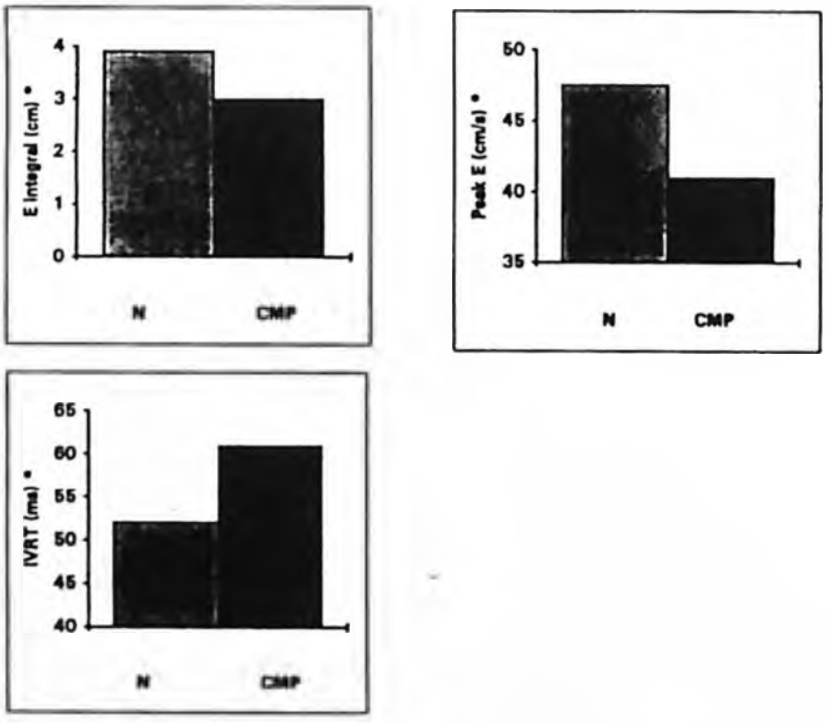

Fig. 3: Right ventricular echocardiographic parameters of control subjects (N) and cardiomyopathy (CMP) group. In the cardiomyopathy group, peak early filling velocity (Peak E) and early diastolic velocity-time integral (E integral) were significantly decreased, while isovolumic relaxation time (IVRT) was significantly increased, when compared with normal subjects. * $p < 0.05$.

Table III: Right Ventricular Diastolic Parameters in Normal Subjects and in Patients with Cardiomyopathy

	Cardiomyopathy	Normal	p Value
Peak E (cm/s)	41 ± 7.9	47.5 ± 8.8	< 0.05
Peak A (cm/s)	35.9 ± 6.8	37.9 ± 7.1	NS
Peak E/A ratio	1.16 ± 0.27	1.25 ± 0.22	NS
E integral (cm)	3.0 ± 1.0	3.87 ± 1.1	< 0.05
A integral (cm)	2.17 ± 0.72	2.23 ± 0.51	NS
E/A integral	1.46 ± 0.46	1.67 ± 0.71	NS
AT (ms)	73.6 ± 16.8	71.2 ± 13.7	NS
DT (ms)	81.8 ± 27.7	92.9 ± 20.8	NS
TDF (ms)	272.6 ± 89.1	296.9 ± 52.4	NS
EF Slope (cm/s^2)	5.0 ± 2.0	4.17 ± 0.86	NS
OE Slope (cm/s^2)	7.15 ± 2.2	7.71 ± 2.55	NS
IVRT (ms)	60.6 ± 16.3	52.2 ± 12.8	< 0.05

Abbreviations as in Table II and methods section.

Discussion

The use of transmitral Doppler recordings in assessing left ventricular diastolic function has received increased attention in clinical cardiology. Hence, several studies in adult patients with dilated cardiomyopathy recently demonstrated diastolic abnormalities as being responsible for symptoms during periods of clinical deterioration[10-13]. Three distinct filling patterns have been described. The first, characterized by a prolonged isovolumic relaxation time, slow deceleration of early filling velocity and a decrease in E/A ratio, is associated with "impaired relaxation". The second type, called the "restrictive" pattern, is characterized by a short isovolumic relaxation time, rapid filling in early diastole (high E velocity), reduced atrial filling velocity (a velocity) and an increase in E/A ratio. The third pattern is called a "normalized" or "pseudonormal" pattern[14]. The restrictive pattern is usually seen in patients with an advanced form of disease, while the impaired relaxation is usually associated with a mild form of the disease[14, 15].

In this study in the cardiomyopathy group we detected significant reductions in left ventricular early filling velocity, early velocity-time integral and in E/A ratio, whereas a significant increase was detected in isovolumic relaxation time. These changes were in accord with impaired relaxation of left ventricle. All our patients were clinically stable during the study period; therefore; an abnormal relaxation pattern was compatible with the clinical status of our patients. Our findings are also in agreement with previous studies, demonstrating abnormal relaxation patterns in adults with a mild or moderate form of dilated cardiomyopathy. However, in the only other study performed in six children with dilated

cardiomyopathy, Riggs[16] reported an increased rather than decreased early to late peak filling velocity (peak E/A) ratio in the cardiomyopathy group. The finding of a higher peak E/A ratio was supposedly related to the increased left atrial pressure due to mitral insufficiency present in most of their patients.

Unlike the case with left ventricular filling, right ventricular diastolic filling has not been studied extensively in either adults or in children with dilated cardiomyopathy. In one study assessing right ventricular diastolic function in patients with dilated cardiomyopathy by cinemagnetic resonance, Suziki et al.[17] demonstrated impairment of diastolic function of the right ventricle. Their findings were consistent with the notion of the existence of impaired early diastolic filling of the right ventricle in patients with dilated cardiomyopathy. In the only other study performed in children by Doppler echocardiography[16], the E/A integral, peak early velocity and peak E/A ratio were each lower in the cardiomyopathy group, while the peak A velocity was higher. Isovolumic relaxation time was not evaluated in this investigation. In the present study, peak E velocity and the corresponding velocity-time integral were significantly lower, and the isovolumic relaxation time significantly higher, in the cardiomyopathy group. Our findings are in favor of impaired relaxation of the right ventricle and suggest that the cardiomyopathic process is a diffuse process associated with impairment of both right and left ventricular diastolic filling.

Study Limitations

The most important limitation of the study lies in the complexity of the physiology that is evaluated. Diastole represents a complex interplay of multiple factors, including ventricular and atrial systolic and diastolic properties and loading conditions, and the pattern of filling may not reflect all diastolic properties. Heart rate, respiration, age, and valvular regurgitation are some of the factors that could also alter diastolic filling[16, 18-22]. In the current study, end expiratory values of diastolic indices were used in order to minimize the effects of respiration, and patients with valvular regurgitation were not included in the study. Also, there were no significant differences in age, heart rate, or systolic blood pressure between the control group and the group with dilated cardiomyopathy. Therefore, we think that these variables were unlikely to have had significant effects on our results.

Lack of an invasive hemodynamic assessment of the patients could be considered as a limitation. However, the purpose of the study was to evaluate diastolic filling by Doppler echocardiography, which is a noninvasive method. Furthermore, previous studies with simultaneous Doppler echocardiographic and hemodynamic measurements have demonstrated that the findings were closely correlated with each other[14, 23].

In conclusion, this study shows that in addition to left ventricular diastolic dysfunctilon, abnormalities of right ventricular diastolic filling may also occur in children with dilated cardiomyopathy. In light of these findings, we recommend Doppler echocardiographic assessment of left and right ventricular diastolic filling in every patient with dilated cardiomyopathy.

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The Turkish Journal of Pediatrics 1998; 40: 347-355

DNA DIAGNOSTIC TESTS IN Xp21 DYSTROPHY FAMILIES FOR PRENATAL DIAGNOSIS*

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SUMMARY: Dinçer P, Topaloğlu H, Ayter Ş. (Department of Medical Biology, Hacettepe University Faculty of Medicine, Ankara, Turkey). DNA diagnostic tests in Xp21 dystrophy families for prenatal diagnosis. Turk J Pediatr 1998; 40: 347-355.

Duchenne and Becker muscular dystrophies are X-linked genetic disorders characterized by dystrophin gene defects. We have studied 250 families with Duchenne and Becker muscular dystrophies (D/BMD) by molecular genetic methods since 1992. Nineteen exons of the dystrophin gene were analyzed for deletion. In families with no deletion, linkage analysis was performed to follow the inheritance of mutant alleles in the affected families. Twenty of these families requested prenatal diagnosis. Six mothers were found to be non-carriers (99% accuracy), thus fetuses were examined in the remaining 14. Two fetuses were affected and terminated. We report our experience and our current clinical practice in providing prenatal studies for D/BMD. *Key words: Duchenne/Becker muscular dystrophies, deletion, linkage analysis, prenatal diagnosis.*

Duchenne and Becker muscular dystrophies (D/BMD) are allelic X-linked genetic disorders[1]. These disorders arise as a result of mutations affecting the dystrophin protein. The majority of gene mutations consist of deletions (65%) or duplications (5%) which are clustered in two hot spots[2-3]. Ninety-eight percent of these deletions can now be detected by polymerase chain reaction (PCR)[4]. For the remaining DMD cases, carrier detection and prenatal diagnosis depend on DNA linkage analysis[5-6].

Genetic counseling and prenatal diagnosis in families with D/BMD are crucial. Females at risk of carrying D/BMD should be counseled before they undertake pregnancies. However, it depends on the availability of information on the carrier status. To date, linkage analysis in D/BMD families without detectable deletions has been performed using diallelic RFLP markers flanking and within the DMD gene by Southern blot analysis[6]. Today, not only RFLPs[7] but also other linkage markers[8-10] can be easily assayed by PCR.

In this study, we report our experience in the molecular approach of D/BMD families who applied for prenatal diagnosis and who were studied at the Medical Biology Department, Hacettepe University Faculty of Medicine, Ankara.

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Material and Methods

Patients

Patients were diagnosed at the Child Neurology Department of Hacettepe University. Diagnosis for DMD was based on family history and pedigree analysis, clinical findings, high creatine phosphokinase levels (CPK), electromyographic findings, DNA studies, and dystrophin immunocytochemistry of muscle biopsy where necessary.

DNA Studies

DNA was isolated by standard methods from leukocytes[11, 12], or from amniotic[13] or chorion cells[14]. PCR deletion analysis of different exons of the dystrophin gene was performed according to Chamberlain et al.[15-16] and Gibbs et al.[17], with modifications described by Abbs et al.[18].

5'DYS1-5'CA[9], short tandem repeats (STR 44-STR 45-STR49-STR50[8], J66 (Yau SC. unpublished data), and 3'CA[10] markers were analyzed at DMD locus in families to detect the mothers' haplotype, for carrier detection of females and to determine the genetic status of the fetus. The methodology for the molecular analysis of D/BMD cases was applied for both pre-and postnatal diagnostic approaches.

To date, we analyzed 250 D/BMD families. In 145 of these (58%), deletion was detected[19]. We studied 105 families by linkage analysis.

Prenatal Diagnosis

We used the X-Y homologous amelogenin gene primers (AMEL) (20) to detect the sex of the fetus for noninformative families. Multiplex PCR and linkage analyses were performed on the DNAs extracted from chorionic villus sample (CVS)[13] or amniocytes[14].

Only a few of our D/BMD families applied to our laboratory for genetic analysis prior to pregnancy; the majority of mothers came eight to ten weeks pregnant. Under these circumstances, deletion screening was done in the male patient. If deletion was detected, a chorionic villus sample was taken for determination of sex and of deletion status of the fetus. If there was no deletion in the male patient, we proceeded with linkage analysis of the family. Twenty families requested prenatal diagnosis. Deletions within the D/BMD gene were found in 12 of the index cases of these families; linkage analyses were carried out in the remaining eight families with an accuracy of 99 percent. Six pregnant women were found to be non-carriers (99% accuracy), thus fetuses were examined in the remaining 14.

Results

Over the past four years, 20 families asked for prenatal diagnosis. We applied the same methods used for the molecular diagnosis of 250 D/BMD families. Deletions were identified in 12 of them; eight families were studied by linkage analysis (Fig. 1). Six mothers were diagnosed as non-carriers, thus we did not proceed with prenatal diagnosis. In the remaining 14 families in which prenatal diagnosis was carried out, two fetuses were found to be affected and were terminated.

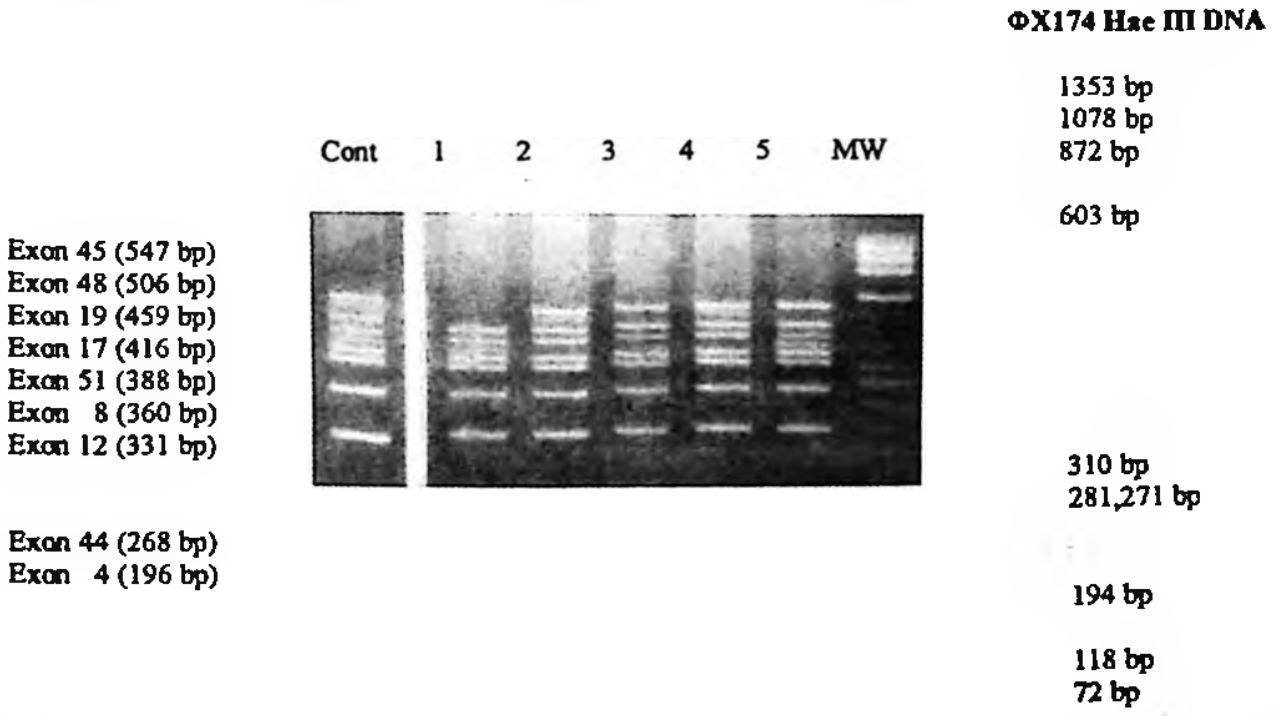

Fig. 1: Deletion analysis of the dystrophin gene by multiplex PCR. The exons represented by each PCR product and product sizes are indicated on the left and MW marker is on the right. Lane 1: deletion of exons 45-48; lanes 2-4: deletion of exon 51; lane 3: deletion of exons 48-51; lane 5: deletion of exon 48. Cont: indicates nonaffected male control subject. MW: molecular weight marker (ϕX174 Hae III DNA) 3% Nu Sieve + 1% agarose gel (05 µg/ml etidium bromid), 120 V, 1.5 hour.

Here, we present several examples of the combined use of dystrophin flanking and intragenic PCR based markers in DMD families, to whom we have provided prenatal diagnosis.

Family 1 : Here, the index case is sporadic. His pregnant sister is asking whether she is a carrier. Highly polymorphic markers showed that she is not a carrier. The affected male (II3) was shown to be deleted for exon 50. STR haplotyping confirmed the deletion, since STR49 failed to amplify while STR 50 was detected. The mother (I1) was heterozygous at STR49 so her carrier risk was therefore reduced to the level of probable mosaicism. Proband's sisters (II2, II4) were heterozygous at STR49, therefore, pregnant sister (II2) did not need to apply for prenatal diagnosis (Fig. 2).

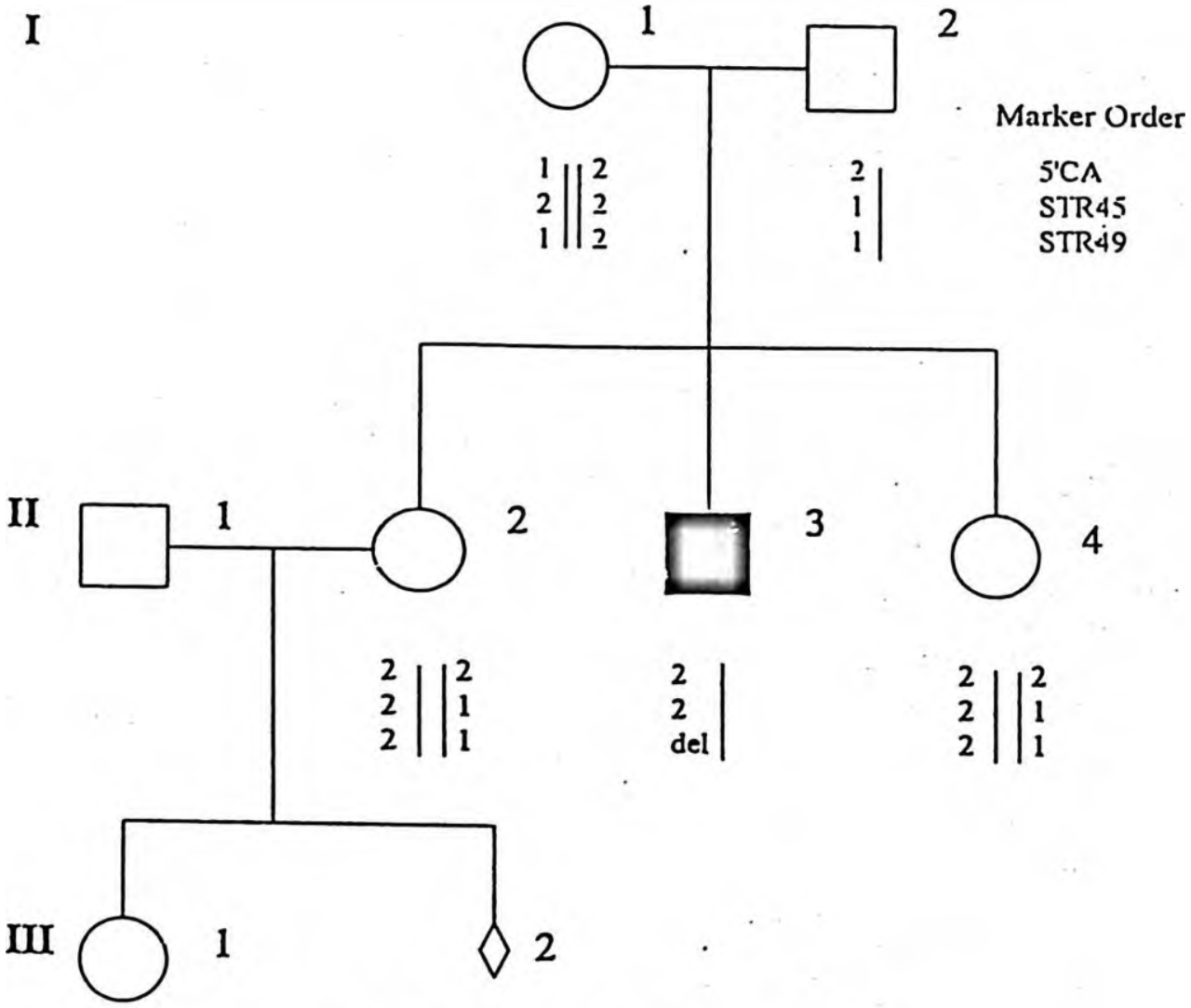

Fig. 2: Pedigree of DMD family 1. del: deletilon.

Family 2: The index case has a deletion. The abnormal X chromosome has a grandpaternal origin which is demonstrated by linkage analysis. This male patient (III4) was shown to have no deletion in the dystrophin gene using the multiplex PCR. He inherited grandpaternal X chromosome at the intragenic marker STR45-9, 5' flanking marker 5'CA and 3' flanking marker 3'CA (recombination error about 1%). His sisters (III3, III5), who inherited the same X chromosomes at these markers and have very high CPKs, are carriers of DMD. The mutation is therefore either grandpaternal in origin or it arose in his mother (II4). A prenatal diagnosis for the exclusion of the grandpaternal X chromosome was offered. After sex determination and genetic analysis, the fetus (III6) was found to be a carrier female (Fig. 3).

Family 2

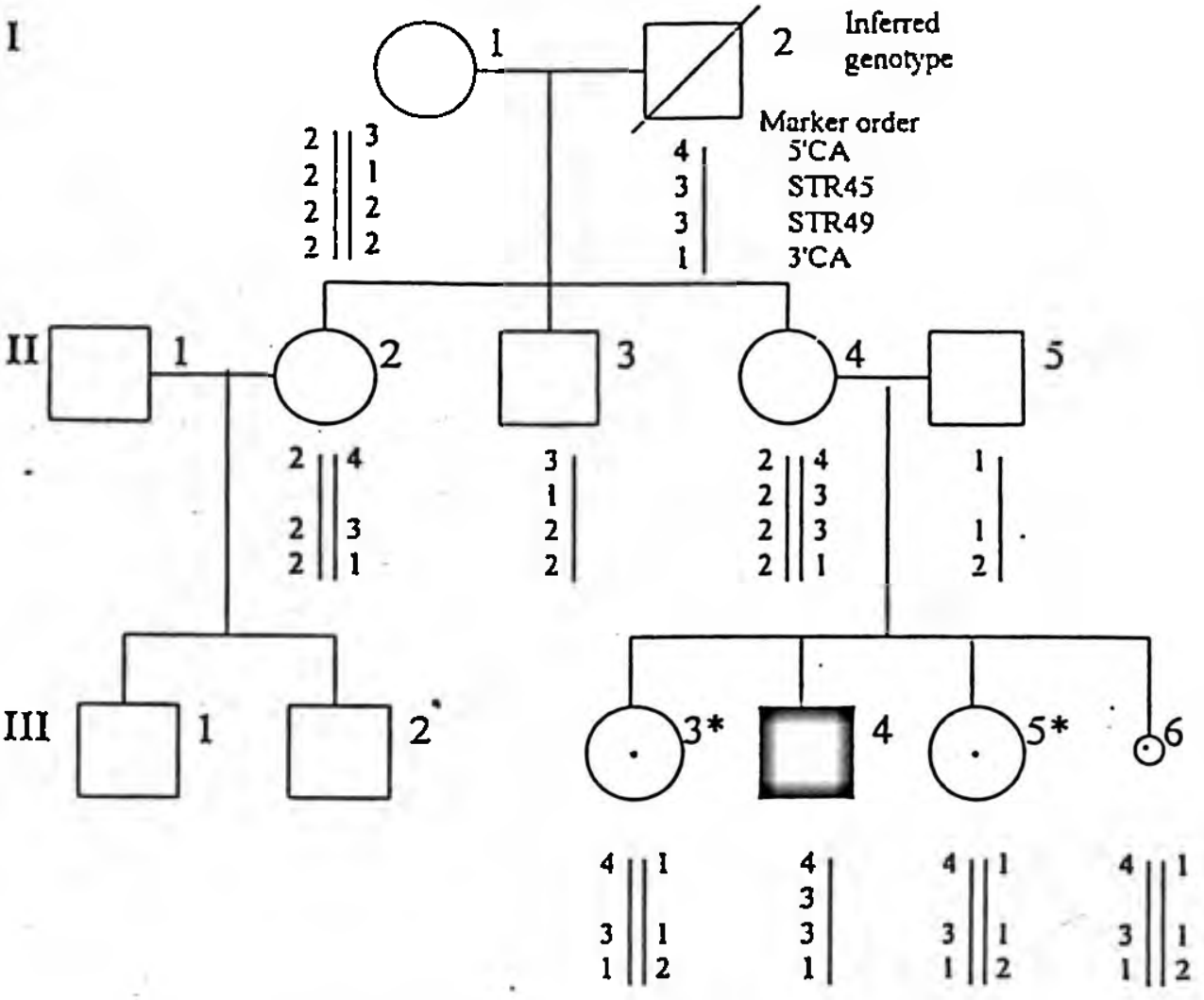

Fig. 3: Pedigree of DMD family 2. *elevated CPK.

Family 3: This example shows the correlation between the biochemical and genetic data. Deletion could not be found in the affected son (III5). The PCR results for the polymorphisms of the whole family are shown in Figure 4. The male patient (III5) inherited the opposite maternal allele from his sisters (III2, III4). This result, combined with the mother's high CPK results, shows that the mother (II4) is a carrier. The CPK results of the sisters were normal. The pregnant sister (III2) is therefore not a carrier (less than 1% recombination error).

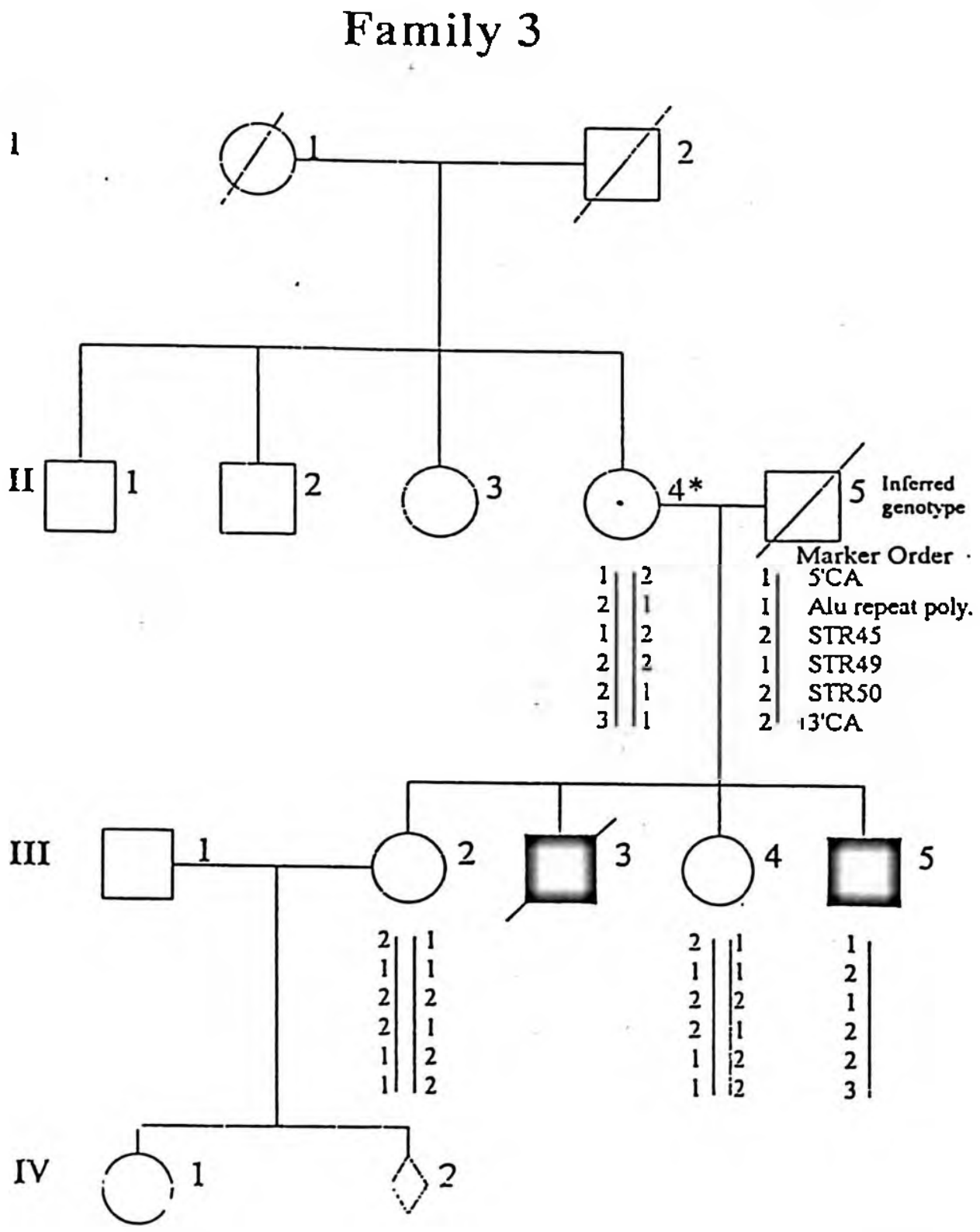

Fig. 4: Pedigree of DMD family 3. * elevated CPK.

Family 4: Deletion studies in the male patient of this family were negative. The two sons (II1, II2) of the pregnant mother (I1) had inherited the opposite alleles from their mother. This increases the mother's carrier risk. Prenatal diagnosis

could be performed for this mother using 5'CA, STR45, STR49, STR50 markers with a one-to-two percent recombination error. For 3' flanking marker of the gene, none of the markers was informative. There is only a one-to-two percent recombination error between intron 50- 3' end of the dystrophin gene[21] (Fig. 5). Fetus has not been worked yet. If the fetus is a male and if he is inheriting the same allele as his unaffected brother, he will be healthy (99% accuracy).

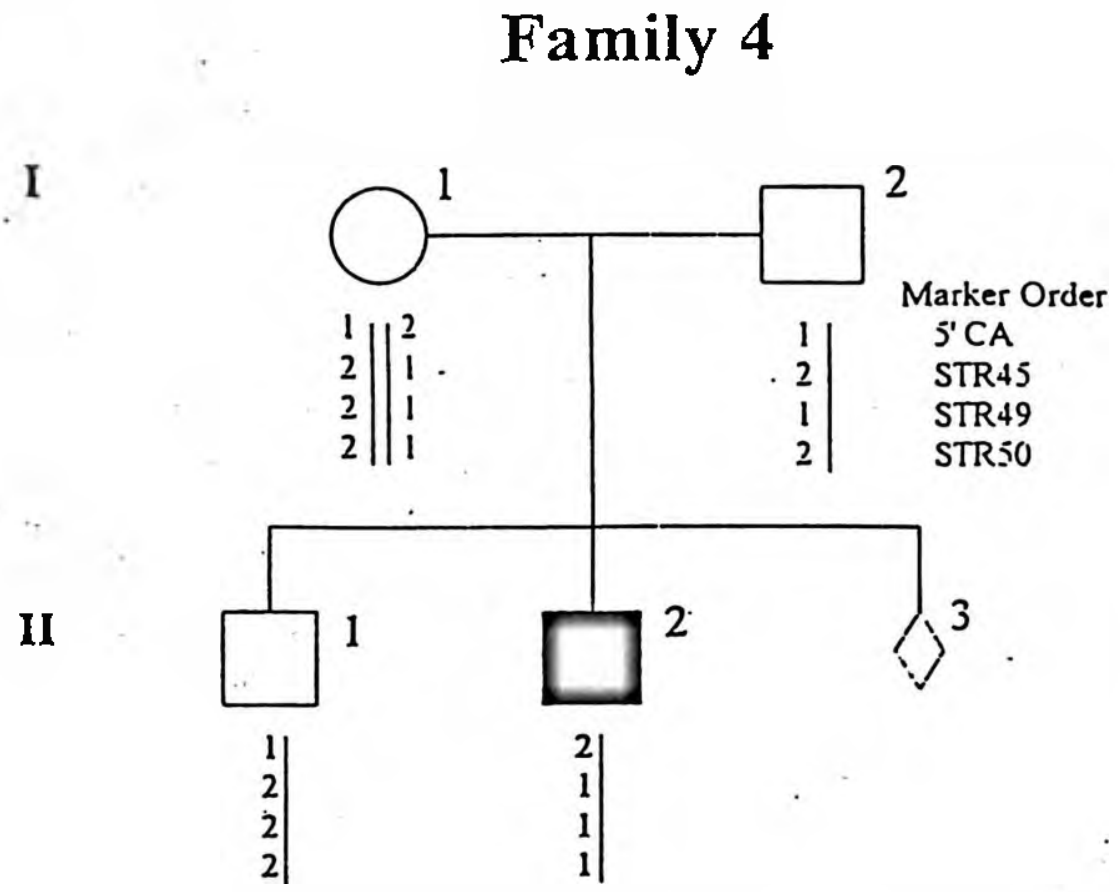

Fig. 5: Pedigree of DMD family 4.

Discussion

There is still no treatment for Duchenne and Becker muscular dystrophies. Genetic counseling is therefore very important to females at risk. There are two means for pre- and postnatal diagnosis. These methods are universal. First, deletion studies must be done. In cases where deletion cannot be demonstrated, a linkage analysis should follow. In our routine experience, we use the same approach. Detection of a deletion in affected children provides a very accurate prenatal test for the future pregnancies. Our deletion rate of 58 percent is nearly the same as other major laboratories[19]. When a deletion is not recognized, we try to track the disease allele through a family by linkage analysis. The problems in using linkage analysis for prenatal diagnosis are the same as for genetic

counseling. The mother must be informative at a suitable locus, and family studies are needed, preferably before prenatal diagnosis. However, we occasionally see mothers already pregnant with no initial DNA studies done. Thus, rapid detection methods should be available, and highly polymorphic and easy-to-study markers should be tried.

Due to the very large size of the gene, analysis of flanking markers is required to avoid diagnostic errors due to intragenic meiotic recombination. When the 5'-3' flanking markers and an intragenic marker are informative and show inheritance of an intact haplotype, the error rate due to recombination is less than one percent[22-23].

As a standard, highly polymorphic microsatellite markers are used in most laboratories. These markers (5'-3' CAs, STRs, DMD1c-2) seem to be efficient for nearly all D/BMD families. In this way, we offer carrier detection and prenatal diagnosis with an accuracy of 99 percent. STR markers lie within the distal deletion hot spot of the dystrophin gene. They provide direct diagnosis of deletion carriers and help to define the mutant X chromosome in affected individuals. These advantages make STRs an ideal intragenic marker for the analysis of D/BMD families. For a single STR marker, the heterozygosity frequency was around 50 percent in our population. When four markers are used in combination, the chances of being informative are tremendously increased. Among the 105 families in which we carried out linkage analysis, only one was found to be non-informative with the STR markers we used. This is very helpful during routine practice of analyzing families.

In conclusion, this combined approach, i.e deletion and linkage analysis, is a safe and reliable method to study D/BMD families in busy clinics serving a large number of families.

Acknowledgements

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PRO – INFLAMMATORY CYTOKINES, LYMPHOCYTE SUBSETS AND INTRAVENOUS IMMUNOGLOBULIN THERAPY IN GUILLAIN – BARRÉ SYNDROME*

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SUMMARY: Tekgül H, Kütükçüler N, Çağlayan S, Tütüncüoğlu S. (Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey). Pro-inflammatory cytokines, lymphocyte subsets and intravenous immunoglobulin therapy in Guillain-Barré syndrome. Turk J Pediatr 1998; 40: 357-363.

Both cell-mediated immunity and humoral factors are involved in the pathogenesis of Guillain-Barré syndrome (GBS). Intravenous immunoglobulin (IVIG) has been reported to be a practical, effective and safe treatment in childhood GBS, although the mode of action of IVIG remains uncertain. We studied pro-inflammatory cytokines (interleukin-2, interleukin-1α and tumor necrosis factor-α) in plasma and cerebrospinal fluid (CSF) and lymphocyte subsets in peripheral blood both in the acute phase and in the recovery period in six children with GBS treated with IVIG. Flow cytometry was used to determine the subsets of lymphocytes in peripheral blood, and cytokines analyses were performed by using ELISA technique. Results were compared with a control group of 20 healthy children. A standard protocol of IVIG (400 mg/kg/day for 5 days) was administered to all the patients. Plasma interleukin-2 concentrations and the number of HLA DR+ active T cells in peripheral blood were significantly higher in the acute phase of the disease than in the recovery period and in healthy controls. There was no significant difference in the other cytokine concentrations in plasma and CSF or in the other lymphocyte subsets in peripheral blood. Our data indicate that IVIG may provide its possible theurapeutic effect by acting in the cell-mediated immunity in GBS patients. *Key words: Guillain-Barré syndrome, cellular immunity, lymphocyte subsets, cytokines, intravenous immunoglobulin.*

Guillain-Barré syndrome (GBS), a demyelinating disease of peripheral nerves, results from abberant immune responses directed against components of the peripheral nerve[1]. Both cell-mediated immunity and humoral factor are involved in the pathogenesis of the disease[1-5]. The close histological resemblance between experimental allergic neuritis and GBS suggests a common autoimmune pathogenesis[2]. The therapeutic efficacy of plasmapheresis treatment and intravenous immunoglobulin (IVIG) and the presence of circulating antibodies directed to the peripheral nerves also evidence an immune-mediated disorder of peripheral nerves[6-8]. The release of pro-inflammatory cytokines such as interleukin-1α (IL-1α) and tumor necrosis factor-α (TNF-α) from macrophages may have a myelinotoxic effect in the peripheral nerves[3-5].

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In this study, we the plasma and cerebrospinal fluid (CSF) concentrations of cytokines (interleukin-2, IL-1α and TNF-α) and lymphocyte subsets in peripheral blood of patients with GBS both in the acute phase and in the recovery period of the disease. The objectives of the study were (1) to investigate the role of cell-mediated immunity in the pathogenesis of GBS, (2) and to determine the effect of IVIG treatment on the cell-mediated immunity and pro-inflammatory cytokines.

Material and Methods

Six children with GBS diagnosed by using NINCDS'78 criteria[9] were included in the study. Their ages were between 2.5-13 years, (8.2 ± 3.8) and four of them were girls. Four patients had a history of acute respiratory infectilon; two had no history of any acute infection. The clinical manifestations were motor weakness (6/6), cranial nerve involvement (1/6) and disturbance of sensation (1/6). One of the patients with maximal motor deficit required ventilation assistance. All the patients showed albuminocytologic dissociation features and a reduction in nerve conduction velocity.

Intravenous immunoglobulin (Sandoglobulin, Sandoz Pharmaceuticals, 400 mg/kg/day for five days) was administered to all the cases. The patients started recovering within four days and the mean time to recovery of independent walking was 14 days. Three were followed for a 12-month period in the outpatient clinic and no relapse was observed. Peripheral blood and CSF were taken before the administration of IVIG (acute phase) and also when the patients reached unaided walking (recovery period).

Twenty healthy children (10 girls, 10 boys) with an age range of two to 17 years (mean 12.4 ± 8.4) were included as a control group for peripheral blood lymphocyte subsets and plasma cytokine concentrations.

Enumeration of peripheral blood mononuclear cell subsets: the percentage of total T (CD3+ cells) and B (CD19+ cells) lymphocytes, T helper/inducer (CD4+ cells) and T suppressor/cytotoxic (CD8+ cells) lymphocytes, natural killer cells (CD16+56+ cells) and HLA-DR+ active T cells were analyzed with monoclonal antibodies labeled with either fluorescein isothiocyanate (FITC) or phycoerthrin (PE) (Becton-Dickinson, Belgium) and with flow cytometry (FACSCAN, Becton-Dickinson). Briefly, peripheral blood cells and each monoclonal antibody were incubated for 30 min at room temperature in the dark and red blood cells were then lysed with FACS lysing solution (Becton-Dickinson). The cells were then washed with phosphate-buffered solution and were analyzed by flow cytometry. Absolute numbers of lymphocyte subsets were calculated on the basis of total and differential white cell counts and the percentage of total T lymphocytes with T cell markers.

Plasma and CSF cytokine levels: IL-2, IL-1 alpha, and TNF-alpha (Amersham, Aylesbury, UK) concentrations were measured using enzyme-linked immunosorbent assay (ELISA). The minimum detectable concentratilons of IL-2, IL-1α and TNF-α

by this method are 0.6 pg/ml, 0.1 pg/ml and 0.1 pg/ml, respectively. The assays have no cross-reactivity with the other cytokines. Inter/intra-assay variabilities were less than 10 percent. The samples were stored at -30 °C until use.

Results of lymphocyte phenotyping and cytokine concentrations were compared using Mann-Whitney U test.

Results

The percentage and absolute counts of lymphocyte subsets in peripheral blood of patients with GBS and in healthy controls are listed in Table I. No significant difference was found in lymphocyte subsets between patient and control groups except HLA-DR+ active T cells. In the acute phase of GBS, the percentages (11.2 ± 3.0%) and absolute counts (540 ± 200/mm^3) of active T cells were highly elevated when compared with the data obtained during the recovery period (5.3 ± 1.5%, 220 ± 60/mm^3) and in healthy children (4.5 ± 2.4%, 190 ± 80/mm^3) ($p < 0.05$). On the other hand, no significant difference was observed in active T cells in the recovery period and in healthy children ($p > 0.05$). Plasma IL-2 concentrations were found to be significantly higher in the acute phase (3.32 ± 1.74 pg/ml) when compared with the recovery period (0.90 ± 0.62 pg/ml) and healthy controls (non-detectable) ($p < 0.05$) (Table II). In contrast, plasma IL-1α and TNF-α levels in the acute phase and recovery period and in healthy controls were similar ($p > 0.05$) (Table II).

Table I: Percentage and Absolute Counts of Lymphocyte Subsets in Patients with GBS and in Healthy Controls

		I. Acute Phase n = 6	II. Recovery Phase n = 6	III. Control n = 20
Total lymphocyte	(ab)	4.44 ± 0.97	3.90 ± 0.76	4.60 ± 1.27
CD3+	(%)	57.2 ± 8.00	59.0 ± 14.5	73.0 ± 6.50
	(ab)	2.92 ± 0.56	2.58 ± 0.72	3.56 ± 0.76
CD19+	(%)	23.4 ± 5.30	15.0 ± 4.30	14.0 ± 4.20
	(ab)	1.08 ± 0.42	0.80 ± 0.30	0.72 ± 0.28
CD4+	(%)	34.6 ± 10.5	35.0 ± 3.00	44.0 ± 7.60
	(ab)	1.80 ± 0.52	1.42 ± 0.22	2.08 ± 0.43
CD8+	(%)	33.2 ± 11.0	30.0 ± 13.1	33.0 ± 5.40
	(ab)	1.58 ± 0.48	1.26 ± 0.66	1.66 ± 0.32
CD4/8		1.22 ± 0.61	1.30 ± 0.60	1.40 ± 0.20
CD16+56+	(%)	16.4 ± 10.5	23.3 ± 13.6	14.1 ± 4.20
	(ab)	0.72 ± 0.52	0.96 ± 0.64	0.84 ± 0.16
HLA-DR+T cell	(%)	11.2 ± 3.00*	5.30 ± 1.50	4.50 ± 2.40
(active T cell)	(ab)	0.54 ± 0.20*	0.22 ± 0.06	0.19 ± 0.08

ab: absolute count. 103 cells/mm3. * I-II: $p < 0.05$, I-III: $p < 0.05$, II-III: $p > 0.05$.

Table II: Plasma IL-2, IL1α and TNF-α Concentrations in Patients with GBS and in Healthy Controls

	I. Acute Phase n = 6	II. Recovery Period n = 6	III. Controls n = 20
IL-2 (pg/ml)	3.32 ± 1.74*	0.99 ± 0.62	ND
IL-1α (pg/ml)	1.08 ± 0.21	1.14 ± 0.45	2.88 ± 1.67
TNF-α	0.23 ± 0.48	0.20 ± 0.34	2.13 ± 2.28

*I-II. $p < 0.05$, I-III: $p < 0.05$, ND: non-detectable concentrations.

CSF IL-2 concentrations were non-detectable in all the samples, both at the onset of the disease and in the recovery period (Table III). There was no significant elevation between CSF IL-1α and TNF-α levels during the acute and recovery periods of the disease ($p > 0.05$, Table III).

Table III: CSF IL-2, IL-1α and TNF-α Concentrations of Patients with GBS ($p > 0.05$)

	I Acute Phase	II. Recovery Period
IL-2 (pg/ml)	ND	ND
IL-1a (pg/ml)	0.88 ± 0.65	0.93 ± 1.17
TNF-a (pg/ml)	0.42 ± 0.83	0.4 ± 0.7

ND: non-detectable concentrations.

Discussion

In GBS, evidence has indicated probable derangement of T cell subsets and alterations of T lymphocyte functions[10, 11]. However, the neuritogen or pathogen that triggers GBS is still not known. One immunocytochemical study about infiltrating lymphocytes on peripheral nerves revealed a predominance of CD4+ T helper/inducer cells over CD8+ suppressor/cytotoxic T cells and the presence of some B cells[12]. In Winer et al.'s[2] and Iqbal et al.'s[10] studies, CD8+ lymphocytes in peripheral blood were found to be significantly reduced, but total lymphocytes, total T and CD4+ cells were not altered. In contrast to these results, in our study CD8+ cells in peripheral blood of patients with GBS were not altered in the acute phase, nor were CD3+, CD4+, CD19+, CD16+56+ cells (Table I).

In GBS patients, circulating T cells that carry on their surface activation markers HLA-DR and interleukin-2 receptor (IL-2R) were increased in number[11, 13]. Increased plasma concentrations of soluble IL-2R (sIL-2R) indicate the presence of circulating activated T cells. In Hartung et al.'s study[11], higher concentrations of sIL-2R shed from activated T cells were measured in the plasma of patients with GBS and correlated with disease activity. On the other hand, circulating IL-2 concentration has been found in increased amounts in GBS and declined in parallel with clinical recovery[14]. In our study, in the acute phase of GBS, both

an increased percentage of HLA-DR+ active T cells in peripheral blood and increased levels of plasma IL-2 in the acute phase strongly indicate the activation of the cellular immune system. As the patients recovered, active T cells and plasma IL-2 levels declined to normal levels. As was expected, the CSF IL-2 concentrations were non-detectable in all the samples both at the onset of the disease and in the recovery period because lymphocytes do not infiltrate CSF in GBS, but also because IL-2 probably does not tranasfer from peripheral blood to CSF.

In GBS, the mechanisms by which circulating active T cells come to the peripheral nervous system are unknown. However, activated T cells could be relevant to the pathogenesis in several ways. First, activated CD4+ cells of the Th2 phenotype synthesize IL-4, IL-5, IL-6 and may cause B cell proliferation and transformation into plasma cells which produce antibodies against peripheral myelin components[1]. Second, the Th1 phenotype, which synthesizes IFN-γ, IL-2, TNF-α and β, could damage myelin by secreting pro-inflammatory and myelinotoxic cytokines and could operate by recruiting macrophages to exert nerve damage[1]. Macrophages have been identified as major cellular components in the tissue lesion, and their decisive role in mediating autoimmune peripheral nerve injury has been proven in the animal model of EAN[11]. It was shown that macrophages act as antigen presenters and are of pivotal importance in the amplification of immune-mediated demyelination by mechanisms including phagocytosis and the release of pro-inflammatory cytokines[3]. On the basis of these observations, we investigated cytokine production (IL-1α, TNF-α) of macrophages in plasma in order to determine their contribution in the pathogenesis of GBS. Both the plasma levels of TNF-α and IL-1α were not found to be elevated and were at low levels as was observed in healthy children. In Sharief et al.'s study[15], ony 20-50 percent of GBS patients have raised serum levels of TNF-α. The low levels of these pro-inflammatory cytokines in plasma suggested that the production of these cytokines by peripheral blood monocytes and macrophages was inhibited or not effected throughout the course of GBS. The serum levels of inhibitors of these cytokines, such as interleukin-1 receptor antagonist (IL-1ra) and soluble TNF-receptors (TNF-sR), have to be measured because in many autoimmune diseases these inhibitors were found to be elevated furthermore, they are important regulatory components of IL-1 and TNF mediated inflammation and immune response[16].

We also measured CSF concentration of IL-1α and TNF-α and tried to determine if there was a passive transfer or overproduction of these cytokines. Similar to results seen in the plasma, they were not found to be elevated in CSF in the acute period of the disease (Table III). Cellular infiltration including monocytes and macrophages of CSF in GBS is minimal and we did not expect to find elevated cytokine levels. On the other hand, it is not possible to suggest the presence or absence of a passive transfer of IL-1α and TNF-α into CSF because of the very low levels on both sides.

The decline to normal levels for active T cells and plasma IL-2 concentrations may be due to IVIG treatment or it may be just laboratory evidence of a recovery period that can be observed following any type of immunotherapy. However, it would have been important to support this claim by studying another group of patients with GBS but without IVIG. Furthermore, the mode of action of IVIG remains uncertain, although several mechanisms have been proposed. It has been reported that IVIG contains anti-idiotype antibodies against auto-antibodies and also down regulates cytokine production (IL-2, TNF-α and β). In addition, it has an interference effect with superantigen-induced T cell activation by antitoxin antibodies[17].

IVIG therapy shortened the duration of disability in GBS in comparison with standard supportive treatment in a large, open, controlled trial[18, 19]. Although IVIG is potentially a safer treatment, relapse or disease progression after treatment has been reported[18]. In our patients, IVIG shortened the average duration of the disease with no relapse after 12 months of follow up.

In conclusion, elevation of IL2 concentrations and an increased number of activated T-cells in the acute phase and a decline to normal levels in the recovery phase indicate that IVIG may play a role in the suppression of cell-mediated immunity in patients with GBS.

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LONG – TERM FOLLOW – UP OF HEPATITIS B VIRUS CARRIERS WITH NORMAL TRANSAMINASES LEVELS*

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SUMMARY: Koçak N, Özen H, Yüce A, Gürakan F. (Gastroenterology Unit, Hacettepe University Faculty of Medicine, Ankara, Turkey). Long-term follow-up of hepatitis B virus carriers with normal transaminases levels. Turk J Pediatr 1998; 40: 365-372.

There has been very little data recorded on the natural course of chronic hepatitis B virus infection in asymptomatic children. In order to assess the natural course of liver disease in hepatitis B surface antigen (HBsAg) carriers with normal liver tests, 124 such children (81 males, 65.3%) were followed for six to 144 months (mean 36.8 ± 22.8 months). Liver tests and hepatitis B virus (HBV) markers were tested at least every six months.

In the beginning, hepatitis B e antigen (HBeAg) was positive in 61 (53%) of the 115 carriers of HBsAg who were tested. Anti-HBe was positive in 51 (44.3%), and both HBeAg antigen and anti-HBe were negative in three (2.7%) carriers. The prevalence of HBeAg was not affected by the patient's age or sex. During follow-up, 11 patients (18%) lost HBeAg (a mean annual clearance rate of 5.8%), and 12 patients (9.7%) lost HBsAg (a mean annual clearance rate of 3.1%). We found no difference in the clearance of HBsAg and HBeAg by age and sex. The presence of another HBsAg positive person in the family affected HBsAg clearance rate but not HBeAg clearance. Only seven patients (5 HBeAg positive and 2 anti-HBe positive) developed transient elevations in liver transaminases. Three of five HBeAg positive children cleared HBeAg after transaminases elevations. Of the five patients who underwent percutaneous liver biopsies, non-specific changes were found.

It is concluded that hepatitis B carriers with normal liver tests should be followed with liver function tests alone and that long-term prognosis is good. *Key words: hepatitis B virus carrier, transaminases level, long-term follow-up.*

Most carriers of hepatitis B surface antigen (HBsAg), both children and adults, are clinically asymptomatic and serum transaminases levels are normal[1-3]. In most of the patients, HBsAg is detected incidentally or as part of the screening of blood donors and relatives of HBsAg positive individuals. Although the natural course of hepatitis B virus (HBV) infection in adults has been reported from various parts of the world[3, 4], there has been very little data recorded on the natural course of HBV infection in asymptomatic children, particularly in those with normal liver tests. Most of the reports have been based on observations

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of long-term HBsAg carriers with elevated liver enzymes and with chronic liver disease[5-9], whereas the infection in most children is asymptomatic and with normal liver enzyme concentrations[1-2, 10-11].

In order determine the natural course and the frequency of significant liver disease in HBsAg carrier children with normal liver biochemical tests, we followed a series of such patients.

Material and Methods

The patients were enrolled into the study either incidentally, by screening of relatives of HBsAg carriers or by following children with acute hepatitis B infection. HBsAg carrier state was defined in children recruited by screening, as any individual who had two HBsAg positive serum samples obtained six months apart. Only patients with normal tansaminases levels during this period were enrolled into the study; those with abnormal alanine aminotransferase (ALT) values (> 40 IU/L) during the first six months of follow-up were excluded. Patients with acute hepatitis B infection were enrolled into the study after their transaminases levels returned to normal and remained as such for six months. Liver biopsies were performed when fluctuations of transaminases were seen after the first six month period.

Patients were seen regularly at least every six months. Each follow-up visit included both a physical examination and biochemical tests including ALT, bilirubin, albumin and alkaline phosphatase levels. Routine liver functions were studied by sequential multiple autoanalyzer (Hitachi 911 Automatic Analyzer), at 37 °C, with standards supplied by the manufacturer. Markers of HBV, including HBsAg, hepatitis B e antigen (HBeAg), and antibodies against HBsAg (anti-HBs) and HBeAg (anti-HBe), were also determined at each visit by commercially available radioimmunoassay kits. All patients underwent abdominal ultrasonographic examination at least on one occasion. Hepatitis B virus DNA determination was not possible during the follow-up period. Available parents and siblings were also evaluated for HBV markers.

Differences in the proportions were tested with chi-square or Fisher's exact test. Differences between means were tested with Student's t test or Mann-Whitney U test when appropriate[12]. A p value of less than 0.05 was considered as significant. Data were given as mean ± SD.

Results

There were 124 patients (81 males, 65.3%; 102 recruited by screening, 82.2%) who ranged in age from nine months to 17 years (mean, 88.1 ± 45.7 months) at the time of enrollment. They were followed for six months to 144 months (mean,

36.8 ± 22.8 months, 380 patient years). Twenty of them were followed for more than 60 months. The mean age of the patients at the end of follow-up was 124.2 ± 46.6 months (range 18 to 231 months).

One hundred and fifteen patients were tested for HBeAg and anti-HBe. Sixty-one of them (53%) were found to be positive for e antigen and 51 (44%) carried anti-e. The remaining three (2.7%) were negative for both e and anti-e. There was no difference in the prevalence of HBeAg for males and females, 51.3% and 56.4%, respectively (p = 0.6, chi-square test). There was no statistically significant difference in the mean age between HBeAg positive and negative children (89.4 ± 44.1 months vs. 93.7 ± 45.2 months, p = 0.27, Student's t test).

Thirty-one of 66 mothers (46.9%) tested were HBsAg positive. This ratio was 76.7 percent (42/55) and 27.2 percent (15/55) respectively, for siblings and fathers tested. At least one family member was HBsAg positive in 60 out of 84 (71.4%) families tested. The percentages of HBeAg positivity in patients were not different between children whose family members were HBsAg positive and those whose family members were HBsAg negative (52.5% vs. 52.2%, p = 0.98, chi-square test). Table I shows the baseline clinical, serological and epidemiological features of the patients.

Table I: Baseline Clinical, Serological and Epidemiological Features of the Patients (n = 124)

	n (%)
Sex	
Male	81 (65.3)
Female	43 (34.7)
Age (mo)	
at the beginning, range (mean ± SD)	9-204 (88.1 ± 45.7)
at the end, range (mean ± SD)	18-231 (124.2 ± 46.6)
of HBeAg (+) patients (n = 61) (mean ± SD)*	89.4 ± 44.1
of HBeAg (-) patients (n = 54) (mean ± SD)*	93.7 ± 15.2
Duration of follow up	
Range (mo), (mean ± SD)	6-144 (36.8 ± 22.8)
HBeAg/anti-HBe status (n = 115)	
HBeAg (+)†	61 (53.0)
male	39/76 (51.3)
female	22/39 (56.4)
Anti-HBe (+)‡	51 (44.3)
male	35/76 (46.0)
female	16/39 (41.0)
Neither	3 (2.7)
Household HBsAg status (positive/number of studied persons)	
Maternal HBsAg positivity	31/66 (46.9)
Paternal HBsAg positivity	15/55 (27.2)
Sibling HBsAg positivity	42/55 (76.7)
At least one positive family member	60/84 (71.4)

* p = 0.27 † p = 0.6 ‡ p = 0.75

Only 11 of 61 patients (18%) who were HBeAg positive cleared HBeAg from their sera during the study period and all of them had seroconversion to anti-HBe. Since they were followed for a mean of 37 months, the mean annual seroconversion rate was 5.8 percent. The clearance of HBeAg occurred between eight and 60 months (median 12 months). There was no difference between males and females with regard to seroconversion, 17.9 and 9.1 percent, respectively ($p > 0.05$, Fisher's exact test). Although the difference was statistically insignificant, the patients clearing HBeAg were older than those who did not, 97.1 ± 48.2 months vs. 86.9 ± 43.1 months ($p = 0.5$, Mann-Whitney U test). The presence of another HBsAg positive person in the family also did not affect HBeAg clearance rate.

Twelve of 124 patients (9.7%) cleared HBsAg. The mean annual seroconversion rate was 3.1 percent. Clearance was seen more frequently among females than males although it was not statistically significant (13.9% vs. 7.4%, $p = 0.34$, Fisher's exact test). There was no difference in the mean age between the patients who cleared HBsAg and those who did not, 90.8 ± 36.5 months and 90.3 ± 45.5, respectively ($p = 0.97$, Mann-Whitney U test). None of the children (0/60) with a HBsAg positive person in the family seroconverted to anti-HBs, whereas 16.7 percent (4/24) of the patients whose family members were free of HBsAg ($p = 0.005$, Fisher's exact test) seroconverted to anti-HBs. Ten of 12 patients who cleared HBsAg were anti-HBe positive on enrollment. Only two of 11 patients who cleared HBeAg also cleared HBsAg.

During the follow-up, seven individuals (5 HBeAg positive and 2 anti-HBe positive) developed an abnormal ALT on at least one occasion after the first six months of follow-up. None of these patients had antibodies against hepatitis delta or C viruses. Three of five HBeAg positive children cleared HBeAg after ALT elevation. None of the two anti-HBe positive children converted to HBeAg positive state during or after the elevation of ALT. Only five of these patients had unexplained fluctuating ALT concentrations (less than three times normal) on repeat analysis. Liver biopsy was performed on these patients and all biopsies had nonspecific changes, including fatty changes, focal necrosis of hepatocytes, and ground glass hepatocytes.

All the children had normal serum albumin and bilirubin levels. None of the 20 patients who were followed for more than five years had clinical or biochemical indices of chronic hepatitis and/or cirrhosis or had hepatocellular carcinoma.

Discussion

HBsAg positivity indicates that the individual's liver is infected with the HBV. Infection in childhood increases the likelihood of becoming a chronic carrier[13]. In Turkey, the prevalence of HBsAg positivity in children is 5.4 percent, and carriers of HBsAg are usually infected during early childhood[14]. The expenses of treatment and the risks of biopsy in such a large population also require consideration.

The prevalence of HBeAg among HBsAg carriers ranges from zero[15] to > 90 percent[16] and it decreases as the ages of the patients increase[1,7,17]. This prevalence has been reported as higher than 80 percent in studies with children[2,7,8,11]. In our study, however, the prevalence of HBeAg positive children was only 53 percent, lower than the prevalences reported in the above studies. Age did not affect the prevalence of HBeAg positivity in our study, in contrast to some studies[1,7,18]. Similar to other studies[1,18], we could not show any significant effect of sex on the prevalence of HBeAg positivity. The annual clearance rate of HBeAg from HBsAg carrier children ranged from 6.2[17] to 16 percent[19]. Although some factors have been reported to affect the clearance rate of HBeAg in HBsAg carrier children, this has not been confirmed in all studies. Female sex[18], younger age[11,17,18], source of recruitment (whether recruited because the parents seek medical care for their children or by screening)[17], and positive maternal HBsAg status[17,20] have been reported as factors increasing HBeAg clearance rate. In our study, although the patients clearing HBeAg were older than those who did not, the difference was not significant. The presence of a person positive for HBsAg in the family and female sex also did not affect the HBeAg clearance. Similar to our results, sex[1,11,17] and age[1] did not affect the HBeAg clearance rate in some studies. During seroconversion, an increase in ALT activity was observed in most of the children[2,8]. In our study, three of five HBeAg positive children had elevated ALT levels before clearing HBeAg.

The annual clearance rate of HBsAg from carrier children has ranged from zero[1,2] to 1.5 percent[11]. Hsu et al.[19] found that the HBsAg clearance rate was significantly higher in children who had anti-HBe, children who were at an older age on entry, children whose mothers were HBsAg negative, and in children with severe histological changes of the liver detected while they were HBeAg positive. In our study, only children whose family members were HBsAg negative seroconverted to anti-HBs more frequently than those whose family members were HBsAg positive. There was no difference in the mean age and gender between the patients who cleared HBsAg and those who did not. Only two of 11 patients who cleared HBeAg also cleared HBsAg, the remaining 10 children clearing HBsAg were anti-HBe positive on entry. This is also in accordance with Hsu et al.'s[19] study. In their study, the annual clearance rate for HBsAg was 0.6 percent after a follow-up of 4.3 years. This rate was 1.7 percent in children with anti-HBe on entry. This study supports our findings that chronic HBV carrier children rarely lose HBsAg. In our study, the overall annual clearance rate of HBsAg was 3.2 percent and it was 5.7 percent in patients who were anti-HBe positive on entry. The discrepancies in annual HBeAg and HBsAg clearance rates may result from differences in ethnic origin, sources of recruitment, age distribution, or severity of the disease in the liver. In this study, we also observed a familial clustering of HBsAg carrier status that indicates the importance of

horizontal transmission. The prevalence of the presence of another HBsAg carrier in the family was 71.4 percent. This is in accordance with our previous study[21] and other studies in different countries[7, 17].

Long-term follow-up results of HBsAg positive children are controversial. Some reports have indicated occurrence of liver cirrhosis[10, 22, 23] or primary hepatocellular carcinoma[18] in HBV carrier children, whereas in some studies[5, 6] spontaneous complete remission in children with chronic active hepatitis has been reported. Most of these studies are related to children with abnormal transaminases and chronic hepatitis and/or cirrhosis. We could not find any studies with children that included only carrier children with normal liver tests. Koretz et al.[4] followed 54 HBsAg carrier adults with normal liver tests for four to 48 months with monthly liver tests. During follow up, only four patients (7.4%) (all HBeAg negative) developed persisting abnormalities in liver tests. Of the 23 patients who underwent percutaneous liver biopsies, chronic persistent hepatitis histology was found in only three patients. Chronic active hepatitis and/or cirrhosis was not seen in any biopsies. The risk of developing low grade transaminases abnormalities over a three-year period was 16 percent. It was 5.6 percent in our study. Koretz et al.'s[4] literature review showed that the frequency of finding "significant" liver disease (chronic active hepatitis, cirrhosis) in patients with normal transaminases levels was 5/237 (2.1%) compared to 19 percent with abnormal tests. They concluded that HBsAg carriers should be followed with serial liver tests and that those whose tests remain normal should not be considered for liver biopsy. In contrast, Sakuma et al.[3] followed 202 adult carriers for five years. Eighty-three of the carriers had normal liver tests initially and 94.3 percent of the 83 had normal tests continuously. They also found that mortality in carriers with normal liver tests was 44.5 times higher than that in noncarriers with normal tests. They concluded that routine liver function tests appear of limited value in predicting prognosis. There is weak correlation between abnormal tests and subsequent deaths in five years among carriers.

Clinical and biochemical features suggest that the majority of Turkish HBsAg carrier children with normal liver tests have stable liver disease. It is also clear that in Turkish HBsAg carrier children there is little clinical morbidity during childhood. Although this does not imply that chronic HBV infection is always a benign disease in children[18, 34], chronic HBV carriers can be followed with serial ALT levels alone. The incidence of significant liver disease in carrier children with normal transaminases levels is too low to justify the risk and expense of liver biopsy. We agree with those authors who conclude that a biopsy is not warranted in asymptomatic carriers[4, 25].

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DETECTION OF EARLY ANTHRACYCLINE – INDUCED CARDIOTOXICITY IN CHILDHOOD CANCER WITH DOBUTAMINE STRESS ECHOCARDIOGRAPHY*

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SUMMARY: Lenk MK, Zeybek C, Okutan V, Özcan O, Gökçay E. (Department of Pediatrics, Gülhane Military Medical Academy, Ankara, Turkey). Detection of early anthracycline-induced cardiotoxicity in childhood cancer with dobutamine stress echocardiography. Turk J Pediatr 1998; 40: 373-383.

Asymptomatic long-term survivors of childhood cancer treated with anthracyclines may have latent cardiac dysfunction which is undetected by commonly used echocardiographic methods. A more sensitive echocardiographic screening test, dobutamine stress echocardiography, was performed on 22 patients (mean age 9.10 ± 3.79 years) treated with 75 to 450 mg/m^2 of anthracyclines (mean 210.45 ± 127.34) and results were compared with 22 healthy age-matched control subjects. Echocardiographic Doppler studies were performed after each dobutamine infusion of 0.5, 2.5, 5 and 10 µg/kg/min.

Although left ventricular mass was decreased and end-systolic wall stress increased in the patient group when compared with the control subjects ($p < 0.01$ and $p < 0.05$, respectively), no differences were found between shortening fraction and ejection force in control subjects and patients, at rest and during each dobutamine infusion. A decreased mitral E/A ratio (ratio of early-to-late peak filling velocity) was demonstrated in anthracycline-treated patients only during dobutamine infusion ($p < 0.01$).

Our data showed left ventricular diastolic dysfunction during inotropic stimulation with dobutamine, and suggest that dobutamine stress echocardiography is a useful technique for evaluating the cardiac status of anthracycline-treated patients on a long-term basis.
Key words: dobutamine stress echocardiography, anthracyclines, cardiotoxicity, childhood cancer.

The anthracycline antibiotics (doxorubicin and daunorubicin) are known to be effective cancer chemotherapeutic agents, but their high incidence of cardiac toxicities limits their effective usage. These side effects can occur acutely, during or shortly after treatment; subacutely, within days or weeks of treatment; chronically, manifesting weeks or months after drug administration, usually as a cumulative dose response; or as late sequelae, many years after termination of therapy[1-4]. To date, several noninvasive methods, including radionuclide

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scintigraphic monitoring and echocardiographic determinants of systolic and diastolic function[1-6], and invasive methods, such as endomyocardial biopsy[7], have been used to detect these cardiac toxicities. Dobutamine stress echocardiography was reported to be a useful noninvasive technique as it unmasks the echocardiographic abnormalities that are not detected by rest echocardiography in asymptomatic doxorubicin-treated patients[8].

In this study we report the effects of anthracycline antibiotics on systolic and diastolic cardiac function in 22 patients using dobutamine stress echocardiography.

Material and Methods

Study Subjects: The study group consisted of children previously treated with anthracyclines (daunorubicin and doxorubicin) in the Pediatric Hematology and Oncology Unit, Gülhane Military Medical Academy, for childhood cancers. Control subjects were healthy age-matched volunteers. Excluded from the study were patients with cardiac failure or arrhythmias and those taking cardiac drugs or chemotherapeutic agents other than anthracyclines known to be associated with chronic or late cardiotoxic effects. Written informed consent was obtained from parents before the study. During the study, heart rate and rhythm were monitored with simultaneous electrocardiogram; blood pressure was measured every five minutes by sphygmomanometry. Age, weight, height and body surface area were obtained from every subject before the study.

Echocardiographic Studies: Echocardiograms were recorded using Hewlett-Packard Sonos 2500 Color Doppler ultrasound system with 3.5 and 5 Mhz transducers. Echocardiographic measurements were based on measurement of three consecutive cardiac cycles (averaged) in each subject. Recordings were obtained at the rate of 100 mm/s. A baseline two-dimensional echocardiographic study was performed to rule out structural heart disease. M-mode echocardiograms of the left ventricle from the parasternal long axis were recorded as described previously[9]. Apical four-chamber views that provided good visibility of the mitral annulus and leaflets were obtained by placing subjects in the left lateral decubitus position. Doppler examination of mitral flow velocity was performed with pulsed Doppler technique, with the sample volume positioned at the level of the tips of the mitral valve leaflets to record maximum transvalvar velocities[10]. Doppler flow velocities in the ascending aorta and aortic diameter were obtained from the suprasternal notch to assess systolic ventricular function, using the indices of peak velocity (PkV), acceleration time (AT), ejection time (ET), pre-ejection period (PEP), and velocity time integral (VTI)[11].

Dobutamine Infusion: After baseline values were obtained by resting echocardiography, continuous dobutamine infusion was initiated at 0.5 μg/kg/min and increased to 2.5, 5 and 10 μg/kg/min. To attain sufficient dobutamine

concentration, dobutamine was infused for 15 minutes at each rate before the echocardiographic study[12], and the echocardiogram was performed after the 15th minute, within five minutes. The infusion rate of dobutamine was increased after the completion of the echocardiographic study at each dobutamine dose.

Echocardiographic Measurements: Left ventricular cavity and thickness of interventricular septum and posterior free wall at end-diastole (LVIDd, IVSd, LVPWd) and end-systole (LVIDs, IVSs, LVPWs) were measured in cm according to standards recommended by the American Society of Echocardiography[13]. Left ventricular shortening fraction (FS) was evaluated according to the method of Gutgesell et al.[14] with the equation: FS (%) = LVIDd-LVIDs/LVIDd x 100.

Left ventricular volume at end-diastole (LVEDV) and end-systole (LVESV) were calculated according to the Teichholz method[15] with the equation: $V = (7.0/2.4 + D)(D)^3$.

The ejection fraction (EF) was calculated as the FS by substituting volume-derived data for the pure dimension data using the formula: EF (%) = LVEDV-LVESV/LVEDV x 100.

Left ventricular wall mass (LVWM) was calculated by M-mode echocardiography using the formula described by Devereux et al.[16] and indexed by body surface area[17]: $LVWM = 0.80\ [1.04\ (IVSd + LVPWd + LVIDd)^3-(LVIDd)^3]+0.6$.

Left ventricular posterior wall percent thickening (%LPWT) was calculated by the equation[18]: %LVPWT = (LVPWs-LVPWd)/LVPWd x 100.

Left ventricular end-systolic meridional wall stress (LVESS) was calculated in g/cm^2 according to the method of Grossman et al.[19] by the equation: LVESS = [(1.35)(MBP)(LVIDs)]/[(4)(LVPWs)(1+LVPWs/LVIDs)]. In this equation, 1.35 is the factor to convert pressure to g/cm^2 and MBP (mean blood pressure), as an estimate of end-systolic pressure in mm Hg, was calculated by the equation, MBP = (2 Diastolic BP + Systolic BP)/3.

Left ventricular ejection force (F), expressed in kilodynes, was calculated from aortic velocity curves described by Isaaz et al.[20] using the principles expounded in Newton's second law of motion (force = mass x acceleration). The accelerated blood mass that passed through the aortic cross-section was calculated in grams as M = p x CSA x VTI and mean acceleration in cm/s^2 as Mac = PkV/AT, where M was the mass of blood, p the mass density of blood (1.06 g/cm^3), CSA the cross-sectional aortic area [$\pi/4$ x (aortic annulus diameter)^2], VTI the velocity time integral, PkV the peak velocity during acceleration and AT the acceleration time.

Diastolic Function: For the determination of left ventricular filling dynamics, without making specific correction for the respiratory phase, only maximal velocities were measured in all patilents, which in the steady state should negate such a periodic variable. The following parameters were obtained from the spectral display:

Peak early filling velocity (peak E) and peak late (atrial) filling velocity (peak A) were measured according to the method of Nishimura et al.[21], from which a peak E/A ratio was derived. Mitral acceleration time (AT) was measured from the onset of diastolic flow to peak E, and deceleration time (DT) from peak E to the point where the deceleration velocity crossed baseline. Left ventricular isovolumetric relaxation time (IVRT) was measured from the closure of the aortic valve determined from phonocardiogram to the first upstroke of diastolic mitral flow velocity.

Statistical Analysis : All data are reported as mean values ± SD. Data for the study group are compared with those for the control group by using Student's t test.

Results

Patient data are summarized in Tables I and II. The study group was composed

Table I: Patient Data

Patient No.	Age (Year)	Sex	BSA (m^2)	Diagnosis
1	6.5	F	0.81	ALL
2	15.7	M	1.61	ALL
3	8.5	M	1.09	ALL
4	4.2	F	0.7	ALL
5	5.5	F	0.83	ALL
6	11.08	M	1.38	ALL
7	9.8	M	1.12	ALL
8	11.25	F	1.15	Hodgkin's lymphoma
9	15	M	1.31	Hodgkin's lymphoma
10	9.5	M	1.04	ALL
11	12	F	0.98	Leiomyosarcoma
12	3.08	M	0.7	Neuroblastoma
13	11.1	M	1.18	ALL
14	12.5	M	1.54	Hodgkin's lymphoma
15	10	F	1.16	ALL
16	5.5	M	0.79	Ewing's sarcoma
17	3.6	F	0.62	ALL
18	10.1	F	0.98	ALL
19	6.5	F	0.79	ALL
20	3.5	F	0.66	Wilms's tumor
21	13.5	F	1.1	Osteosarcoma
22	12	M	1.28	Osteosarcoma
Mean ± SD	9.10 ± 3.79	1.03 ± 0.28		

ALL: acute lymphoblastic leukemia, BSA: body surface area, F: female, M: male.

Table II: Patient Data (Continued)

Pt No.	Anthracyclines (Total Dose mg/m²)			Age During Rx (Year)	Duration of Rx (Year)	Time Since Rx (Year)
	Doxorubicin	Daunorubicin	Total			
1	–	75	75	2	1.83	2.67
2	75	–	75	12	2.5	1.2
3	–	75	75	6	1.66	0.84
4	–	75	75	1.5	2.58	0.12
5	75	–	75	2	2.66	0.84
6	90	–	90	2	1.08	8
7	90	–	90	4	2.91	2.89
8	160	–	160	8	0.08	3.17
9	160	–	160	11	1.08	2.92
10	–	170	170	4	3.66	1.84
11	180	–	180	10	0.16	1.84
12	–	180	180	2	0.5	0.58
13	120	120	240	10	0.5	0.6
14	240	–	240	9	0.41	3.09
15	120	120	240	9	0.5	0.5
16	240	–	240	3	0.83	1.67
16	240	–	240	3	0.83	1.67
17	120	120	240	2	0.58	1.02
18	90	200	290	3	7.08	0.02
19	295	120	415	2	2.25	2.25
20	420	–	420	2	0.75	0.75
21	450	–	450	11	0.83	1.67
22	450	–	450	11	0.75	0.25

Pt: patient, Rx: therapy.

of 22 patients (11 boys, 11 girls) aged 3.08 to 15.7 years (mean 9.10 ± 3.79, median 9.9), who had been previously treated with anthracycline group antibiotics. Seventeen patients received doxorubicin therapy at a mean cumulative dose of 198.52 ± 131.32 mg/m² (range 75 to 450) and 10 patients daunorubicin at a mean cumulative dose of 125.5 ± 45.05 mg/m² (range 75 to 200). Five patients received both doxorubicin and daunorubicin. The total mean cumulative dose of anthracyclines was 210.45 ± 127.34 mg/m² (range 75 to 450, median 180). The time elapsed since the last dose of therapy ranged from 0.02 to eight years (mean 1.76 ± 1.72, median 1.43). The control group consisted of 22 healthy subjects (13 boys, 9 girls) aged four to 16 years (mean 9.13 ± 3.88). Mean weight was 30.75 ± 11.91 kg for the study group and 31.31 ± 13.43 kg for the control group. Mean body surface area was 1.03 ± 0.28 m² for the study group and 1.05 ± 31 m² for the control group. Age,

weight and body surface area were not significantly different between the patient and control groups. No arrhythmia or symptoms were observed with the increasing infusion rates of dobutamine in any study or control subject.

The mean difference in left ventricular posterior wall end-systolic and end-diastolic dimensions between anthracycline-treated patients and control subjects was only 0.2 and 0.03 cm, respectively, at rest and during each dobutamine infusion (Table III). Though individual changes in percent left ventricular posterior wall thickening from baseline to lower doses of dobutamine infusion (0.5 and 2.5 µg/kg/min) were decreased in patient group when compared with changes in control subjects, the difference in the mean percent left ventricular posterior wall thickening between the two groups was not significant ($p > 0.05$) (Table IV). However, a significant reduction in left ventricular myocardial muscle mass was seen in the patient group ($p < 0.01$) (Table III). The mean left ventricular shortening fraction and ejection force were not different between the patient and control groups at rest and at each dobutamine infusion. Mean end-systolic wall stress was significantly higher at rest and during each dobutamine infusion in the patient group when compared with mean values in the control group ($p < 0.05$) (Table IV). Early (mitral E) and late (mitral A) peak filling velocities in the anthracycline-treated subjects were not different from that in control subjects at rest but demonstrated significantly greater amplitudes compared with those of control subjects during each dobutamine infusion ($p < 0.05$ and $p < 0.01$, respectively) (Table V). The mean mitral E to mitral A ratio was significantly different between the study and control groups only during dobutamine infusion ($p < 0.01$). Although left ventricular isovolumetric relaxation time did not differ significantly between the study and control subjects at rest and at 0.5 µg/kg/min infusion rate of dobutamine, it was significantly decreased in the patient group at 2.5, 5 and 10 µg/kg/min infusion rates of dobutamine ($p < 0.05$).

Table III: Left Ventricular Dimensions and Wall Mass

Dobutamine Dose	LVIDd (cm)	LVPWd (cm)	LVIDs (cm)	LVPWs (cm)	LVM (g)	LVMI (g/m²)
Baseline						
Patient	3.85 ± 0.54	0.47 ± 0.20	2.44 ± 0.44	0.88 ± 0.20	50.20 ± 29.05*	46.34 ± 16.53
Control	4.26 ± 0.38	0.50 ± 0.09	2.21 ± 0.11	1.08 ± 0.12	79.60 ± 23.34	76.53 ± 6.41
0.5 µg/kg/min						
Patient	3.84 ± 0.58	0.46 ± 0.18	2.39 ± 0.47	0.91 ± 0.17	49.44 ± 26.18*	46.26 ± 15.08
Control	4.28 ± 0.31	0.51 ± 0.08	2.52 ± 0.16	1.11 ± 0.10	80.54 ± 20.90	78.22 ± 7.33
2.5 µg/kg/min						
Patient	3.88 ± 0.56	0.48 ± 0.20	2.33 ± 0.55	0.88 ± 0.26	48.55 ± 26.95*	45.22 ± 16.74
Control	4.2 ± 0.29	0.56 ± 0.09	2.49 ± 0.18	1.15 ± 0.09	81.62 ± 20.27	79.52 ± 6.98
5 µg/kg/min						
Patient	3.92 ± 0.51	0.48 ± 0.28	2.23 ± 0.45	0.94 ± 0.22	50.38 ± 32.52*	45.90 ± 16.53
Control	4.11 ± 0.35	0.54 ± 0.08	2.45 ± 0.16	1.21 ± 0.06	77.81 ± 20.67	75.63 ± 8.49
10 µg/kg/min						
Patient	3.92 ± 0.58	0.51 ± 0.25	2.26 ± 0.50	0.99 ± 0.23	51.32 ± 27.75*	47.72 ± 14.22
Control	4.33 ± 0.31	0.58 ± 0.08	2.27 ± 0.15	1.26 ± 0.06	87.24 ± 20.67	85.23 ± 7.86

LVIDd: left ventricular end-diastolic dimension, LVPWd: left ventricular end-diastolic posterior wall thickness, LVIDs: left ventricular end-systolic dimension, LVPWs: left ventricular end-systolic posterior wall thickness, LVM: left ventricular mass, LVMI: left ventricular mass index. *$p < 0.01$ relative to values in control subjects.

Table IV: Systolic Function

Dobutamine Dose	EF (%)	FS (%)	% LVPWT	LVESS (g/cm²)	Ejection Force (Kilodynes)
Baseline					
Patient	66.95 ± 7.12	36.68 ± 5.43	101.22 ± 68.96	58.18 ± 20.55*	24.65 ± 13.65
Control	79.37 ± 3.82	37.42 ± 0.42	121.79 ± 47.40	37.69 ± 7.81	24.98 ± 13.45
0.5 µg/kg/min					
Patient	67.86 ± 8.93	35.76 ± 7.53	110.00 ± 46.73	52.68 ± 17.63*	31.19 ± 19.76
Control	71.67 ± 4.95	36.89 ± 3.47	122.87 ± 42.75	42.96 ± 8.52	31.01 ± 19.25
2.5 µg/kg/min					
Patient	70.65 ± 10.2	34.07 ± 7.94	92.99 ± 49.70	58.63 ± 35.93*	33.09 ± 16.06
Control	71.33 ± 5.39	36.04 ± 3.24	108.30 ± 37.27	40.18 ± 6.11	33.98 ± 18.55
5 µg/kg/min					
Patient	74.63 ± 6.49	33.19 ± 5.57	126.69 ± 87.26	47.78 ± 15.99*	42.29 ± 19.86
Control	71.12 ± 5.15	35.21 ± 0.38	125.52 ± 35.57	36.82 ± 5.90	44.86 ± 20.93
10 µg/kg/min					
Patient	73.61 ± 9.03	36.53 ± 7.41	119.84 ± 90.18	47.68 ± 23.39*	43.04 ± 20.38
Control	78.78 ± 3.88	38.07 ± 3.40	121.38 ± 34.38	31.73 ± 5.87	44.59 ± 20.83

EF: ejection fraction, FS: shortening fraction, %LPWT: percent left ventricular posterior wall thickening, LVESS: left ventricular end-systolic wall stress. * $p < 0.05$ relative to values in control subjects.

Table V: Diastolic Function

Dobutamine Dose	Peak E (cm/s)	Peak A (cm/s)	E/A ratio	IVRT (ms)	DT (ms)
Baseline					
Patient	84.79 ± 12.35	45.82 ± 4.27	1.85 ± 0.25	49.31 ± 21.45	88.40 ± 24.70
Control	81.68 ± 9.95	43.50 ± 5.20	1.88 ± 0.15	50.81 ± 13.99	91.81 ± 23.65
0.5 µg/kg/min					
Patient	90.90 ± 13.15*	62.81 ± 14.29†	1.52 ± 0.44†	52.27 ± 24.23	92.50 ± 26.53
Control	82.36 ± 10.33	41.04 ± 5.26	2.00 ± 0.05	51.54 ± 13.95	95.81 ± 25.93
2.5 µg/kg/min					
Patient	94.10 ± 12.85*	65.73 ± 16.12†	1.50 ± 0.37†	39.75 ± 27.31*	99.31 ± 33.46
Control	82.81 ± 7.98	41.04 ± 3.99	2.01 ± 0.04	49.13 ± 9.98	102.36 ± 33.12
5 µg/kg/min					
Patient	97.20 ± 16.57*	64.40 ± 16.17†	1.58 ± 0.40†	30.90 ± 21.58*	92.04 ± 29.94
Control	84.90 ± 6.61	43.59 ± 5.03	1.96 ± 0.15	46.45 ± 9.73	99.09 ± 29.32
10 µg/kg/min					
Patient	100.37 ± 14.50*	62.75 ± 18.74†	1.71 ± 0.45†	23.00 ± 16.09*	99.09 ± 31.07
Control	91.31 ± 6.64	45.09 ± 3.32	2.02 ± 0.10	45.86 ± 13.70	102.81 ± 30.56

Peak E: peak early filling velocity, Peak A: peak late (atrial) filling velocity, E/A ratio: ratio of early-to-peak filling velocity, IVRT: isovolumetric relaxation time, DT: deceleration time. *$p < 0.05$ and †$p < 0.01$ relative to values in control subjects.

Discussion

The anthracyclines, doxorubicin and daunorubicin, are well-known cancer chemotherapeutic agents with their high incidence of cardiac toxicities[1,2]. The most prominent toxic effect occurs as a cardiomyopathy, resulting from chronic, cumulative, dose-related myocardial injury as demonstrated by abnormal endomyocardial biopsy findings[7]. Endomyocardial biopsy studies have demonstrated histologic changes in almost all patients who had received a total dose of ≥ 240 mg/m^2 of doxorubicin, and histologic damage was reported to be directly proportional to the total cumulative dose of doxorubicin between doses of 100 and 600 mg/m^2[2,22]. However, in accordance with the significant variability in the degree of morphologic changes, severe damage has been reported with cumulative doses as low as 45 mg/m^2. It has also been reported that cardiac

function is not impaired in proportion to the degree of histologic damage, indicating that a critical threshold of myocyte injury is necessary before cardiac function becomes abnormal[22].

The global indices of systolic left ventricular function with echocardiography derived either from M-mode measurements (the left ventricular ejection and shortening fraction), or from the aortic velocity curves. Show that ejection force generally decrease with higher levels of anthracycline doses. Bu'Lock et al.[4] reported that patilents with a shortening fraction < 30 percent at any stage, < 32 percent at > 200 mg/m^2, or those with a fall in shortening fraction of > 2-3 absolute percent per 100 mg/m^2 would have appeared to be at increased risk of significant cardiotoxicity. However, they are reported to be in the normal range unless cardiotoxicity is severe[23]. Although eight of our 22 study patients had a shortening fraction < 30 percent at > 200 mg/m^2, in general they were in normal levels at rest and during each dobutamine infusion. Left ventricular end-systolic wall stress, which better reflects the inotropic state of the myocardium by taking the loading conditions into account, increases in doxorubicin-treated patients[1]. We found this index increased in the study group, at rest and during dobutamine infusion, but the levels were not as high as seen in congestive heart failure secondary to cardiomyopathies[24].

Lipshultz et al.[1] hypothesized that in younger patients, cardiac afterload was more adversely affected by doxorubicin because more myocardial growth is required for young children to reach adult proportions. Furthermore, loss of cardiac myocytes during doxorubicin therapy in childhood might result in inadequate left ventricular mass and clinically important heart disease in later years. The results of our study were consistent with this work confirming the finding of reduced myocardial mass in relation to body surface area in our anthracycline-treated patients.

The reduction in muscle mass may be considered as a pattern of cardiac dysfunction. As a result of reduced muscle mass, left ventricular wall stress significantly increases. Individual changes in left ventricular end-systolic wall stress from baseline to 10 µg/kg/min dobutamine infusion were accompanied by a reduction in myocardial muscle mass in our study group. Our patients can be considered to be in a compensated state before the evident cardiac dysfunction.

Changes in left ventricular diastolic function with normal systolic indexes have been reported in anthracycline-treated patients and prolonged isovolumetric relaxation time and decreased mitral E/A ratio have been shown[5]. Our results of diastolic function demonstrated augmented early and late filling velocity during dobutamine infusion. The decreased early-to-late filling ratio during dobutamine infusion in anthracycline-treated patients when compared to the ratio in control subjects shows diastolic dysfunction in the patient group with dobutamine stress echocardiography.

Congestive heart failure, ventricular tachycardia and sudden death occurring more than five years after doxorubicin treatment have been reported[25]. Recent reports have focused on sex and the dosage of doxorubicin before limiting the cumulative dose of the agent. In children, girls especially were reported to have a greater susceptibility to anthracycline-induced cardiotoxicity[26]. More than 40 percent of patients with significant abnormalities of cardiac function ≥ 15 years after anthracycline treatment has been reported[27]. A more intensive evaluation has identified abnormal cardiac function in up to 74 percent of patients at a mean length of follow-up of only seven years[1] and with dobutamine stress echocardiography at a mean follow-up of only 6.1 ± 3.6 years[8].

Dobutamine is a short-acting $beta_1$ agonist with primarily inotropic action at low doses and seems an ideal drug for evaluating cardioselective responses to catecholamines. Dobutamine stress echocardiography is reported to be a new methodology used to identify early anthracycline toxicity[8]. With this technique, we found the diastolic function minimally affected. Systolic function was preserved in our study populatilon, and they had no clinical manifestations.

The authors could not comment on the suitability of this test for younger cancer patients in a previous study[8], as the youngest study subject was reported as nine years old and as 10 μg/kg/min infusion rate of dobutamine is associated with a high incidence (50%) of adverse effects. In our study, we applied dobutamine to children with a mean age of 9.1 ± 3.79 years, with the youngest being three years old, at 10 μg/kg/min dose for 15 minutes without observing any adverse effects.

Our investigation had an important limitation. As we do not have an extented follow-up time, we cannot derive clearly significant clinical implications from the echocardiographic findings. We believe that future evaluation of the clinical status of these patients is necessary.

Asymptomatic anthracycline-treated cancer patients may have cardiac dysfunction undetected by commonly used rest echocardiography. Left ventricular systolic function was preserved in our patient group. Mitral E, mitral A and E/A ratio were abnormal only during inotropic stimulation by dobutamine in our asymptomatic anthracycline-treated patients. Our data suggest that dobutamine stress echocardiography is a useful technique for monitoring the cardiac functions of these patients in the follow-up period.

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MAGNETIC RESONANCE IMAGING (MRI) FINDINGS IN ISOLATED GROWTH HORMONE DEFICIENCY*

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SUMMARY: Kandemir N, Cila A, Besim A, Yordam N. (Endocrinology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Magnetic resonance imaging (MRI) findings in isolated growth hormone deficiency. Turk J Pediatr 1998; 40: 385-392.

In this study the presence of pituitary-hypothalamic abnormalities was searched by magnetic resonance (MR) imaging in 30 children (18 males and 12 females, aged 7.4 to 23 years) with isolated growth hormone deficiency (IGHD). Small anterior pituitary was demonstrated in 18 patients and ectopic posterior pituitary (EPP) in four of them. Pituitary stalk was found to be thin in two patients with anterior pituitary hypophasia and EPP and was visible only in post-gadolinium images. In one patient, a hypothalamic mass was found and the bright spot of the posterior pituitary was not observed. In three other patients, the absent bright spot of the posterior pituitary was found without diabetes insipidus, possibly due to a variation in the intensity of the bright signal. Eight patients had normal pituitary imaging suggesting functional damage. In all five patients with familial growth hormone deficiency the anterior pituitary was hypoplastic.

We conclude that a high percentage of patients with IGHD had anomalies of the hypothalamo-pituitary region, which could be demonstrated by MR imaging. Furthermore, the low frequency of perinatal abnormalities in these patients suggested developmental defect as the cause of the morphostructural abnormalities. The presence of the familial cases with the same defect pointed to the genetic origin in some instances. *Key words: growth hormone deficiency, magnetic resonance imaging.*

Magnetic resonance imaging (MRI) offers the advantage of revealing the morphological and structural abnormalities of the hypothalamic-pituitary region in patients with idiopathic growth hormone deficiency (GHD).

The reported abnormalities comprise one of three patterns[1-4].

1. Anterior pituitary hypoplasia, absence of the pituitary stalk and ectopia of the posterior pituitary.
2. Isolated anterior pituitary hypoplasia.
3. Normal morphology of the pituitary gland.

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Anterior pituitary hypoplasia and ectopic posterior pituitary (EPP) with unidentified stalk have been found to be associated with multiple pituitary hormone deficiency, whereas normal and hypoplastic anterior pituitary have been shown in isolated growth hormone deficiency (IGHD)[1-4].

The etiology of the neuroradiological abnormalities found in idiopathic growth hormone deficiency is not clearly understood. Several explanations have been proposed[4-6].

1. Mechanical cut of the stalk during breech and foot delivery.
2. Hypoxia-ischemia during difficult delivery which results in interruption or occlusion of the hypophyseal portal system.
3. Dysgenesis or abnormal embryonic development of both adenohypophysis and neurohypophysis.

The aim of this study was to define the abnormalities of the hypothalamic-pituitary region in IGHD using MRI and to assess the relation between endocrine function and morphological findings. In addition to this, MRI findings were compared with perinatal histories.

Material and Methods

We evaluated 30 patients with IGHD (18 males and 12 females) aged from 7.4 to 23 years who were followed in the Pediatric Endocrinology Unit of Hacettepe University, Ankara. At the time of the evaluation, eight patients were prepubertal and the others reached puberty spontaneously.

The diagnosis of IGHD was based on the following criteria:

1. Short stature (height was below 2 SD for chronological age)[7].
2. Decreased growth velocity (below the 10th percentile for age) evaluated over a period of at least six months[8].
3. Delayed bone age, determined by the Greulich and Pyle method[9].
4. Growth hormone response of less than 10 ng/ml during insulin and L-DOPA stimulation tests.
5. Normal levels of the other pituitary hormones such as thyroxin (T_4), prolactin, cortisol, FSH and LH.

Height was measured by Harpenden stadiometer and converted to standard deviation scores (SDS) to allow comparison of data in children, using reference values at different ages[7]:

$$SDS = \frac{X-\bar{X}}{SD}$$

where X = measurement, $\bar{X}$ and SD = the mean and standard deviation, appropriate for age and sex.

Serum growth hormone (GH), triiodothyronine (T_3), tetraiodothyronine (T_4), TSH, free T_3, free T_4, cortisol, prolactin, FSH, LH and plasma ACTH levels were measured using commercial RIA kits. The diagnosis of isolated growth hormone deficiency was confirmed in all patients with normal levels of the other pituitary hormones.

Perinatal histories were reviewed for breech or cesarean section births and for neonatal asphyxia. A history of breech delivery was recorded in three patients and cesarean section in another three. All other patients were born by normal vaginal delivery at term without any perinatal trauma and/or asphyxia.

Children with an acquired GHD such as that resulting from craniopharyngioma, histiocytosis, a cerebral inflammatory disease, or hydrocephalus and those who received irradiation for tumors were excluded.

Magnetic resonance imaging studies were performed with a 0.5 T Unit (gyroscan T5 II; Philips, The Netherlands). Spin echo T1-weighted sagittal and 3D T1-weighted gradient echo coronal images were obtained in every patient and repeated after gadolinium-DTPA injection. Sella size was evaluated in sagittal plane. Height of the gland was measured on coronal images and the gland was considered hypoplastic if the maximum height was ≤ three mm. Bright spot of the posterior pituitary was located in both coronal and sagittal images. It was considered ectopic if located outside sella turcica. Since all of our patients had received Gd-DTPA injection, stalk continuity was determined in post-contrast images.

Results

The auxological data and MRI findings of 30 children at the time of the diagnosis are shown in Table I.

At the time of the diagnosis, the mean (SD) chronological age was 10.9 (2.8) years and the mean bone age was 7.7 (3.0) years. The height SDS for chronological age was below -2 in all patients and the mean value was -4.07 (1.09). The mean pretreatment growth velocity was 3.3 (0.6) cm/yr. The mean peak GH level was 2.9 (2.3) ng/ml during stimulation test with L-DOPA and 2.3 (2.0) ng/ml with insulin. At the time of the evaluation, the mean chronological age was 13.6 (3.1) years.

Of three patilents who had breech presentation at birth, one boy had pituitary hypoplasia, a thin stalk and EPP. The other two (a girl and boy) had normal pituitary imaging except in one of them the bright spot of the posterior pituitary was not observed. Of three patients born by cesaren section, one had pituitary hypoplasia and two had normal MR imaging.

In 12 patients from 10 families, consanguinity was present between parents (35%) and in five offspring of three families, a familial form of IGHD was diagnosed.

Table I: MR Imaging and Auxological Data of Patients with Growth Hormone Deficiency

Patient No.	Sex	CA (yr)	HSDS-CA	BA (yr)	GV (cm/yr)	Peak GH (ng/ml) L-DOPA	Insulin
Pituitary hypoplasia							
1	M	6.2	-3.26	3	4	2.4	1.7
2	F	10.3	-3.93	5	3	2.1	0.5
3	M	12.5	-5.88	7.5	3	1.1	1.3
4	F	8.7	-4.18	8	2.5	< 0.2	0.9
5	F	8.4	-4.20	6	2.6	1.7	0.7
6	M	15.9	-6.61	14.5	3.5	1.1	0.7
7	M	5.9	-5.76	5	3.5	0.05	0.86
8	M	4.4	-4.31	2.5	3.5	< 1.5	< 0.5
9	M	9.4	-3.75	6	3	6.9	2.7
10	F	7.4	-4.59	2	4	1.7	1.5
11	M	13	-4.53	10	2.8	1.8	1.6
12	F	11.7	-4.72	9	2.5	3.1	0.8
13	F	10.2	-2.60	8	3.5	4.4	2.0
14	M	11	-5.23	5.5	3	0.7	1.3
Pituitary hypoplasia with thin pituitary stalk and EPP							
15	F	8.6	-4.58	4	3.2	2.3	3.2
16	M	9.7	-5.54	4	2.6	2.1	2.1
Pituitary hypoplasia with thick pituitary stalk and EPP							
17	M	12	-2.91	11.5	4	7.2	4.4
Normal pituitary							
18	M	10	-3.14	7	4	7.2	1.8
19	M	13.4	-2.54	10.5	3.5	2.5	2.4
20	M	15.2	-4.26	11	4	1.2	1.7
21	F	12.2	-2.75	9	4	2.2	6.7
22	F	11.5	-3.13	9.5	3.5	6.2	3.4
23	M	13	-4.77	8	2.2	0.8	0.4
24	M	16	-3.80	11	3	3.9	4.2
25	F	12	-3.26	10	4	3.8	1.3
Normal pituitary with absent bright spot of the posterior pituitary							
26	M	15	-5.47	11	4	1.3	1.5
27	F	11	-2.50	8	4	2.4	1.4
EPP with normal anterior pituitary							
28	M	13.4	-2.99	10	4	7.9	5.9
Pituitary hypoplasia with absent bright spot of the posterior pituitary							
29	F	11	-3.51	9	3	1.6	9
Hypothalamic mass							
30	M	9.5	-3.43	6	2.2	7.2	3

The height of the anterior pituitary gland was less than three mm in 18 of 30 children (60%); five patients with a familial form of IGHD were in this group (Fig. 1). In the study group four patients had ectopic posterior pituitary (EPP). In three of them the anterior pituitary gland was hypoplastic and in one of them it was normal. The bright spot indicating posterior pituitary was observed in median eminence (Fig. 2) in two cases, in midstalk (Fig. 3) in one case and in the lower part of the stalk in the other. In the two patients with hypoplastic anterior pituitary and EPP, stalk was found to be thin and was visible only in post-gadolinium images (Fig. 2).

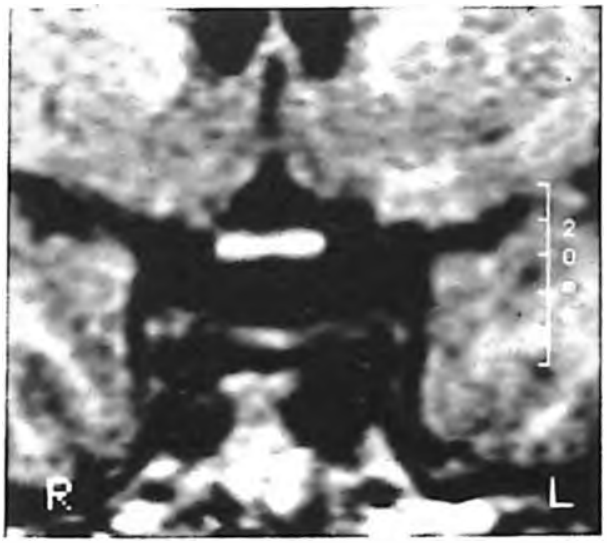


Fig. 1: 15-year-old male. Anterior pituitary gland height of two mm confirming gland hypoplasia.

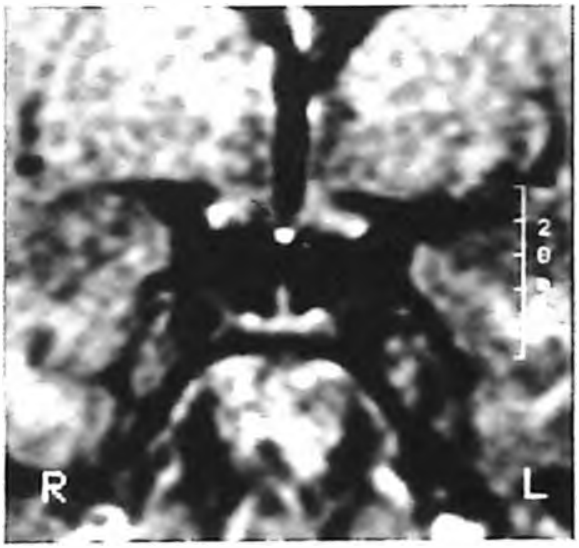
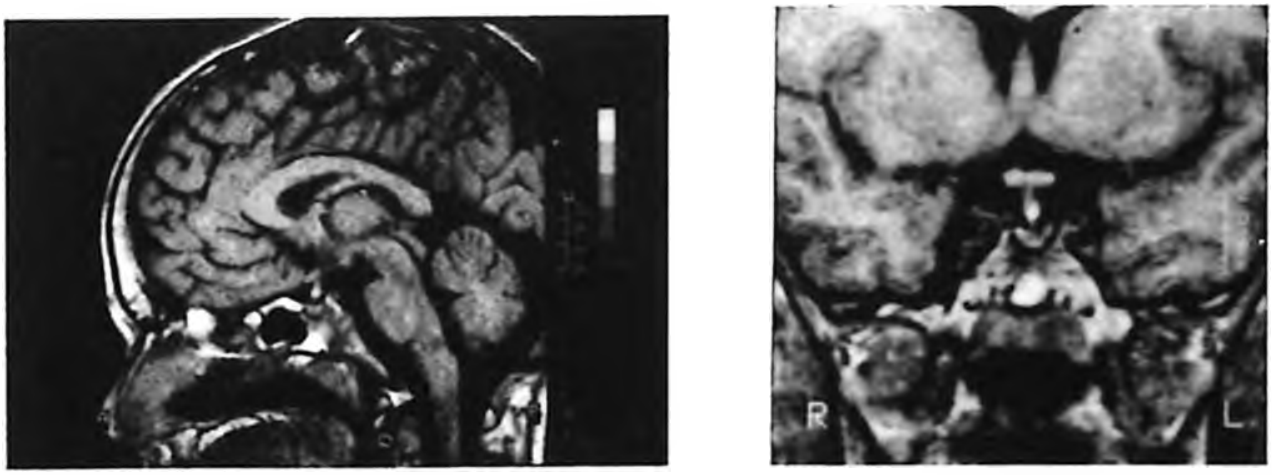


Fig. 2: 14-year-old male. Thickness of the pituitary was less than one mm. The stalk was very thin. Bright spot was located at the median eminence just below hypothalamus (arrow).



(a) (b)

Fig. 3: 13-year-old male. a) Sagittal. b) Coronal. Sella turcica was small and shallow in sagittal image. Thick stalk with bright spot of neurohypophyseal hormones located at the middle of the stalk can be seen in both planes.

In one patient, a hypothalamic mass was found and the bright spot of the posterior pituitary was not observed. In three other patients absent bright spot of the posterior pituitary was found, together with pituitary hypoplasia in one patient and with normal pituitary imaging in two. None of the eight patients with ectopic or absent bright signal of the posterior pituitary had polyuria or polydipsia. Diabetes insipidus was excluded in these patients by water deprivation test results.

Discussion

The most prominent reported MRI finding in IGHD is hypoplasia of the anterior pituitary gland without neuroradiological abnormalities[10, 11]. A small anterior lobe of the pituitary was seen in 60 percent of our cases. The cause of pituitary hypoplasia in growth hormone deficiency is not known. Huot et al.[12] hypothesized that since growth hormone secreting cells are the most abundant cell population in the pituitary gland, reduced pituitary volume can be an expected finding in GHD. However, in many studies no correlation was found between the pituitary size and the hormonal status[1, 13].

High incidence of breech and foot delivery has been reported in patients presenting with pituitary hypoplasia, EPP and stalk transection[1, 4, 14]. Traumatic transection of the stalk results in a newly formed small ectopic posterior lobe at the proximal stump of the transected stalk in both experimental animals and in patients[1-4]. However, the traumatic hypothesis cannot explain the findings in the relatively high percentage of patients with normal delivery[2, 3, 10]. For sample, in our study traumatic delivery history was found in 10 percent of all patients whereas 60 percent had pituitary hypoplasia. Another hypothesis is reduced hypothalamic stimulation or a diminished blood supply consequent to the absence of the hypothalamic and hypophyseal portal system. The association of a transected pituitary stalk with a hypoplastic pituitary gland supported this hypothesis. However, in this study and in others many patients had pituitary hypoplasia without stalk abnormalities. Marwaha et al.[10] reported a thin pituitary stalk with normal-sized pituitary in two patients and suggested that the size of the stalk does not indicate the height of the pituitary. Recently Maghnie et al.[15] showed a collateral arterial system which is easily recognized after gadopentetate dimeglumine (Gd-DTPA) administration both in IGHD or multiple pituitary hormone deficiency (MPHD). Therefore, decreased pituitary perfusion and ischemia are unlikely to cause pituitary dysfunction. In recent studies the association of GHD with congenital facial and central nervous system abnormalities suggested congenital dysembryogenetic anomaly is responsible for the abnormal development of the pituitary gland and stalk[2, 3, 6, 11, 16]. In one of our patients, midstalk localization of the posterior pituitary supported the possibility of defective neuronal migration during embryogenesis as a cause of EPP. In addition, familial occurrence of pituitary hypoplasia suggested a genetic origin.

Ultmann et al.[17] showed that nonvisibility of a stalk was associated with complete lack of anterior pituitary function, while visibility of the stalk was associated with preservation of some anterior pituitary function. In this study, we demonstrated the continuity of the stalk in all cases with IGHD.

A high percentage of normal magnetic resonance images was found in subjects with IGHD, suggesting purely functional damage[18]. Nonvisibility of the posterior lobe in four patients without diabetes insipidus was possibly related to the normal variation of the intensity of the bright signal, as reported previously in some healthy subjects[19, 20].

In conclusion, MR imaging demonstrated a high incidence of morpho-structural abnormalities of the hypothalamic-pituitary area in patients with IGHD. The lack of a significant increase in the frequency of traumatic or hypoxic deliveries in the children with these abnormalities suggested a developmental defect, which in some cases can have a genetic origin.

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PLAQUE RADIOTHERAPY IN THE MANAGEMENT OF RETINOBLASTOMA*

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SUMMARY: Kıratlı H, Bilgiç S, Atahan İL. (Departments of Ophthalmology and Radiation Oncology, Hacettepe University Faculty of Medicine, Ankara, Turkey). Plaque radiotherapy in the management of retinoblastoma. Turk J Pediatr 1998; 40: 393-397.

Retinoblastoma is the most common intraocular malignant tumor in childhood and is radiosensitive. We treated five patients with solitary intraocular retinoblastoma using iodine-125 plaque radiotherapy as a primary procedure. Tumor basal diameters ranged from six to 16 mm and thickness ranged from three to 11 mm. The radiation dose delivered to tumor apex ranged from 3500 to 5000 centigrays. All tumors regressed considerably within a follow-up ranging between seven to 12 months. There was no tumor recurrence or any complications related to plaque brachytherapy within this early period. We concluded that plaque radiotherapy is an effective method in local tumor control and allows eye preservation in patients with retinoblastoma. *Key words: retinoblastoma, brachytherapy, iodine-125.*

Retinoblastoma is the most common intraocular malignant tumor in childhood[1]. Current treatment options of retinoblastoma include external beam radiotherapy[2], enucleation[1], laser photocoagulation[3], cryotherapy[4] and plaque radiotherapy[5]. More recently, a combination of chemotherapy, cryotherapy and laser therapy has been introduced into clinical practice to avoid external beam radiotherapy[6]. Some important clinical variables that determine the specific treatment are patient age; unilateral versus bilateral disease; hereditary versus non-hereditary disease; size, number and location of the tumors; and potential for useful vision.

There is a growing emphasis on organ preservation in selecting appropriate treatment for patients with retinoblastoma, and episcleral plaque brachytherapy (EPB) is a serious consideration in an effort to salvage the eye. In this study, we evaluated our short-term experience in the management of retinoblastoma using iodine-125 radioactive plaque brachytherapy.

Material and Methods

Patients treated with solitary plaque radiotherapy for intraocular retinoblastoma at the Ocular Oncology Unit, Hacettepe University Hospital, were selected for this study. Patients were initially examined under general anesthesia and the

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tumor size (base and thickness) was estimated by indirect ophthalmoscopy and ultrasonography (A-and B-mode). Iodine-125 radioisotope was used exclusively because of its ease in individualizing plaque designs and radiation safety. All calculatilons were made on USC 1.50 radioactive plaque software. The plaque was surgically applied to the sclera over the tumor base and was removed after the calculated radiation dose was delivered usually after three to five days. All surgical procedures were carried out with the patient under general anesthesia.

The age of the patient at the time of treatment, the eye involved, the location of the tumor (inferior, superior, medial and lateral fundus), the thickness of the tumor in milimeters measured by B-mode ultrasonogram (Fig. 1), and the radiation dose at the apex of the tumor in centigray (cGy) were tabulated. The outcome of the treatment, in terms of tumor status and follow-up period, was recorded. The tumor was considered regressed if it was smaller than at the time of plaque placement. Data on the fellow eye and systemic metastases were also reviewed.

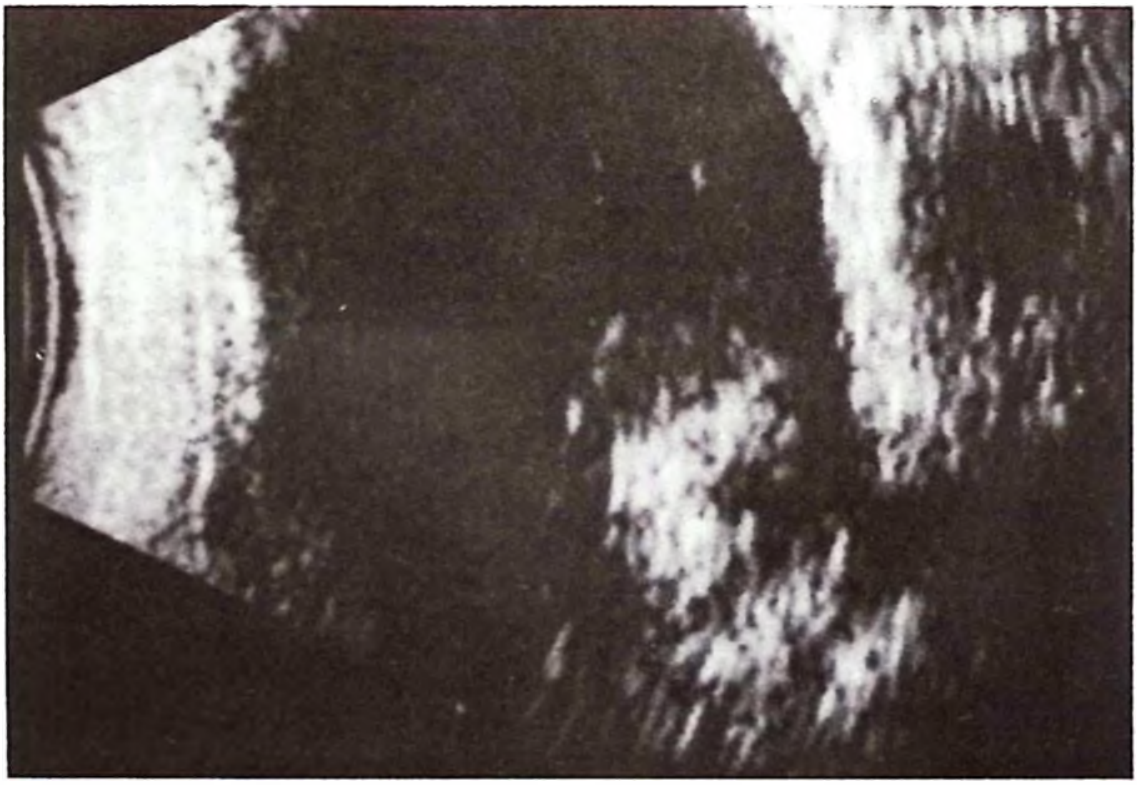

Fig. 1: Preoperative B scan ultrasonogram of the right eye of patient 4, showing the centrally calcified tumor with a thickness of 7.5 mm.

Results

Between January 1996 and January 1997, EPB was used in five patients (5 eyes) with retinoblastoma as the initial primary treatment. Table I summarizes the data on these five patients. All eyes that received EPB initially had solitary tumors

with maximum basal diameters ranging from six to 16 mm. All the tumors were at least five mm away from the fovea and/or optic disc and there were no vitreous seedings. The radiation dose at the tumor apex ranged from 3500 to 5000 cGy and at the tumor base from 12000 to 33000 cGy. Custom-made round plaques with 10 to 18 mm diameters were employed.

Table I: Data on Patients Treated with Episcleral Plaque Brachytherapy

Patient	Age (years)	Tumor Quadrant	Max. Basal Diameter (mm)	Thickness (mm)	Apical Dose (cGy)	Outcome	Follow-up (Months)	Fellow Eye
1	2	lateral	16	11	5000	regressed	7	normal
2	1	superior	6	3	3500	regressed	9	enucleated
3	2.5	inferior	8	4.5	4000	regressed	11	enucleated
4	1	inferior	9	7.5	5000	regressed	12	EBR
5	2	nasal	11	6	4500	regressed	11	enucleated

Max: maximum, cGy: centigray, EBR: external beam radiotherapy.

Patient follow-up ranged from seven to 12 months. The tumor regressed dramatically in all eyes within the first three to four weeks following EPB (Fig. 2). During the follow-up period there was no recurrence in the irradiated tumors. However, in three patients, new tumor foci were discovered in other quadrants and were immediately treated by cryotherapy. To date, there have been no acute or short-term complications related to EPB. Metastatic work-up was negative in all cases.

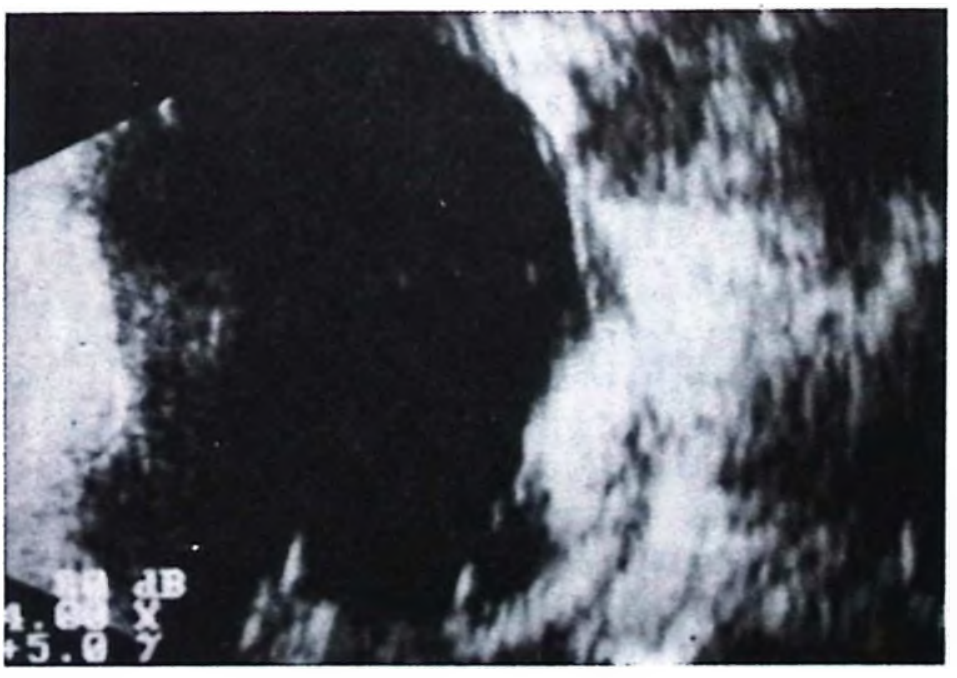

Fig. 2: 7 months after the radioactive plaque treatment, B scan ultrasonogram demonstrates that the tumor has shrunken to 2.5 mm in thickness with almost total calcification.

The fellow eye was affected in four patients. In three instances the eye had to be enucleated; one eye was managed by external beam radiotherapy. None of the four patients with bilateral disease had a family history of retinoblastoma.

Discussion

Despite the fact that enucleation is still an apropriate method for many cases with advanced or unilateral retinoblastoma, there is a definite trend toward more conservative treatments[7,8]. It has been observed that whether the patient was treated conservatively or enucleated, there was a five percent chance of metastatic disease developing; conservative modalities did not adversely affect the prognosis.[8]

Foster Moore[9] is cited as the first to succeed in destroying intraocular retinoblastoma and conserving the eye by inserting radon seeds within the tumor, in 1929. Encouraged by early results, several ocular oncology groups started to employ different radioisotopes in EPB. Recently, iodine-125 (emits gamma radiation with 27-35 keV energy) has become the choice of most centers by virtue of its appropriate tissue penetration, easy shielding and facilitation of individualized palque designs[10].

Relative indications for EPB in retinoblastoma include tumors with a basal diameter less than 15 mm and thickness less than nine mm[5,11]. Juxtapapillary and foveal tumors can also be treated using EPB[12]. In a study of 103 patients treated with plaque radiotherapy, the tumor was controlled by a single applicatilon of EPB in 87 percent of cases, whereas in 13 percent of eyes subsequent treatment was necessary[5,11]. Tumor recurrence was seen at a mean interval of five months after plaque radiotherapy[11]. We did not encounter recurrence in the irradiated tumors in our patients. EPB can also be used successfully in patients with residual or recurrent tumors after failure of one or more treatment modalities. In an analysis of 91 such patients in whom enucleation was considered to be the only remaining option, tumor regression was achieved in 89 percent of eyes with use of EPB[13].

Under proper indications, it is clear that plaque radiotherapy has the advantages of preserving the eye and of serving as an alternative to enucleation. Compared to external beam radiotherapy, which has an established role in the treatment of retinoblastoma, EPB offers the advantages of a focused radiation dose and decreased consequent side effects[10]. These complications include dry eyes, cataract, retinopathy, optic neuropathy and poor orbital bone development[10,14]. A more serious long-term risk associated with external beam radiotherapy is the development of secondary malignancies within the radiation field[15]. It has been reported that children with bilateral retinoblastoma treated with external beam radiation have a 35 percent cumulative risk of a second malignancy in the irradiated area, whereas this risk is six percent in similar patients treated with other methods[15].

The shortcomings of this descriptive case series are the small number of patients, the relatively short follow-up period and the lack of comparable information on the results of alternative therapies. Thus, one cannot weigh the relative effectiveness and benefits of plaque radiotherapy over other methods with our results. Still, our data lends strong evidence to the current belief that episcleral plaque brachytherapy as a primary treatment is very effective in the management of selected retinoblastomas, with a high rate of tumor control thus contributing to organ preservation.

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URINARY CALCIUM EXCRETION OF CHILDREN LIVING IN THE EAST REGION OF TURKEY*

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SUMMARY: Selimoğlu MA, Alp H, Bitlisli H, Orbak Z, Energin M, Karakelleoğlu C. (Department of Pediatrics, Atatürk University Faculty of Medicine, Erzurum, Turkey). Urinary calcium excretion of children living in the east region of Turkey. Turk J Pediatr 1998; 40: 399-404.

We screened 1647 randomly selected Turkish primary school children to detect the prevalence of hypercalciuria. Ninety-seven children had hypercalciuria, with a prevalence of 5.88 percent. Mean Uca/Ucr ratio was 0.135 ± 0.108; mean Uca/Ucr value for girls was 0.139 and for boys 0.130 ($p > 0.05$). Mean Uca/Ucr of boys with hypercalciuria was 0.341 ± 0.09 and of girls 0.327 ± 0.08 ($p > 0.05$). Of these 97 children, all investigations could be completed in only 40 and these cases were considered to be idiopathic. Twelve children (21%) had a family history of consanguinity and 17 (29.8%) of renal stones. The relation between blood parathormone and Uca/Ucr ratio was not statistically important ($p > 0.05$). *Key words: hypercalciuria, prevalence, children.*

Idiopathic hypercalciuria is characterized by a urinary calcium excretion exceeding 4 mg/kg/day or by a calcium/creatinine ratio exceeding 0.21, and is one of the most common causes of renal stones at any age[1-5]. Excretion of abnormal amounts of calcium interferes with the metabolism of calcium, parathormone, 1.25 dihydrocholecalciferal and calcitonin, and increases the risk of stone formation[2, 5-9]. Although urolithiasis is endemic in Turkey, epidemiological and etiogenic investigations are not satisfactory[7]. This study was performed to screen 1647 Turkish primary school children living in the east region of Turkey for hypercalciuria and to determine its prevalence.

Material and Methods

We included 1647 randomly selected Turkish primary school children in this study. Since it has been demonstrated that 24-hour calcium excretion is best represented by second morning urine, these samples were collected[4]. Urine calcium (Uca) and creatinine (Ucr) concentrations were determined by the O-Cresolphthalein and Jaffe methods, respectively, on a Hitachi-717 autoanalyzer[4]. In children with a Uca/Ucr ratio above 0.21, parameters were restudied; those

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with high levels recorded again were considered as having hypercalciuria. These students were asked to complete a questionnaire providing information on history of urinary tract infections, renal stones, hematuria, abdominal pain, dysuria, polyuria, pollakuria, day and night enuresis, polydipsia and renal stones or renal disease in their families. Ninety-seven children with hypercalciuria were invited to hospital for further investigations. Weight, height and blood pressure measurements were recorded. Specific gravity, pH and protein were measured, and urine culture, microscopic examination, as well as tests to determine blood urea nitrogen, creatinine, uric acid, calcium, phosphate, alkaline phosphatase, sodium, potassium and parathormone levels were performed. Abdominal radiography and ultrasonography were performed. For statistical analysis, Student's t test and regression analysis were used.

Results

Of 1647 primary school children, 847 (51.5%) were boys and 800 (48.5%) were girls. Ninety-seven children had hypercalciuria (prevalence 5.88%). Mean Uca/Ucr ratio was 0.135 ± 0.108; mean Uca/Ucr value of girls was 0.139 and of boys 0.130 ($p > 0.05$). Table I shows the number of each age group and the mean Uca/Ucr values, no statistical difference was detected ($p > 0.05$). Figure 1 shows the relation between age and Uca/Ucr ratio ($r = 2.601$, $p < 0.005$). Figure 2 shows percentile curve of Uca/Ucr values. Of 97 cases with hypercalciuria, 63 (65%) were boys and 34 (35%) were girls. Mean Uca/Ucr of boys was 0.341 ± 0.09 and of girls 0.327 ± 0.08 ($p > 0.05$). Of these children, 57 accepted the invitation to hospital; all investigations could be completed. In only 40 (25 boys and 15 girls). Figure 3 includes the clinical symptoms of the 57 children. Twelve children (21%) had a family history of consanguinity; 17 (29.8%) of renal stones. Weight, height and blood pressure values were within normal limits. In the 40 cases, aside from Uca/Ucr ratios, no abnormality was detected in other parameters. These cases were considered to be idiopathic. The relation between blood parathormone and Uca/Ucr ratio was not statistically important ($p > 0.05$). Abdominal radiograms and ultrasonographs (14 cases) were normal.

Table I: Mean Uca/Ucr Values of Each Age Group

Age (Years)	Mean Uca/Ucr
6 (n = 28)	0.138 ± 0.121
7 (n = 255)	0.145 ± 0.111
8 (n = 287)	0.138 ± 0.121
9 (n = 376)	0.131 ± 0.104
10 (n = 402)	0.128 ± 0.121
11 (n = 299)	0.130 ± 0.098

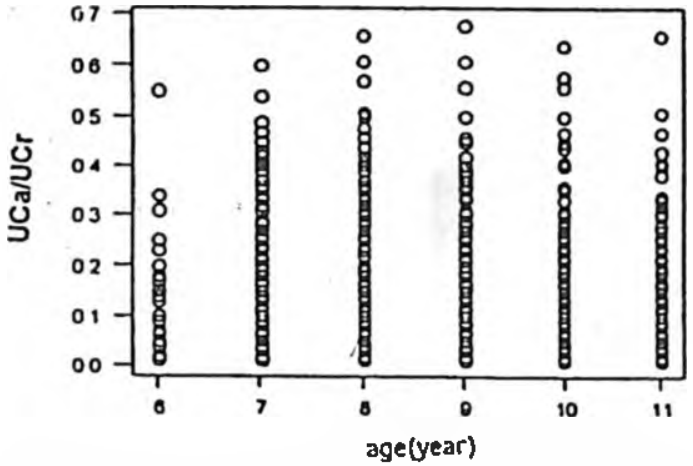

Fig. 1: Correlation between age and Uca/Ucr value.

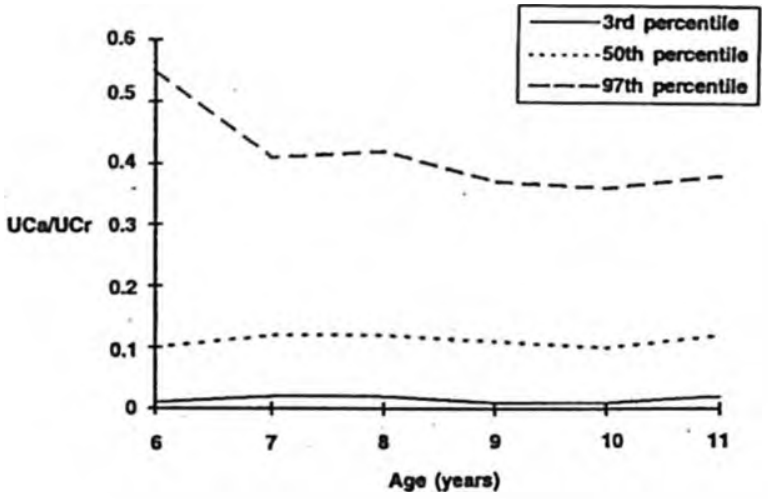

Fig. 2: Percentile curve of Uca/Ucr value.

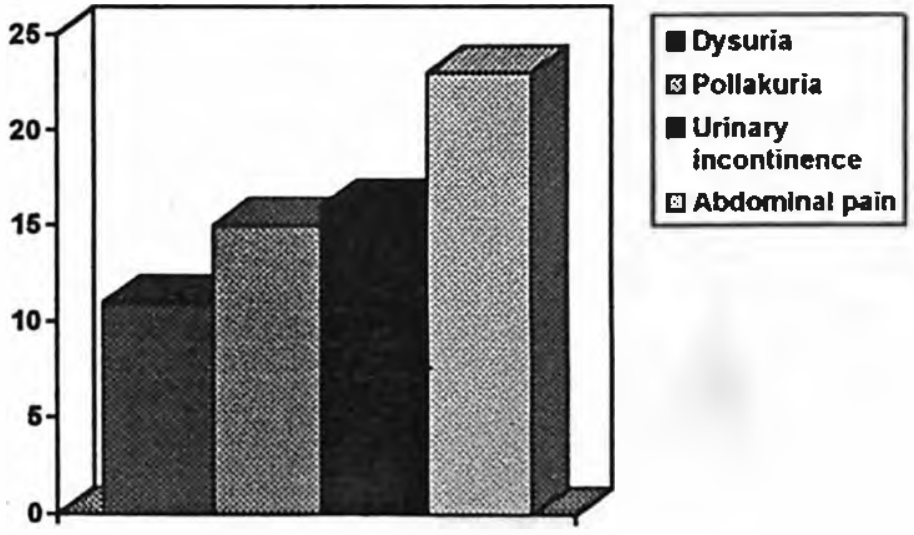

Fig. 3: Symptoms of children with hypercalciuria.

Discussion

Asymptomatic idiopathic hypercalciuria was first reported in 1978[10-12]. In literature, the prevalence is reported to be 2.9-6.2 percent[9, 10, 12, 13]. Our hypercalciuria prevalence was 5.88 percent, which is similar to that of Moore et al.[12]. The prevalence in Ankara and İstanbul were reported as 4.2 and 2.5 percent, respectively[9, 10]. The differences between regions of Turkey may speculatively be attributed to different water calcium concentrations. While the mean Uca/Ucr values of girls and boys were similar to those of Ghazali et al.'s[4], they were higher than those reported by others[12, 14]. This higher mean Uca-Ucr value may be the cause of endemic urolithiasis in Turkey[7].

Consistent with findings of other studies, except that of Moore et al.[12], was found that mean Uca/Ucr values did not differ with sex and age[4, 13-16].

We analyzed urinary samples for calcium content while the children were on an unrestricted diet. Several studies demonstrated that restriction of diet did not influence the results, but oral calcium loading did[4, 9, 12, 13, 17]. Distinguishing between renal and absorptive subtypes of hypercalciuria is important because of their different management. This distinction was made with an oral calcium loading test established by Pak et al.[17]. The variation in Uca/Ucr levels between samples from the same individual obtained at different times is helpful in the diagnosis of subtypes of hypercalciuria. Pak et al.[16] and Stapleton et al.[13] demonstrated in their studies that mean fasting value of Uca/Ucr was lower in absorptive hypercalciuria, but in renal hypercalciuria, there was no difference in the value following a meal. Forty children with hypercalciuria were considered to be renal subtype since there was no difference in the Uca/Ucr values after a meal.

Hypercalciuria may lead to resorption of calcium from bones resulting in secondary hyperparathyroidism. Coe et al.[18] and Moore et al.[19] found hyperparathyroidism in 2/3 and 42 percent, respectively, of cases with idiopathic hypercalciuria. In contrast, Stapleton et al.[2] found no hyperthyroidism among their cases with hypercalciuria. Our parathormone results were consistent with those of Stapleton et al.[2].

It has been stated that idiopathic hypercalciuria (IHC) may be familial with autosomal dominant trait[10, 11, 16, 20, 21]. In the study of Coe et al.[16], 26 of 73 relatives of nine patilents with hypercalciuria also had hypercalciuria. Likewise, Beilin and Clayton[22], Stapleton[2], and Carvera et al.[21] reported hypercalciuria in siblings and parents of children with hypercalciuria. Buckalew et al.[23] detected IHC in 13 of 64 members of a family. In 1988, Buyan et al.[20] described two forms of IHC, familial and non-familial. They stated that the familial form is inherited as autosomal dominant. In their study, urolithiasis was reported in 26.3 percent of the family members of patients with IHC[20]. We found urolithiasis in 29.8 percent

of first-and second-degree relatives of children with hypercalciuria. All these results suggest that children with hypercalciuria may unfortunately develop urolithiasis in the future.

Management of IHC is still controversial. Drug therapy is not recommended unless dysuria, recurrent gross hematuria, polyuria, recurrent urinary tract infection or urolithiasis is present. In most patients, adequate fluid intake and sodium restriction are sufficient[3, 5, 11, 19, 21, 22, 24, 25]. Because of the absence of the symptoms mentioned above in our cases, we also recommended only fluid intake.

Idiopathic hypercalciuria must be considered an important entity because of its onset in childhood, of its being asymptomatic in 80-90 percent of patients and of the risk of stone formation. Early diagnosis and management may reduce the incidence and morbidity, particularly in countries like Turkey where urolithiasis is endemic[7].

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PERONEAL NERVE INJURIES AS A COMPLICATION OF INJECTION*

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SUMMARY: Kırdı N,Yakut E, Meriç A. (School of Physical Therapy and Rehabilitation, Hacettepe University, Ankara, Turkey). Peroneal nerve injuries as a complication of injection. Turk J Pediatr 1998; 40: 405-411.

Ten children (8 males, 2 females) diagnosed with peroneal nerve injury as a complication of injection were included in this study. The age of the children ranged between four to seven years (mean 6.5 ± 1.25 years). Physiotherapy and rehabilitation protocol included superficial heat, neuromuscular electrical stimulation (either galvanic or faradic current according to the response elicited), electromyographic biofeedback, exercises (passive, active-assistive and active), and orthotic support. Before treatment, foot-drop and steppage gait were observed in all the patients; both were remedied. The post-treatment muscle strength and electrodiagnostic test results showed statistically significant improvement when compared with pretreatment values ($p < 0.05$). We believe that our relatively favorable results in this study, manifested as shorter recovery time with no residual deficits, may be related to early intervention with an extensive physiotherapy program. *Key words: post-injection peripheral nerve injury, physiotherapy.*

The common peroneal nerve aries from the division of the sciatic nerve in the popliteal fossa. It bears a close relationship with the head of the fibula as it winds anteriorly. It divides into superficial and deep branches, as well as giving off a purely sensory branch which forms the sural nerve, mediating sensation from the dorsum and lateral aspect of the foot[1-3].

The anatomical and topographical situation of the common peroneal nerve is very important. As the area is frequently exposed to trauma, paralysis is not rare[3]. Disc herniations; fracture of the pelvis, femur or caput fibulae; hip dislocation; orthopedic surgery; injection of drugs into or close to the nerve; and toxic infections may cause paralysis[2,3].

A number of antibiotic, antifungal and antituberculous drugs have been known to cause peripheral neuropathy in a small percentage of cases. Chloramphenicol, for example, can cause a mild, primarily sensory, peripheral neuropathy after long-term use of the drug at relatively high doses[4,5].

Most drug-induced neuropathies are reversible after discontinuation of treatment but recovery may be slow, especially when intoxication was prolonged[5].

Symptoms that appear in the nerve paralysis change in respect to the place of the lesion[1-3]. Injury to the sciatic nerve is often the result of injections into

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the nerve or in its vicinity. Paralysis may affect the whole territory of the sciatic nerve or only one of its two main divisions, most commonly the peroneal nerve. Foot-drop, amyotrophy and growth disturbances of the leg are the most frequent manifestations[5].

If the lesion is just above the popliteal fossa, total paralysis becomes evident. Dorsiflexion and evertion of the foot and extension of the fingers are lost. The patient walks with a "steppage gait" (walking with exaggerated knee and hip flexion). Sensory loss involves the dorsum and outer aspect of the foot. Partial common peroneal nerve palsies are common with very selective muscle weakness[1].

In general, the physiotherapy and rehabilitation protocol for peripheral nerve injuries resulting from different causes include heat; electrotherapeutic modalities, such as neuromuscular electrical stimulatilon and electromyographic biofeedback; therapeutic exercises (passive, active-assistive, active and resistive), orthotic devices; and training of the patient to best perform functional activities. Modalities are commonly used with the aim of delaying atrophy, improving the circulation in the muscles and maintaining normal range of motion[6-10].

We found two studies in the literature related to post-injection neuropathy, but did not come across a study reporting a specific physiotherapy rehabilitation protocol for the treatment of peripheral nerve injuries due to complications of intramuscular injections.

This report was undertaken with the aim of discussing a detailed and sequential physiotherapy program which can be implemented in such cases. It is a case report on 10 patients and for ethical reasons lacks a control group. The efficacy of the treatment protocol is evaluated within this limitation.

Material and Methods

This study was performed on 10 children who were diagnosed as having peroneal nerve paralysis at the Department of Pediatric Neurology in Hacettepe University Faculty of Medicine between 1992-1996. In all 10 cases the peroneal nerve was affected as a complication of intragluteal injection. The EMG evaluations were done at the Neurology Department of Hacettepe University following the 30th day of injury to the nerve and showed severe axonal degeneratilon. The patients were referred to the Physiotherapy Department of the School of Physical Therapy and Rehabilitation.

The age of the children (8 males, 2 females) ranged between four to seven years (mean 6.5 ± years. The peroneal palsies began after multiple injections of medication (penicillin, semisynthetic penicillin) and were noted immediately after a buttock injection. In four cases penicillin was used and in the remaining children semisynthetic penicillin was used.

The rehabilitation program started two to six weeks (mean 4.00 ± 1.83 weeks) after foot-drop was observed during gait.

All patients were assessed before and after the physiotherapy program through range of motion, manual muscle testing, posture and gait analysis, electrodiagnostic tests and sensorial evaluation. Range of motion was determined by goniometric measurements[11]. Muscle strength was assessed by Dr. Lovett's[12] manual muscle test on a scale of zero to five. Five shows normal muscle strength where the patilent can perform the activity of the muscle against gravity and hold against resistance given at the end of the range. Zero shows absence of muscle contraction.

Postural faults were determined from anterior, posterior and lateral posture analysis while standing. Gait analysis was performed by observation[12, 13].

For electrodiagnostic evaluation, the faradic excitability test was applied to the motor points of m. tibialis anterior and m. peroneus longus. In this test, the response of these muscles, to the same intensity of faradic current, was compared with the sound side. If there was not even minimal contraction response to the faradic current, it showed inexcitability and total degeneration. If the response was less than the sound side, it showed hypoexcitability and regeneration. Eliciting a more obvious contraction on the affected side showed hyperexcitability and degeneration[7].

Control EMGs were conducted every month as required. The EMG results were in accordance with clinical manifestations showing reinnervation at the time improvement of muscle strength was observed.

None of the patients complained of pain; sensorial evaluation was performed for discrimination of light touch and temperature.

After this evaluation, patients were treated with superficial heat (infrared) for 20 minutes followed by electrical stimulatilon (either galvanic or faradic current according to the response elicited) and a possive range of motion exercises daily, for five days a week. In order to localize the current to the muscles, a small active electrode was applied to the motor point of the muscle, the circuit being completed with the same size dispersive electrode sited at a proximal area. The muscles were stimulated for a total of 20 minutes, with a rest of five minutes between two 10 minute periods.

These modalities are commonly used for improving the circulation, delaying atrophy in the muscles and maintaining the normal range of motion[6, 8, 9, 10, 13].

During the treatment, static foot-drop orthoses were used to maintain the foot in normal alignment and to improve function[14]. The static orthosis was used until active contraction was achieved. When the patient was able to actively contract his/her muscles, electrical stimulation was discontinued, and reeducation treatment was begun with electromyographic biofeedback and active-assistive and active exercises.

Electromyographic biofeedback was applied by the use of surface electrodes for 20 to 30 minutes, three days a week.

The outcome of treatment was considered successful when electrodiagnostic tests showed regeneration: the patients were able to dorsiflex and evert their foot against gravity and walk a distance of 50 meters without foot-drop or a compensating steppage gait.

The measurement of the pre-to post-treatment values were evaluated statistically by the Wilcoxon signed rank test.

Results

The physical features and treatment results of the children are shown in Table I. Clinical manifestations of peroneal nerve damage varied considerably. For this reason, total time of treatment sessions ranged between 14 to 47 sessions (mean 20.1 ± 10.25 sessions) (Table I). Recovery took place between two to six months (mean 4.00 ± 1.83 months). Range of motion and sensory assessments showed no limitations or loss before or after treatment.

Table I: Physical Features of Cases

Case No.	Age (Years)	Sex	Primary Disease	Neurological Sign at Time of Diagnosis	Time When Physiotherapy Started	Length of Treatment	Residual Signs	Recovery Time
1	6	M	Prophylactic antibiotics after appendectomy		4 weeks	43 sessions		6 months
2	4	M	Acute tonsillitis	..	2 "	29 "		5 "
3	7	M	Infection of upper part of the respiratory tract		2 "	21 "		3 "
4	7	M	"	Foot-drop	4 "	32 "	No	5 "
5	6	M	Acute tonsillitis	No sensory loss	2 "	14 "	residual	2 "
6	6	F	Cellulitis		2 "	22 "	signs	3 "
7	4	M	Bronchopneumonia		4 "	20 "		3 "
8	6	F	Infection of upper part of the respiratory tract		6 "	47 "		6 "
9	6	M	"		2 "	28 "		5 "
10	7	M	"		2 "	14 "		2 "

M: Male, F: Female.

An increase in muscle strength took place with therapy. The difference was significant in favor of post-treatment values ($p < 0.05$) (Table II).

Table II: Pre-and Post-Treatment Values of the Manual Muscle Testing Scores (n = 10)

	Pre-Treatment Values	Post-Treatment Values
Sound Side	4.70 ± 0.45	4.80 ± 0.40*
Affected Side	0.40 ± 0.66	3.30 ± 0.48**

Number are mean ± SD.
* not significant.
** $p < 0.05$.

Before treatment, foot-drop and steppage gait were observed in all the patients; both were remedied.

The post-treatment electrodiagnostic test results of faradic excitability values ranged from hyperexcitability to hypoexcitability, with treatment showing regeneration, and statistically significant improvement ($p < 0.05$) (Table III).

Table III: Pre-and Post-Treatment Values of the Electrodiagnostic Test Results (Faradic Excitability) (n = 10)

		Pre-Treatment Values (mA)	Post-Treatment Values (mA)
M. Tibialis Anterior	Sound Side	5.40 ± 0.79	5.17 ± 0.50*
	Affected Side	3.78 ± 5.32	11.68 ± 4.25**
M. Peroneus Longus	Sound Side	5.31 ± 1.28	5.03 ± 1.03*
	Affected Side	4.35 ± 3.12	12.15 ± 7.06**

Number are mean ± SD.
* not significant.
** $p < 0.05$.

Discussion

There are few studies in the literature concerning post-infection peripheral nerve injuries. Many pathogenic mechanisms have been postulated for the palsies appearing in infants, children and adults in cases of intramuscular injection therapy. These mechanisms include allergic peripheral neuritis, puncture of the nerve, ischemia of the nerve, and direct neural damage by drugs[15, 16].

It is well recognized that peripheral nerve paralysis may occasionally occur in nerves distant from the injection site, and an allergic mechanism has been postulated. In Gilles's and French's[15] study, their cases had no clinical evidence of hypersensitivity to the medication being used. They observed that the majority of 21 patients suffering from sciatic palsy were infants. In our cases, there was also no clinical evidence of hypersensitivity to the medication. All our patients were pediatric and had peroneal palsy.

In spite of the widespread parenteral use of penicillin in various situations and in various concentrations, there appears to be but one report suggesting a toxic effect on the peripheral nervous system[16].

Broadbent et al.[16] reported four cases in whom there developed one sciatic, one ulnar and two radial peripheral nerve lesions immediately following the administration of penicillin into or about a peripheral nerve. In our study, four of the 10 children had received penicillin and the remaining had received semisynthetic penicillin. The peroneal nerve lesion had developed immediately following the administration of the medication.

Casey et al.[17] recorded clinical and electrophysiological observations in seven patients treated with vincristine for between six months and two years, and in six patients treated for two to three months. Reflex loss occurred early, followed by mild sensory disorder. Mild weakness of finger and/or toe extensors then developed, which in some patients became severe. The neuropathy was largely reversible, either when the dose was reduced or the drug stopped. The recovery time was between five to 32 months in this study.

Gilles and French[15] reported the results of 21 patients in whom foot-drop developed after intragluteal injection. Fifteen of the patients recovered without being treated. Recovery time ranged between 24 hours and 13 months. The remaining six were with sequelae with permanent foot-drop and had to use foot-drop orthoses during gait.

Broadbent et al.[16] assessed degeneration and regeneration in affected muscles by means of response to faradic stimulation in four cases of intramuscular injection complications. No treatment was administered, recovery took place between 10 to 18 months, and only one out of four cases recovered without residual signs. We also utilized the faradic excitability test in our patients (Table III). Recovery time ranged from two to six months and all our patients recovered without residual deficits.

After injury to the peripheral nerve, it is important to maintain the functional integrity of muscles and joints. With this objective, an appropriate physiotherapy rehabilitation program should be implemented to shorten recovery time and to ensure recovery without residual impairment. Such a program should encompass muscle reeducation. Various research studies have reported electromyographic biofeedback as the most effective form of muscle reeducation[9, 10, 18, 19].

Although there are only a few studies in the literature with which we can compare our results, we believe that our relatively favorable results, manifested as shorter recovery time with no residual deficits, may be related to early intervention with an extensive physiotherapy program.

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THE ROLE OF PAF AND LEUKOTRIENES IN BIOINCOMPATIBILITY OF CUPROPHANE MEMBRANES IN HEMODIALYSIS*

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SUMMARY: Kabasakal C, Mir S, Gousseinov A, Cura A, Betin N, Çoker I. (Department of Pediatrics, Ege University Faculty of Medicine, and Biochemistry Research Laboratory, Tepecik Social Security Hospital, İzmir, Turkey). The role of PAF and leukotrienes in bioincompatibility of cuprophane membranes in hemodialysis. Turk J Pediatr 1998; 40: 413-420.

Inflammatory lipid mediators, PAF and leukotrienes (LTs), are thought to have an important role in biocompatibility in hemodialysis. PAF, LTB4 and LTC4 were studied both in controls (n: 12) and in 11 children on regular hemodialysis (150 minutes) with cuprophane dialyzers. Blood samples were collected initially (0'-precapillary), at first minute (1'-postcapillary) and at one hour after the hemodialysis sessions (210'-venous). Presence of LTs and high levels of PAF in 0' samples compared to levels in controls and significant increases in 1' samples suggested the alterations in PAF and LTs likely originated from the peripheral leukocyte activation. In 210' samples, PAF and LTs levels were decreased but still higher than the levels in 0' samples. This study suggested that PAF and LTB4 may be the control elements in biocompatibility in hemodialysis with cuprophane membranes, and demonstrated that the effects of activation last until the following session. *Key words: hemodialysis, children, biocompatibility, PAF, leukotrienes.*

Clinical hemodialysis (HD) with cellulose-based membranes, especially cuprophane, causes marked alterations in leukocyte and platelet functions and kinetics, and production phenomena that likely contribute to the morbidity of these procedures and to clinical immunosuppression in dialysis patients. A complex array of inflammatory mediators are generated as a consequence of blood contact with HD membranes. Besides complement activation, other mediators involved in cell activation are thought to possibly be responsible for early- and long-term changes in immunity, hypercatabolism, hemostatic mechanisms and susceptibility to infections[1-7].

Recent studies have established that cell membrane-derived new lipid mediators, such as leukotrienes (LTs) and platelet activating factor (PAF), might be generated by complement dependent or independent mechanisms of cell interaction with hemodialysis membranes[8-11]. LTs are biologically active 5-

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lipoxygenase products of arachidonic acid metabolism, and are involved in the mediation of various inflammatory disorders. Of these, LTB4, a potent chemoattractant for leukocytes, and the sulfidopeptide-leukotrienes LTC4, LTD4 and LTE4 which increase vascular permeability and constrict smooth muscle, exert their biological action through specific ligand-receptor interactions[12, 13]. PAF is the common name given to 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphoryl-choline, a biologically active phospholipid. PAF intermits signals between cells and thus functions in a similar fashion to hormones, cytokines, interleukin and other signaling molecules[14-16]. It is now known that LTs and PAF are produced by a wide variety of isolated human cells, upon different immunologic and nonimmunologic stimulation[17, 18].

In this study, we have measured inflammatory lipid mediators just before, at the very beginning of hemodialysis and after the end of the session to clarify the effects of LTs and PAF on bioincompatibility of cuprophane HD membranes.

Material and Methods

Patients: Eleven patients (5 girls and 6 boys) with a mean age of 13.0 ± 3.6 years were included in this study. The patients studied were on regular bicarbonate-buffered HD with cuprophane membranes three times per week, and the sessions were 150 minutes using Gambro AK100 HD machines. The surface area of the dialyzers used (Renak E 08 U) was identical (0.8 m^2). All of the patients included gave informed consent to this study. Twenty healthy children with similar age and gender distribution were studied as the control group.

None of the patients had a history of anaphylactic reaction, autoimmune disease or clinical evidence of acute inflammation, and none had received medical treatment, such as angiotensin converting enzyme (ACE) inhibitors, corticosteroids, benzodiazepines or anti-inflammatory agents, known to have an effect on leukocytes or lipid mediators.

Study Protocol: In patients, blood samples were collected via sampling port proximal to the dialyzer initially (0'), distal to the dialyzer at the end of the first minute (1') of dialysis (to sample blood at first pass before it effects the endothelium and the vascular system), and from a vein an hour after the end of the session (210') to determine LTs, PAF and hemoglobin (Hb), leukocyte count, platelet count, urea, creatinine and C3 levels.

Sample Procedure: Whole blood count was developed by Sysmex K-1000 hemocounter. Plasma creatinine and urea levels were determined by autoanalyzer (Falcon 300), and nephelometry was used to determine serum C3 levels by Backman-Array Protein System analyzer.

PAF Extraction and Purification: PAF was extracted with solid-phase extraction method by XAD-2 Ambetlit columns. PAF was purified with HPLC method by Silasorb Si60, 5μ analytic (250x2.0 mm) columns.

RIA for PAF: The specificity and quantitative measurement of PAF from dried samples were checked using the platelet activating factor (PAF) (^{3}H) scintillation proximity assay system (TRK-990) from Amersham Life Sciences (UK). The dried PAF samples were reconstituted with 100 ml of kit working assay buffer and wirled intermittently in a vortex for about 10 minutes, before measurements. All determinations were done in duplicate.

PAF was measured according to the procedure recommended by the manufacturer. The labeled PAF which remained unbound at the end of the reaction was determined by a β-liquid scintillation counter (TRI-CARB-1600 TR, LSA Packard, Camberra Company) and results expressed as ng PAF/ml plasma. To know the recovery of PAF with the solid-phase extraction method (XAD-2 columns) and HPLC purification, plasma was obtained from five normal subjects and eight patients, mixed with 0.1 ml 0.1 nCi PAF (^{3}H) (Amersham, UK, TRK 743) and processed as described above. The recovery rate was 84.6 ± 2.3 percent (average of 13 experiments).

Extraction and Purification of LTs: LTs were extracted by using Sep-Pak C18 cartridge with solid-phase extraction method. LTs extracted from the plasma samples were separated from the eluent through Ultraphaser ODS C18 (5 micron) analytic column (250x4.6 mm ID) at 280 mm UV in Waters HPLC system.

RIA for LTs: The dried samples were separately solved for LTC4 and LTB4 in a 100 microliter kit buffer. The sensitivity of used kits was 10 pg/ml plasma for LTC4 and 5 pg/ml plasma for LTB4. LTs were measured by RIA kits according to the procedure recommended by the manufacturer. The concentrations of unlabeled LTs in samples were determined by a β-liquid scintillatilon analyzer system (TRI-CARB-1600 TR, LSA Packard, Canberra Company) and results were expressed as ng-ml plasma. The total recovery values of plasma LTs were 78.0 ± 3.6 percent for LTC4 and 84.1 ± 3.2 percent for LTB4. All assaying procedures were performed in duplicate.

Statistical Analysis: Statistical analyses were performed using paired t test to compare the consecutive data of the patients and using independent t test with controls. Data were given as mean ± SD.

Results

The levels of the inflammatory lipid mediators determined in controls and patients at 0', 1' and 210' are given in Table I. PAF levels were found higher in 0' samples compared with controls. PAF levels significantly increased in 1' samples compared with 0' samples, and slightly decreased (210' samples) one hour after the end of the HD sessions when compared with 1' samples. Nevertheless, PAF levels were still higher than controls and 0' samples (Fig. 1).

Table I: Levels of Lipid Mediators in Patients and Controls (ng/ml; mean ± SD)

	PAF	LTB4	LTC4
Control group	0.2 ± 0.1	*	*
0'	1.2 ± 0.7[a]	0.04 ± 0.018[a]	0.038 ± 0.023[a]
1'	4.8 ± 1.4[ab]	0.554 ± 0.296[ab]	0.23 ± 0.139[ab]
210'	3.3 ± 1.9[abc]	0.082 ± 0.068[ac]	0.097 ± 0.058[abc]

a: $p < 0.05$ compared to controls, b: $p < 0.05$ compared to 0', c: $p < 0.05$ compared to 1'.
* less than 0.002 ng/ml.

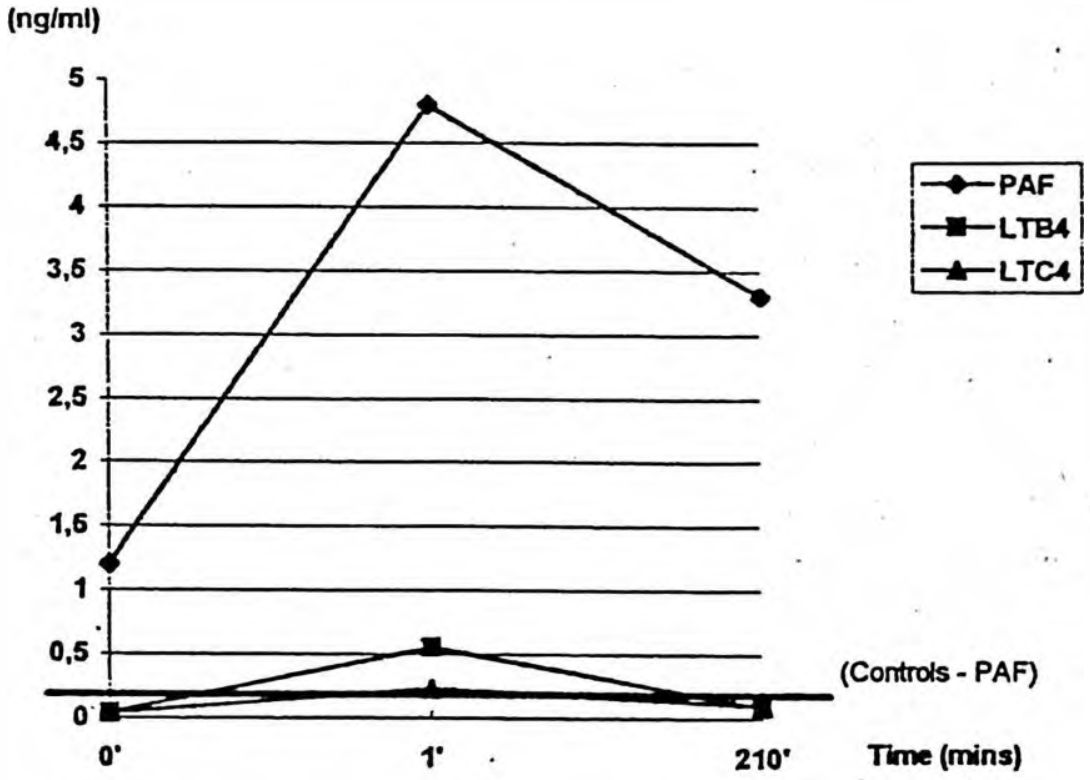

Fig. 1: Graphics of lipid mediators in patients and controls.

LTB4 and LTC4 were measured at detectable levels in 0' samples against the controls. LTs were significantly increased in 1' samples and then decreased to levels higher than the original baselines (Table I) (Fig. 1).

The average C3 level of the control group was significantly higher when compared to the values of patients at all stages. Although a slight decrease was detected in the mean C3 level in 1' samples compared to the initial value, there was no significant difference. C3 levels were found slightly over the initial levels (Table II).

The mean leukocyte count showed a non-significant reduction at 1' compared to 0' samples and controls. However, a rebound leukocytosis was detected in 210' samples, and this increase was statistically significant, (Table II).

Table II: C3 Levels and Leukocyte and Platelet Counts in Controls and Patients Before, During and After the Hemodialysis Session

	C3 (mg/dl)	PNL ($x10^3/mm^3$)	Platelets ($x10^3/mm^3$)
Control group	128.7 ± 29.5	7.7 ± 1.6	275 ± 90
0'	81.9 ± 18.2[a]	6.1 ± 1.7	212 ± 53
1'	77.0 ± 15.0[a]	5.1 ± 0.8[a]	176 ± 51[ab]
210'	84.9 ± 26.5[a]	6.5 ± 2.1[c]	244 ± 54[bc]

a: $p < 0.05$ compared to controls, b: $p < 0.05$ compared to 0', c: $p < 0.05$ compared to 1'.

A significant decrease in platelets in 1' samples occurred when compared to initial count (0') and controls. Similar to leukocytes and C3 levels, the platelet count was increased in 210' samples over the initial levels (0'). The rebound detected in the platelet count was also significant, (Table II).

Discussion

It is known that several reactions are activated during hemodialysis when blood is exposed to artificial surfaces. Cuprophane hemodialysis membranes have been shown to activate several pathways of inflammatory response, as well as cellular elements such as granulocytes, monocytes and platelets. To clarify the bioincompatibility of cuprophane membranes, changes in some biological markers, such as activation of the complement system and degree of leukopenia, are studied as parameters. However, the assessment of bioincompatibility is complex. The role of new lipid inflammatory mediators originated from cell membrane, PAF and LTs, has been studied more recently.

The important role of the complement system in bioincompatibility was noted as the probable cause of granulocytopenia during hemodialysis[4, 19, 20]. The detection of activation of the complement system with C3a and C5a but without C4a, generated from the classical pathway, suggests the activation occurred via an alternate pathway[4, 21]. Maximum complement activation takes place at 15' and lasts till 90' especially with cellulosic membranes[22]. In this study, only C3 levels were determined; the changes we noted during and after the HD sessions were nonsignificant. Since the complement system activation has been well known with cellulosic membranes, the observations in this study may suggest that C3 activation was minimal at the beginning of HD and had finished before the 210' samples were collected. Thus, C3 levels could not be used as an early index of membrane biocompatibility.

Leukopenia, mainly with a reduction in granulocytes and monocytes, also takes place at 15' maximally and may be followed by a rebound during dialysis with incompatible membranes[22, 23]. In this study, a slight decrease in leukocytes was observed at 1' compared to 0' samples, though it was not significant. In 210' samples there was a minimal increase in the leukocyte count. It is likely that

a notable reduction in leukocytes, reported in literature with cellulosic membranes, might have happened after we had bled the patients at 1'. The data we observed showed that neither serum C3 levels nor leukopenia could be an early index to evaluate the membrane biocompatibility.

Although there is some conflicting data in literature, platelet counts have frequently been reported as decreased with cellulosic membranes[24, 25]. We observed significant changes in the mean platelet count at 1' and 210'. Considering the data on cellulose membranes in the literature and the results of this study, the effects of the contact between blood and cuprophane membranes on platelets might begin even in the first minute of contact.

It is known that LTs and especially PAF are generated by complement dependent or independent mechanisms after cell interaction with HD membranes[7]. Furthermore, lipopolysaccharides originated from dialysate solutions may generate PAF via monocyte adhesion to membranes[26, 27]. The data on effects of dialyzers on PAF and LTs is limited to the 1st, 5th and 30th minutes. Alterations in PAF levels were first reported by Ward (1991)[28] within the first five minutes, and at first contact by Tetta et al. (1993)[8]. In this study, the presence of LTs and significantly high levels of PAF in 0' samples compared to controls and the significant increases of LTB4 and PAF in 1' samples compared to 0' samples were noted. An almost ten-fold increase in LTs in the 1' samples, collected subsequent to blood-dialyzer contact and prior to return to the patients' vascular system, suggests cuprophane HD membranes activate the peripheral leukocytes. Since the only contact of 1' samples was with HD membranes and no other contact had taken place with other lipid mediators releasing tissues as endothelium, the detected alterations in PAF and LTs likely originated from the activation of peripheral leukocytes.

Although the levels of the inflammatory lipid mediators were lower in 210' samples compared to the previous samples, they did not return to baseline levels. Therefore it is likely that the lipid mediators activate endothelial cells via three possible pathways: i) by circulating lipid mediators, ii) by lipid mediators in activated leukocytes, iii) by factors, such as PAF and endothelin, released from activated endothelium.

The results we observed in the study may suggest that inflammatory lipid mediators are generated first by peripheral leukocytes after cell interaction with HD membranes and then by endothelium activation. It is likely that endothelium activation continues till the following HD session though it is tapering off. The data in this study suggests that PAF and LTB4 released from stimulated leukocytes may be the control elements in bioincompatibility in HD with cuprophane membranes, and that the effects of activation last at least until the next session.

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INTERMITTENT – LOW DOSE G – CSF TREATMENT IN A PATIENT WITH CHRONIC NEUTROPENIA*

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SUMMARY: Yetgin S, Özbek N. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Intermittent-low dose G-CSF treatment in a patient with chronic neutropenia. Turk J Pediatr 1998; 40: 421-424.

Chronic neutropenia in childhood has many definable causes and thus a clear cause cannot be identified in a large group of patients. Since the committed stem cell is involved in this disorder, growth factors such as granulocyte colony-stimulating factor (G-CSF) may play an important role in the treatment of severely affected children. Because of the side effects and cost, the use of G-CSF should be restricted to a minimum dose. Here we report a child with chronic neutropenia in whom intermittent-low doses of G-CSF were successfully used over a long period. *Key words: chronic neutropenia, intermittent-low dose G-CSF.*

Chronic neutropenias (CN) of childhood are a group of uncommon disorders which involve committed myeloid stem cells with normal numbers of red cells and platelet precursors. Decreased or ineffective production of neutrophils has been suggested as a mechanism for this disorder. Spontaneous remissions in some children with CN, usually around the age of two to four, may occur. In the majority of patients, there is no genetic pattern. However, a rare, autosomal dominant form has been described[1]. Chronic neutropenia may present with recurrent infections in severely affected children. The use of recombinant human granulocyte colony-stimulating factor (rhG-CSF) may be an effective therapy in these patients. However, there is a risk of malignant transformation and of some other side effects with G-CSF therapy in patients with different forms of neutropenia[2]. Therefore, the use of G-CSF in these patients should be restricted to a minimum.

Here we report a child with CN with recurrent infectilons who responded to intermittent-low doses of G-CSF therapy.

Case Report

A two-year-old boy was admitted to our hospital with complaints of recurrent bronchopneumonia and cough. On physical examination, breath sounds were decreased on the left side and there was minimal hepatosplenomegaly. A chest radiograph revealed cardiac enlargement and pleural effusion on the left side.

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The blood picture was compatible with absolute neutropenia (< 500/mm^3). Bone marrow examination disclosed 24 percent erythroid cells, 46 percent myeloid cells, 6 percent monocytoid cells and 24 percent lymphoid cells. However, no myeloid element was noted beyond the myelocytic stage. Echocardiogram showed pericardial effusion, and a chylous-like fluid was obtained by pericardiocentesis. Surgical pericardiectomy was performed successfully. Pathologic examination showed that chylous pericarditis was due to intrathymic ectatic lymphangiomatous malformation. The patient was neutropenic during recovery and for three months follow-up thereafter with recurrent infections. No cyclic pattern was observed during the follow-up period.

Because of his recurrent infections, persistence of a neutropenic state and past history, the patient was given G-CSF therapy. All the doses of G-CSF were administered subcutaneously. Initially, he was started on 5 µg/kg/day, subcutaneously. The dose was tapered down to 2.5 µg/kg/day after a week, 1 µg/kg/day after 20 days and then to 1 µg/kg/day every other day for 27 days with normal neutrophil counts. He did not maintain this neutrophil count with a G-CSF dose of 0.5 µg/kg/day every other day. However, reintroduction of 1 µg/kg/day G-CSF was successful in maintaining normal neutrophil counts for 20 days. Intermittent therapy was started with 1 µg/kg/day G-CSF being given every other day. The patient was then started on a three-month course with 1 µg/kg/day G-CSF every three days (Table I). At the writing of this report, he has maintained a neutrophil count > 500/mm^3 and no severe infections have occurred over the last year.

Table I: Neutrophil and White Blood Cell (WBC) Counts During Follow-up

Day	WBC Count (per mm^3)	PNL Count (per mm^3)	Treatment*
0	7800	468	–
7	4800	480	–
15	5200	884	–
25	5800	406	–
40	3200	128	–
55	4000	480	–
70	5000	250	–
80	5600	280	–
90	5000	250	–
90-97**	21000	15170	5 µg/kg/day
97-104**	20300	14120	25 µg/kg/day
104-124***	7700 (6000-8400)	5467 (4734-6200)	1 µg/kg/day
124-151***	8100 (7700-8500)	3350 (3000-3700)	1 µg/kg/day, every other day
151-171***	4000 (2800-5700)	360 (235-435)	0.5 µg/kg/day, every other day
171-191***	6000 (5800-6600)	3240 (2800-3900)	1µg/kg/day
191-231***	4600 (4400-4800)	1656 (995-2098)	1 µg/kg/day, every other day
231***	4800 (3000-5700)	1228 (522-1680)	1 µg/kg/day, every three days

* All the doses of G-CSF were given subcutaneously.

** White blood cell and PNL counts were obtained at the end of the week.

*** White blood cell and PNL counts were obtained every week. All the numbers represent the mean of these values. Numbers in the parentheses represent minimum and maximum values for each treatment period.

Discussion

Granulocyte colony-stimulating factor is a hematopoietic growth factor which increases the number of hematopoietic progenitor cells in the bone marrow and peripheral blood of normal subjects and of patients with various types of neutropenia. It also controls activation and late maturation stages of neutrophil development and synergises with GM-CSF and IL-3 in inducing proliferation of primitive hematopoietic progenitor cells in vitro[3]. In a recent article, a defect in the endogenous G-CSF production at the post-transcriptional level was suggested to be an etiologic factor in CN[4]. Therefore, G-CSF treatment is a good aproach for maintaining a normal neutrophil count in CN patients with infections. However, the use of G-CSF has been associated with a number of side effects, such as severe bone pain, splenomegaly, Sweet's syndrome, leukocytoclastic vasculitis and disseminated intravascular coagulation[5-7]. Leukocytoclastic vasculitis was shown to be dose dependent, with the lesions disappearing at doses of < 1 mg/kg-day and recurring at 2 mg/kg/day. Malignant disorders such as aplastic anemia and Kostmann syndrome[8] have been reported in some patients with neutropenia receiving G-CSF therapy. In some cases with severe congenital neutropenia, mutations in the gene for the G-CSF receptor were found. It is suggested that these mutations interrupted signals required for the maturation of myeloid cells. However, the mechanism of malignant evolution is not clear. Possible propensity toward malignant disease in these disorders or G-CSF therapy itself may be the cause of malignant transformation.

In our patient, neutrophil counts over 500/mm^3 were achieved with low and intermittent-low doses of G-CSF. However, the minimum dose to which the patient responded was 1 µg/kg/day. With lower doses of G-CSF the neutrophil count decreased below 500/mm^3. Interestingly, further treatment with 1 µg/kg/day G-CSF dose and intermittent treatment with this same dose was successful. No severe infections or adverse reactions due to G-CSF treatment occurred for a year. In a recent article, Jayabose et al.[9] reported successful results with a three-day-a-week G-CSF regimen in a patient with cyclic neutropenia.

In conclusion, intermittent doses of G-CSF may be used in patients with various types of neutropenia. However, it is suggested that the dose be tapered to a minimum that maintains normal neutrophil counts and that dosages be determined on an individual basis. This approach may be useful in diminishing side effects as well as the cost of treatment.

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HYDATID CYST MIMICKING PULMONARY HEMATOMA IN A PATIENT WITH HEMOPHILIA A*

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SUMMARY: Olcay L, Besim A, Şenocak ME, Göçmen A, Büyükpamukçu M, Kale G, Çetin M, Gürgey A. (Departments of Pediatrics, Radiology and Pediatric Surgery, Hacettepe University Faculty of Medicine, Ankara, Turkey). Hydatid cyst mimicking pulmonary hematoma in a patient with hemophilia A. Turk J Pediatr 1998; 40: 425-429.

We present a patient of 2.5 years of age with hemophilia A and a pulmonary hydatid cyst. A chest x-ray taken by chance showed a paracardiac opacity resembling an intrapulmonary hematoma which did not reduce in size after infusions of fresh frozen plasma and factor VIII but rather enlarged. Transabdominal ultrasound, colored echocardiography, thoracic computed tomography and magnetic resonance imaging findings were consistent with a cyst that was firmly attached on the border of the right atrium and also indented it; the wall was remarkably thick with no internal echoes. Hydatid cyst was dagnosed after thoracotomy. *Key words: haydatid cyst, hemophilia A.*

In countries with endemic regions, the diagnosis of "hydatid cyst" is easily made and treated[1-3]. Nevertheless, in a patient with hemorrhagic diathesis like hemophilia, presence of a pulmonary opacity suggests hemorrhage rather than a hydatid cyst and thus there may be some problems in the differential diagnosis.

Here in we present a hemophiliac patient with a pulmonary opacity which was suggestive of a hemorrhage at first but diagnosed as hydatid cyst postoperatively.

Case Report

A 2.5-year-old boy was admitted to our hospital because of bleeding of a cut on his foot. His past history revealed the diagnosis of hemophilia A at eight months of age, which had been followed in our hospital. He was hospitalized

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as the bleeding did not stop despite regular fresh frozen plasma infusions. A routine chest x-ray revealed a right paracardiac opacity. At that time, the borders of the opacity were not clear. Therefore, and in view of the patient's history, this opacity was thought to most probably represent an intrapulmonary hematoma. The patient was infused with fresh frozen plasma and factor VIII for seven days. Chest x-ray taken at the end of this therapy revealed that this opacity showed no reduction in size. On the contrary it had enlarged and this time with clear borders (Fig. 1). Transabdominal ultrasound and colored echocardiography

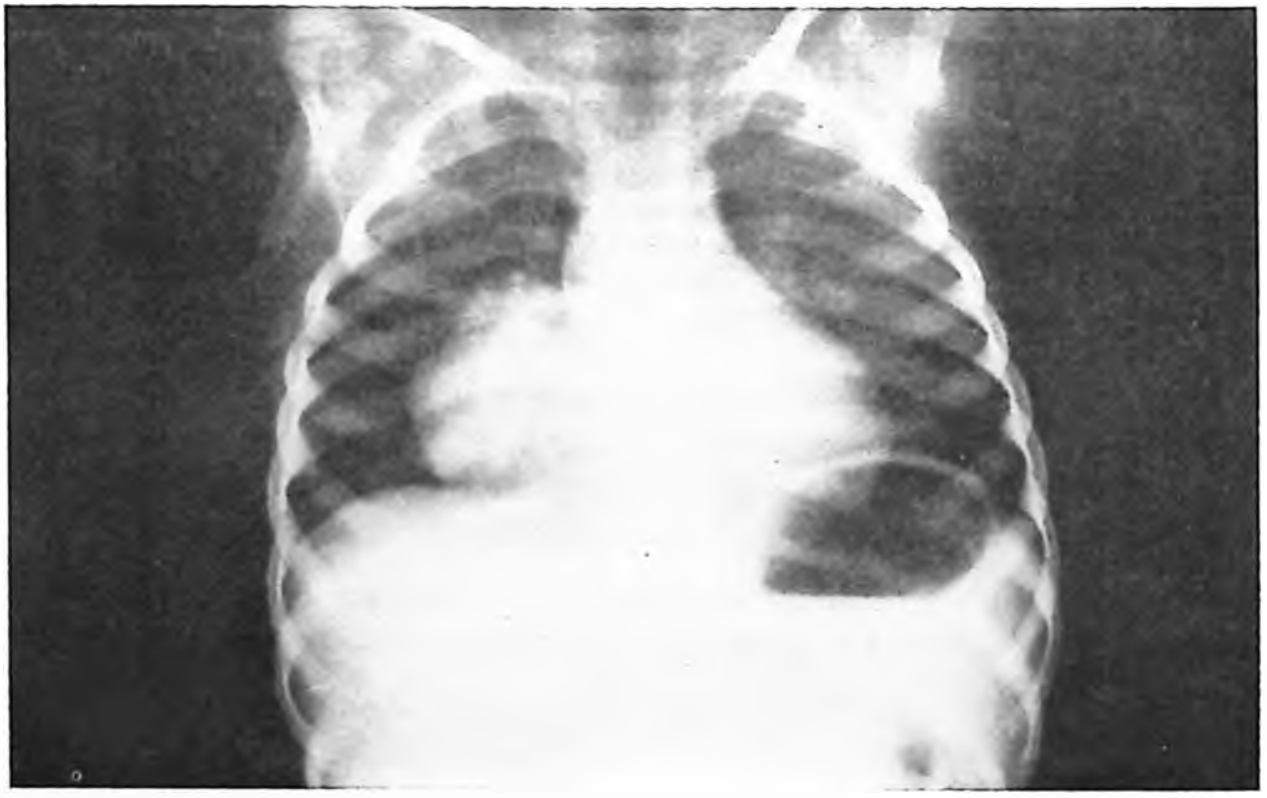

Fig. 1: The chest x-ray done after factor VIII and fresh frozen plasma therapy. Enlargement of the opacity, this time with clear borders, is seen.

revealed a pure cystic structure without internal echoes and with a thick wall. Thoracic computed tomograpy (CT) and magnetic resonance imaging (MRI) findings were also consistent with a cyst (Figs. 2 and 3) which was firmly attached on the border of the right atrium and also indented it. The wall of the cyst was remarkably thick. The patient's peripheral blood smear revealed eosinophils 27 percent anti-echinococcal antibody IgM and IgG 1/100, and Echinococcus specific IgE negative. In differential diagnosis, Echinococcus, pericardial cyst, thymic cyst bronchogenic cyst were considered.

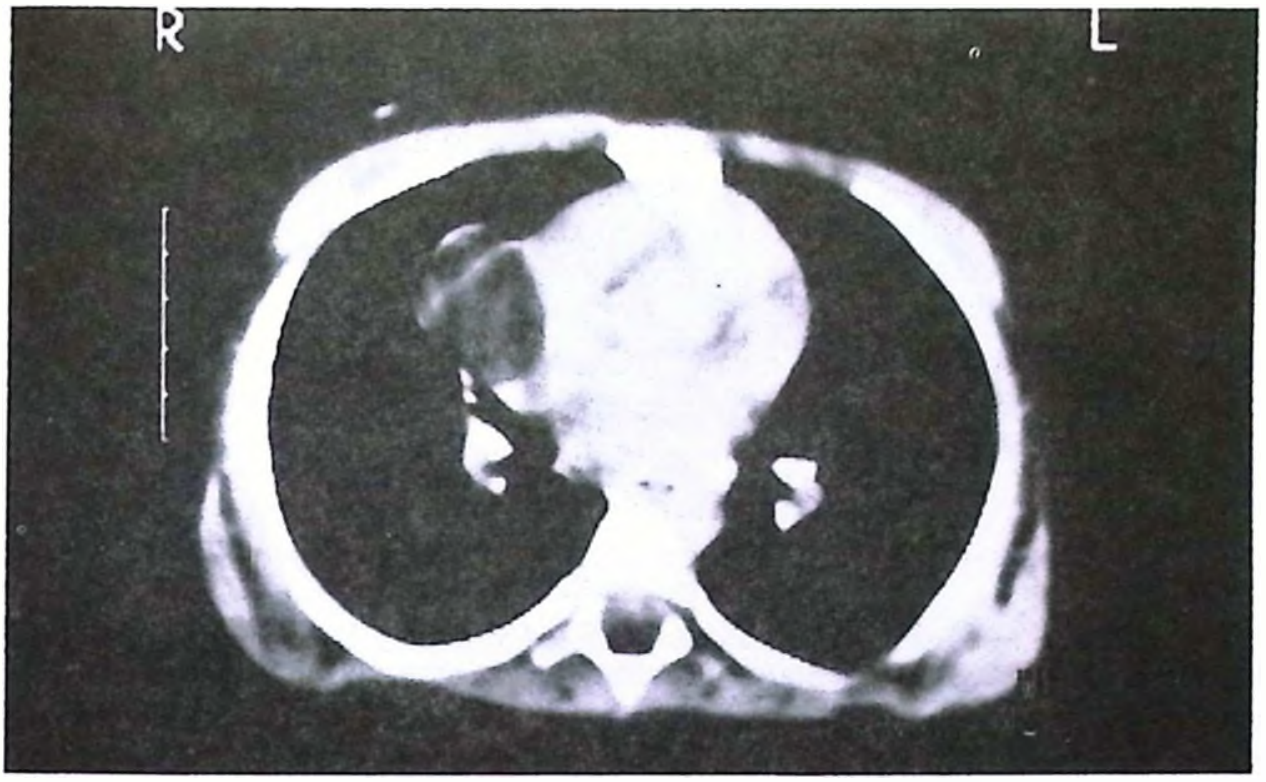

Fig. 2: Thoracal CT revealing presence of a cyst.

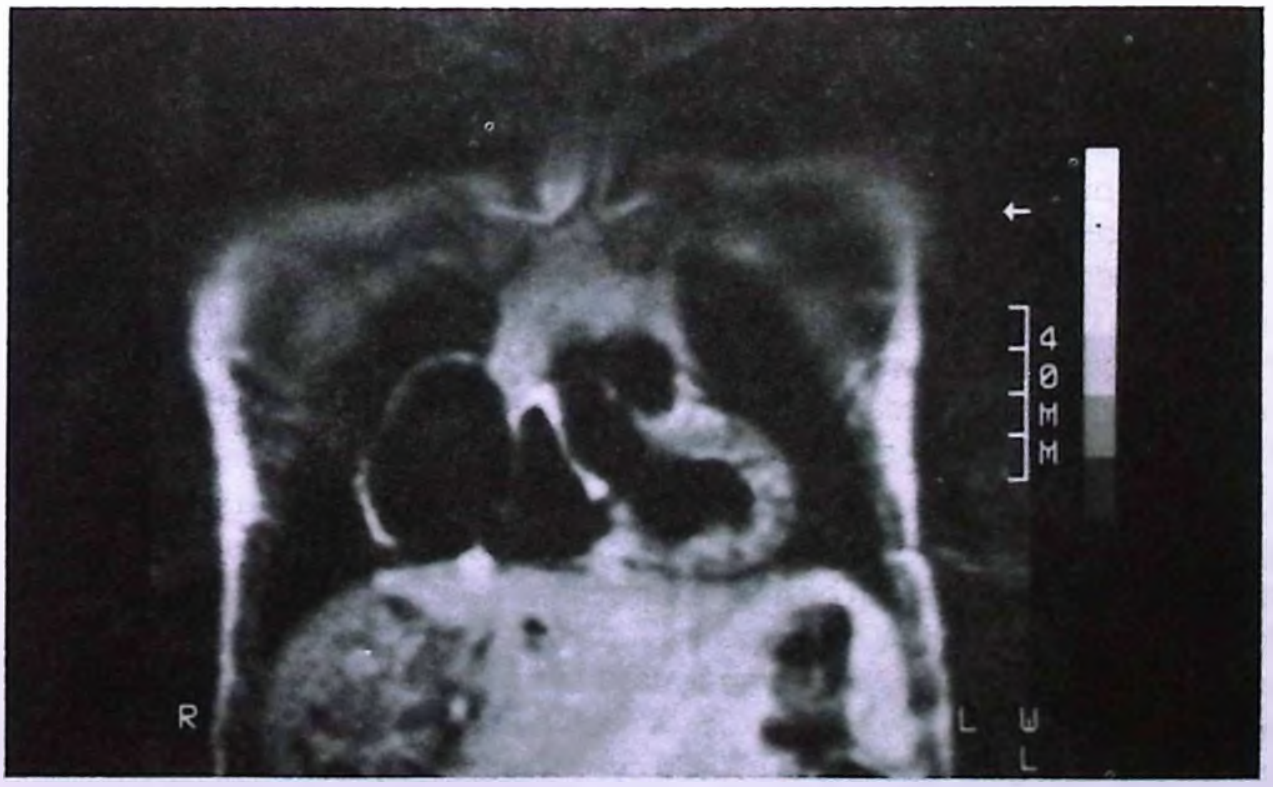

Fig. 3: Thoracal MRI showing a cyst firmly attached on the border of right atrium and indenting it. The wall of the cyst is remarkably thick.

Exploratory thoracotomy was performed after raising the factor VIII level over 60 percent. A hydatid cyst of 5 X 5 X 5 cm, with live scoleces on the right middle lobe, was found and the germinative membrane was explored. The inside of the cyst was washed with saline solution (10%), and the anterior wall of the cyst was then removed completely and the excision border was oversewn with an absorbable suture. Histological examination of the specimen also confirmed the diagnosis as a hydatid cyst. Albendazole treatment was then started at a dose of 10 mg/kg/day in two divided doses. On the 75th day of treatment, serum transaminases levels elevated mildly. The dose was tapered down by half and the drug was stopped at the third month when serum transaminases levels were normal. Chest x-ray taken 10 months after the operation was normal. The patient is fine and has been followed up at six month intervals.

Discussion

In the present case the most likely presumptive diagnosis was pulmonary hematoma. However, enlargement of the lesion instead of a reduction after appropriate fresh frozen plasma and factor VIII replacement suggested that the diagnosis of hematoma was unlikely. The symptom-free progress of the patient with such a large lesion, which was discovered by chance, is inconsistent with an abscess, or a primary or secondary malignant tumor. The radiographic appearance was not consistent with pulmonary tuberculosis or hamartoma. Although bronchogenic cysts and congenital arterio-venous fistulae could not be eliminated, as the symptoms may be delayed until adulthood[4], we did not stress them because of their rarity and focused instead on hydatid cyst.

The hydatid cyst disease is suspected with an history of residence in an endemic area. The patients are asymptomatic unless there is pressure of the enlarging cyst on the bronchial tree or pleura, secondary infection or cyst rupture. Our patient had no history of contact with possibly infected dogs; however, such history has been reported in only 29-48 percent of cases. Laboratory tests, other than radiographic examinations, are generally unreliable. The radiographic study, which is the main diagnostic tool, is accurate in 98-100 percent of cases, unless the cysts are ruptured or infected[5].

Ultrasonography distinguishes cystic lesions from solid tumors. If a desquamated germinative membrane, hydatid sand (debris) or daughter cyst (s) is seen, the diagnosis of hydatid cyst can be made confidently. These findings are pathognomonic for hydatid cyst and can be demonstrated by CT and MRI. It is striking that in ultrasonography, CT and MRI, some hydatid cysts, like in our case, might be seen as just simple cysts and nothing else. In such cases, the thickness of the wall, as in our case, can be suggestive of hydatid cyst. Thoracotomy was performed for both exploration and to prevent an anaphylactoid reaction and pneumothorax which could be fatal in the case of rupture of a possible hydatid cyst[6].

Mebendazole or albendazole is used in medical treatment of hydatid cysts[5]. In our patient, albendazole was started, as one-third of surgically treated patients reportedly had recurrences[7].

In conclusion, in hemophiliac patient such as ours, if a pulmonary lesion enlarges rapidly instead of getting smaller in spite of appropriate factor VIII replacement therapy, the diagnosis of hydatid cyst should be suspected and the appropriate therapy should be rapidly started. The diagnosis of hydatid cyst should especially be considered in areas with non-hygienic environmetal conditions.

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COMPLETE ATRIOVENTRICULAR HEART BLOCK IN CONGENITAL HYPOTHYROIDISM*

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SUMMARY: Olguntürk R, Tunaoğlu FS, Oğuz D, Cinaz P, Bideci A. (Departments of Pediatric Cardiology and Endocrinology, Gazi University Faculty of Medicine, Ankara, Turkey). Complete antrioventricular heart block in congenital hypothyroidism. Turk J Pediatr 1998; 40: 431-435.

Severe hypothyroidism in children is known to produce cardiac abnormalities such as asymmetric thickening or hypertrophy of the interventricular septum, smaller internal dimensions of the left ventricle, a smaller left ventricular outflow tract, and less systolic septal excursion. In this report, we present a 1.5-year-old boy who was admitted to our hospital because of growth retardation. According to the clinical and laboratory findings, congenital hypothyroidism, dilated cardiomyopathy (DCMP), atrioventricular complete heart block and secundum type atrial septal defect were diagnosed. *Key words: AV block, hypothyroidism, congenital.*

Severe hypothyroidism has long been known to produce abnormalities of cardiac structure and function[1]. Morphologic abnormalities such as asymmetric thickening or hypertrophy of the interventricular septum, smaller internal dimensions of the left ventricle, a smaller left ventricular outflow tract, and less systolic septal excursion have been reported in hypothyroid adults and children[2-4]. However, there is much less reported and contradictory data about disturbances of cardiac function of infants with hypothyroidism[5, 6]. In this report, we present a 1.5-year-old boy with congenital hypothyroidism who had dilated cardiomyopathy (DCMP), atrioventricular complete heart block and secundum type atrial septal defect.

Case Report

A 1.5-year-old boy was admitted to Gazi University Hospital because of growth retardation. His mother was a healthy woman. His parents were consanguineous and had a five-year-old healthy son. His physical examination revealed weight

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6100 g (< 3rd percentile), length 70 cm (< 3rd percentile), coarse facial appearance, large tongue, skin mottling, hypotonia, umbilical hernia, and pes equinovarus deformity. He was inactive, unsmiling and reluctant to contact, and was crying hoarsely. His skin was smooth and his hair was not coarse. His hematologic values were: hemoglobin 10.6 g/dl, ferritin 5.14 µg/L and hypochromia in blood smear. He had iron deficiency anemia. His chest x-ray showed cardiomegaly, and ECG showed atrioventricular complete heart block, ventricular rate 44/min and low voltage (Fig. 1a). Echocardiographic and angiocardiographic examinations displayed secundum type atrial septal defect, (QP/QS = 1.42) tricuspid insufficiency (1°) and dilated cardiomyopathy, but no pericardial effusion. His echocardiographic measurements are shown in Table I. His bone age was three months according to his extremity x-rays. Thyroid function tests were performed by RIA and TSH was performed by IRMA methods. The serum thyroxine (T4) was 1.74 µg-dl (N: 4.5-1.25 µg-dl), T3 0.44 ng/dl (N: 0.52-1.75 ng/dl), and TSH 50 mIU/ml (N: < 5.5 mIU/ml).

Based on his physical and laboratory examinations, he was diagnosed as having hypothyroidism and treated with gradually increasing doses of thyroxine starting from 0.025 mg daily, up to 0.100 mg daily.

After one month of thyroxine therapy he was smiling, and he began to sit and play. His echocardiographic measurements improved (Table I). His heart rate was 75/min, and he still had atrioventricular complete heart block (Fig. 1b). Thyroid function test results were: T4 value 10.62 µg/dl, T3 value 0.68 ng/dl, free T4 1.65 ng/dl (N: 0.73-1.95 ng/dl), free T3 2.67 pg/ml (N: 2.2-4.7 pg/ml), and TSH 6.69 mIU/ml.

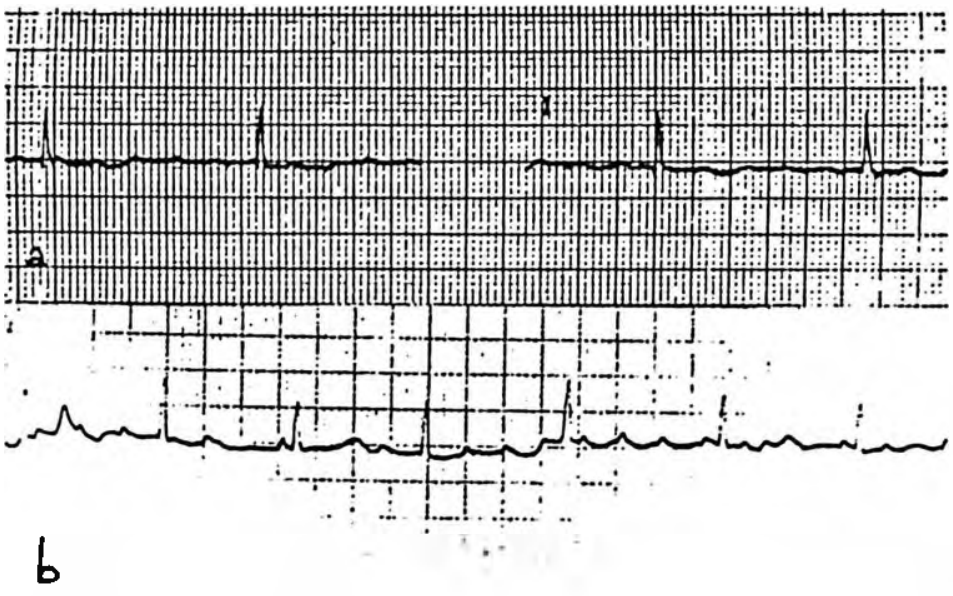

Fig. 1: a) ECG, pretreatment. b) ECG, one month after the start of therapy.

Table I: Echocardiographic Measurements

Values	Before Therapy	First Month of Therapy	Normal Values (Min-Max)
LVDd (cm	3.90	3.50	2.20-2.70
LVDs (cm)	3.00	2.20	1.30-1.90
LVFS (%)	23	37	0.28-0.44
LVVd (mm^3)	9.74	10.81	
LVVs (mm^3)	6.53	4.24	
LVEF (%)	33	61	64-83
PWd (cm)	0.30	0.50	0.4-0.55
VSd (cm)	0.30	0.50	0.4-0.5
PWs (cm)	0.60	0.80	
VSs (cm)	0.70	0.80	
PEP (msc)	56.0	64.0	$< 131 \pm 13$
ET (msc)	240	267	$< 415 \pm 11$
PEP/ET (%)	23	24	0.30-0.39

LVDd : left ventricular dimension in diastole.
LVDs : left ventricular dimension in systole.
LVFS : left ventricular shortening fraction.
LVVd : left ventricular diastolic volume.
LVVs : left ventricular systolic volume.
LVEF : left ventricular ejection fraction.
PWd : left ventricular posterior wall dimension in diastole.
VSd : ventricular septum dimension in diastole.
PWs : left ventricular posterior wall in systole.
VSs : ventricular septum dimension in systole.
PEP : pre-ejection period.
ET : ejection time.

Discussion

Our patilent's clinical and laboratory findings revealed the diagnosis of dilated cardiomyopathy, and his systolic time intervals were also abnormal. The latter findings have been previously reported in children and adults with hypothyroidism[6,7]. Though there were no reports on dilated cardiomyopathy in children with hypothyroidism, Ladenson et al.[8] had reported a young man with hypothyroidism and DCMP who showed marked improvement in left ventricular ejection fraction (LVEF), (LVIDd), and cardiac index. They suggested that the alteration in gene expression had caused the dilated myopathic heart. In our patient, the investigation with gene expression was not performed; it can be suggested that the long duration of the hypothyroidic state was the cause of DCMP.

The complete atrioventricular block was the other unusual finding in our patient. Alterations in heart rate, first degree heart block and bundle branch block are the conduction disorders so far defined in hypothyroidism[9,10]. recently accumulating data indicates that atrioventricular block is a complication of amiodarone-induced hypothyroidism[11]. Electrophysiological studies suggested the presence of dual atrioventricular nodal pathways in a patient with hypothyroidism[12]. We reached no conclusion about AV nodal pathways, as the electrophysiological study was not performed on our patient. Syed[13] reported

an infant with congenital hypothyroidism and atrioventricular block whose conduction disturbance persisted despite therapy. This is the only case with AV block we found reported in the literature. Baysal et al.[14] reported that echocardiographic pathologic findings of children with hypothyroidism returned to normal with thyroxine therapy. In our patient, we observed significant improvements in echocardiographic measurements during thyroxine therapy, but atrioventricular block was continuing. It has been suggested that cardiac function disturbances in adults with hypothyroidism are exaggerated when associated with other pathologies, like atherosclerosis[15]. Our patient had secundum type atrial septal defect, but it is known that there is no association between atrial septal defect and complete heart block.

In our patient, the improvement of cardiac function with thyroxine therapy indicates that hypothyroidism was responsible for his dilated cardiomyopathy. His conduction disturbance may be congenital, or due to his prolonged hypothyroidic state. We can also assume that the effects of hypothyroidism worsened the conduction disturbances as is the case with adults[16].

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The Turkish Journal of Pediatrics 1998; 40: 437-440

BALLOON ATRIAL SEPTOSTOMY UNDER ECHOCARDIOGRAPHIC GUIDANCE*

Case Report

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SUMMARY: Öztunç F, Saltık İL, Batmaz G, Erek E. (Pediatric Cardiology Unit, İstanbul University Institute of Cardiology, İstanbul, Turkey). Balloon atrial septostomy under echocardiographic guidance: case report. Turk J Pediatr 1998; 40: 437-440.

A seven-days-old male neonate was transferred to our institution in critically ill condition. Echocardiographic (ECHO) examination revealed the transposition of the great arteries (TGA) with a small ventricular septal defect. In the laboratory examination, arterial oxygen saturation was 29 percent and pH was 7.16. The poor condition of the neonate led us to decide to perform an immediate bedside balloon atrial septostomy (BAS) in the intensive care unit (ICU) with ECHO guidance. The umbilical vein was cannulated with a 5 Fr. Miller BAS catheter. Four balloon passes were performed resulting in large atrial septal defect. After the procedure, arterial oxygen saturation was measured at 40 percent. In TGA, the baby may present with severe hypoxia and may need management in the ICU. Emergency BAS may improve the clinical condition of the patient. Transferring the baby, who is mechanically ventilated (and is in openbed), to the catheterization laboratory takes time and can be harmful for him, and carries risk of extubation and heat loss.

The limitations of transthoracic ECHO guidance of BAS include the possibility of a poor ECHO window in an ill neonate on assisted ventilation and possible interference with maneuverability for both echocardiographer and catheter operator. It also carries the risk of contamination of the sterile field. When the advantages and disadvantages of transthoracic ECHO guidence are considered, transferring the baby to the catheterization laboratory can cause problems and time loss. Thus, ECHO-guided BAS at bedside is an efficient and good alternative. The transumbilical approach may be easier in the first few days of life. *Key words: balloon atrial septostomy, echocardiography.*

Balloon atrial septostomy (BAS) is a standard palliative procedure to improve intracardiac blood mixing in neonates with transposition of the great arteries[1]. It has traditionally been performed in the cardiac catheterization laboratory under fluoroscopic guidance[2,3]. The cross-sectional echocardiography (ECHO) has been used to help position the balloon catheter during the procedure. The advantages given for this method are the increased speed in performing the procedure, the reduced risk of trauma to atrioventricular valves and other vital

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structures, and the reduced exposure to radiation[4,5]. The procedure has been performed only with echocardiographic guidance, especially in the critically ill neonates in the intensive care unit (ICU)[2,3,5,6].

Case Report

A seven-day-old male neonate weighing 2000 g was transferred to our institution in critically ill condition. The patient was accepted to the ICU for evaluation of the cyanosis. In the physical examination, tachypnea, intercostal retractions and 2/6 systolic murmurs in the left sternal border were noted.

Echocardiographic examination revealed transposition of the great arteries with three mm ventricular septal defect in the midtrabecular interventricular septum and a small patent ductus arteriosus. There was a bidirectional shunt through the ventricular septal defect. There was no interatrial septal defect or shunt. In the laboratory examination arterial oxygen saturation was 29 percent and pH was 7.16.

The neonate was immediately placed on an infusion of prostaglandin E_1, but no improvement in oxygen status or acidosis was noted. After the placement arterial cut-down, the maximal arterial pressure was measured as 35 mmHg and the patient was then entubated. Because of the reduction of the urinary output a peritoneal dialysis was placed. The poor condition of the neonate led us to decide to perform an immediate bedside balloon atrial septostomy in the ICU with echocardiographic guidance. The umbilical vein was cannulated with a 5 Fr. Miller septostomy catheter. It was advanced to the left atrium through ductus venosus, right atrium and foramen ovale. Four balloon passes were performed resulting in an atrial septal defect of five mm with unrestrictive left-to-right shunt (Figs. 1 and 2). After the procedure, arterial oxygen saturation was measured at 40 percent. In spite of intensive care and treatment, the baby died after 10 hours.

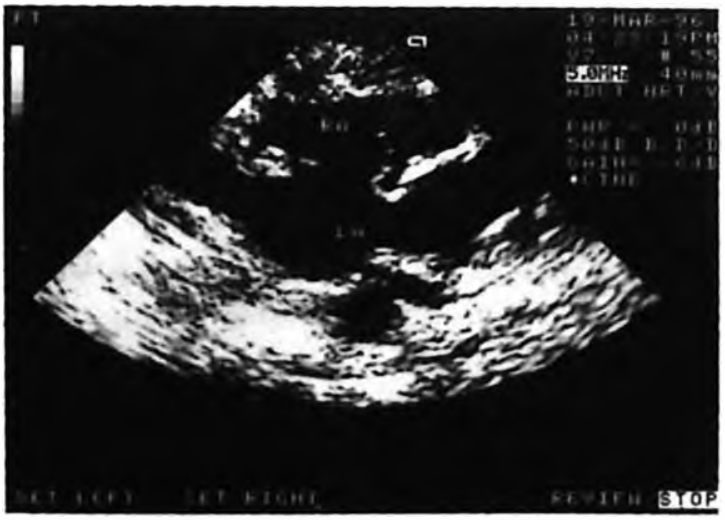

Fig. 1: Subcostal view showing the adequate size of interatrial communication.

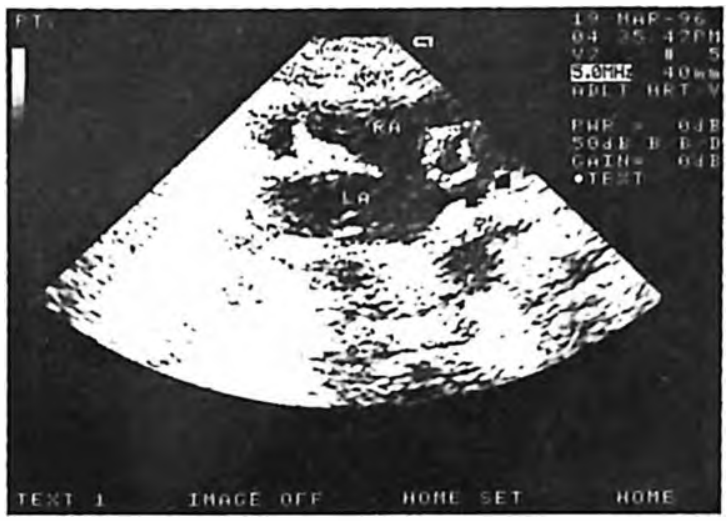

Fig. 2: Subcostal view showing the balloon that is being pulled from the left atrium to the right atrium.

Discussion

Balloon atrial septostomy (BAS) has been traditionally performed under fluoroscopic guidance, but the use of two-dimensional echocardiography is a method that augments the safety of the procedure[7]. The advantages of this method can be listed as fllows: it increases the speed of the procedure, reduces risk of trauma to atrioventricular valves and other vital structures, and reduces exposure to radiation[1, 2, 4, 5, 7, 8]. Two-dimensional echocardiography also enables the operator to directly assess the adequacy of the septostomy by measuring the diameter of the interatrial communication[5, 8].

Because of restrictive interatrial communication, the baby may present with severe hypoxia and acidosis and may need management in the ICU. In spite of beginning prostaglandin infusion and medical therapy for the acidosis, the hypoxemia may not improve. Emergency BAS may improve the clinical condition of the patient, with augmented mixing of the saturated and unsaturated blood[3]. Transferring the baby, who is mechanically ventilated (and is in openbed), to the catheterization laboratory takes time and can be harmful for him, and carries risks of extubation and heat loss[2, 4]. BAS has been performed recently in such babies with only ECHO guidance. This method is also effective and is less time consuming under these circumstances[4]. Thus, we decided to perform BAS in our patilent in the ICU because of his poor clinical condition.

When the femoral vein is used (in patients older than three days of age), it is possible to insert a sheath, change the catheters through it and measure the pressures and oxygen saturation after the procedure. However, it may be difficult to find a femoral vein in a neonate, thus consuming time, and it also removes the usage of the femoral vein for other invasive procedures[9]. Furthermore, in the first few days of life, because of the incomplete closure of the ductus venosus,

the transumbilical approach is a good alternative[1,9]. When the umbilical vein is used as the site of entry, the control of the pressures and oxygen saturation is not possible after BAS.

The occurrence of iliac vein or inferior vena cava thrombosis following BAS via the femoral vein is not a rare complication[1]. The umbilical venous approach may be complicated by the dislodging of a thrombus through the ductus venosus to the circulation[7].

The limitations of transthoracic ECHO guidance of BAS include the possibility of a poor ECHO window in an ill neonate on assisted ventilation and posible interference with maneuverability for both echocardiographer or catheter operator. In babies in the ICU, there was the risk of contamination of the sterile field because of the difficulties in maneuverability[10].

When the advantages and disadvantages of transthoracic ECHO guidance are considered, transferring the baby to the catheterization laboratory can cause problems and time loss. Thus, ECHO-guided BAS at bedside is an efficient and good alternative. The transumbilical approach may be easier in the first few days of life.

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OCCULT PNEUMOCOCCAL SEPTICEMIA AND PNEUMOCOCCAL PNEUMONIA COMPLICATING PNEUMATOCELES IN A PREVIOUSLY HEALTHY CHILD*

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SUMMARY: Aslan Y, Orhan F, Dinç H, Soylu H, Erduran E. (Departments of Pediatrics and Radiology, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey). Occult pneumococcal septicemia and pneumococcal pneumonia complicating pneumatoceles in a previously healthy child. Turk J Pediatr 1998; 40: 441-445.

Fulminating pneumococcal septicemia without an obvious focus of infection is very rare in previously immunocompetent children older than two years. Furthermore, pneumatocele formation in pneumococcal pneumonia is uncommon. The majority of pneumatoceles are self-limited and disappear spontaneously. Here, we report a six-year-old healthy child with occult pneumococcal septicemia and pneumococcal pneumonia secondary to septicemia. Giant pneumatoceles causing respiratory insufficiency formed secondary to the pneumococcal pneumonia and were aspirated via needle under fluoroscopic guidance. *Key words: pneumococcal septicemia, pneumatoceles.*

Pneumococci are important agents of pneumonia, sinusitis, and otitis media, and are a syndrome of occult bacteremia and pyogenic meningitis in children[1]. Occult pneumococcal bacteremia is very rare in healthy children older than two years, but severe septicemia occurs in patients with functional or anatomical absence of the spleen[2].

Pneumococci are common causes of bacterial pneumonia over three months. They produce lobar involvement and may cause pleural effusion and sometimes lung abscesses. Pneumatoceles, however, are uncommon and have been discussed in only three reports to date[3-5].

We report a six-year-old previously immunocompetent child with pneumococcal septicemia and secondary pneumococcal pneumonia with pneumatocele formation.

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Case Report

A six-year-old boy was admitted to our clinic with the complaints of fever, tachypnea, abdominal pain and confusion, which had been present for one day. Medical history indicated a previously healthy child. On physical examination body temperature was 38.9 °C, pulse rate 128/min, respiratory rate 56/min and blood pressure 60/30 mmHg. He was hyperpneic, pallid and confused. Common moderate rales were heard on both lung fields. The spleen and liver were not palpable. Cutaneous petechiae and ecchymoses, peripheral cyanosis and coldness were noted. Other than lethargy, his neurologic findings were normal and his neck was not stiff. Laboratory studies revealed hemoglobin 8.2 g/dl, white blood cell count 1600/μl (63% polymorphonuclear neutrophils, 22% band neutrophils, 15% lymphocytes, immature/mature + immature neutrophil ratio = 0.26), and platelet count 97,000/μl. The peripheral smear showed toxic granulation in neutrophils. Prothrombin time was 35 seconds and partial thromboplastin time was 92 seconds. The erythrocyte sedimentation rate was 55 mm/h. Fibrinogen was 69 mg/dl (N: 125-300) and fibrin split products were present. Neutrophil functions, serum immunoglobulin levels, CD4/CD8 ratio and CH50 were normal. Sickledex test yielded negative result, and function of the spleen, as documented by radionuclide liver-spleen scans, was normal. Biochemical analyses yielded serum urea 28 mg/dl, creatinine 1.1 mg/dl, AST 198 U/L, and ALT 213 U/L. Streptococcus pneumonia was grown in blood culture. Arterial blood gasses showed metabolic acidosis. Chest x-ray, and abdominal and renal ultrasound were normal. The diagnosis of pneumococcal septicemia was made and the patient was treated with ceftriaxone (75 mg/kg/day) and amikacin sulfate (15 mg/kg/day), with adequate fluid replacements and monitoring of pulse, respiration, blood pressure and urine output.

On the fourth day of admission, he developed cough and dyspnea. On physical examination, breath sounds were diminished on the right hemithorax and there were rales on the left hemithorax. Plain chest x-ray revealed air bronchograms. consolidation in the right upper lung fields, right-sided pleural fluid and pneumatocele formation in the right paracardiac and peripheral lung fields (Fig. 1). Examination of pleural fluid revealed a cloudy appearance, pH 7.25, specific gravity 1019, glucose 46 mg/dl, protein 3 g/dl, lactate dehydrogenase (LDH) 1260 U/L, pleural fluid LDH/serum LDH ratio 18, WBC 1200/μl and pneumococci in gram staining. Streptococcus pneumoniae isolated in pleural fluid culture. An expectorant agent was added to the therapy and the dose of the ceftriaxone was increased to 100 mg/kg/day.

During the five-week period of hospitalization, his clinical findings (other than tachypnea and moderate dyspnea), and hematological and biochemical disorders improved, but arterial blood gasses showed moderate hypoxemia,

and pulmonary function tests showed restriction pattern. Plain x-ray and computed tomography of the chest showed multiple large pneumatoceles on the right middle and lower lung fields and there was also compressive atelectasia at the medial sites of the pneumatoceles (Figs. 2a, b).

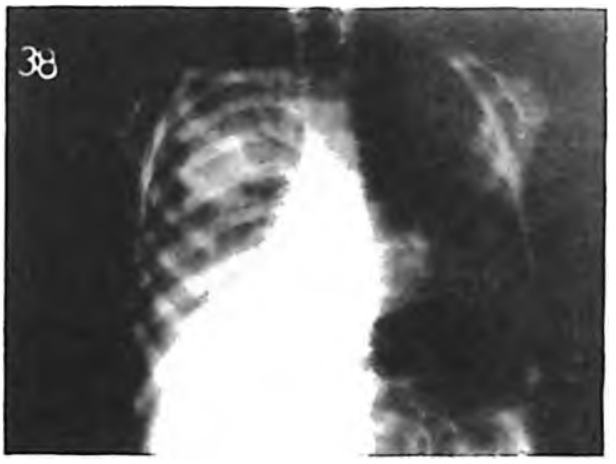
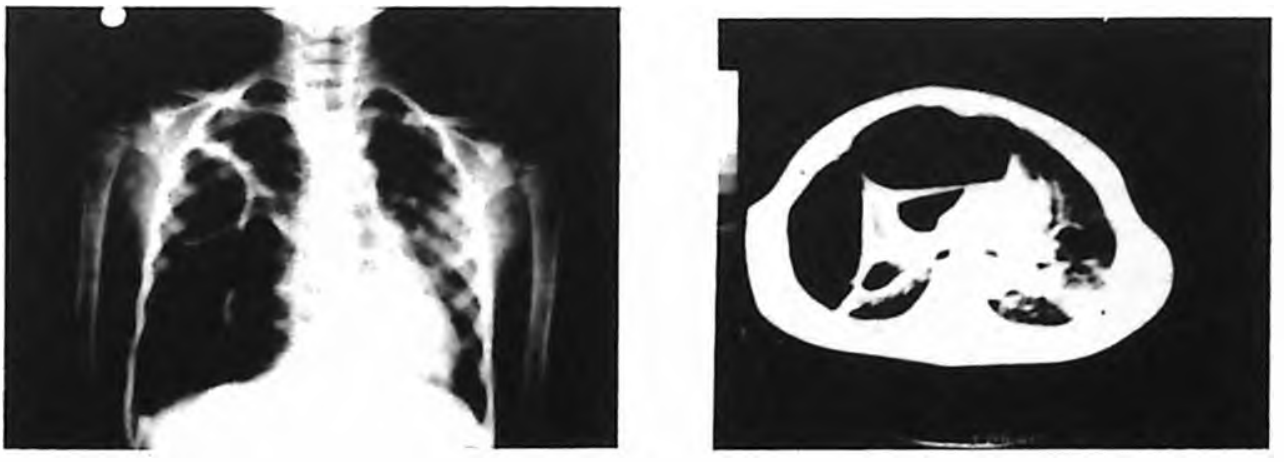


Fig. 1: Plain chest x-ray at fourth day of admission shows air bronchograms, consolidation in the right upper lung fields, right-sided pleural fluid, and multiple pneumatoceles in the right paracardiac and peripheral lung fields.



(a) (b)

Figs. 2a, b: Plain x-ray (a) and computed tomography (b) of the chest at the end of the fifth week of treatment and before needle aspiration show multiple large pneumatoceles in the right middle and lower lung fields, and compressive atelectasia at the medial sites of the pneumatoceles.

At the end of the five-week therapy, 18 Gauge intracat needles were inserted into the pneumatoceles by fluoroscopic guidance and the air in the pneumatoceles was aspirated by syringes, because respiratory difficulty persisted. Decompression via needles resulted in improved respiration, pulmonary functions and blood gasses. Immediately after the drainage, plain chest x-ray showed disappearance of pneumatoceles and reexpansion of the right lung field (Fig. 3).

Fig. 3: Plain chest x-ray after the needle aspirations shows disappearance of the pneumatoceles and reexpansion of the right lung field.

Discussion

Previously immunocompetent nonhospitalized patients may develop community-acquired bacteremia and/or sepsis from extension of local tissue infections, such as pneumonia due to streptococcus pneumoniae and Hemophilus influenzae type b, gastroenteritis due to salmonella, pyelonephritis due to Escherichia coli, etc. Alternatively, colonization and local mucosal invasion by a particularly virulent pathogen in a previously normal host may produce primary bacteremia and sepsis[6]. Occult bacteremia (without an obvious focus of infection) due to streptococcus pneumonia occurs in relatively well-appearing children between three and 24 month of age. The increased incidence of bacteremia among three to 24-month-old children may be due in part to a maturational immune deficiency in the production of opsonic IgG antibodies to the polysaccharide antigens present on these encapsulated bacteria[7].

Severe pneumococcal septicemia is extremely rare in healthy children older than 24 months[2, 6]. However, pneumococcal septicemia occurs in children with sickle cell anemia, asplenia, agammaglobulinemia, AIDS, etc.[2, 7-9]. This six-year-old case was previously healthy, and he doses not have immunodeficiency, splenic dysfunction, sickle cell disease, and/or local tissue infection.

Pulmonary pneumatoceles are uncommon but generally benign, thin-walled parenchymal air collections arising in association with acute pneumonia[10]. Although pneuomatoceles can be seen in the course of staphylococcal pneumonia, they also occur in pneumonia caused by other bacteria such as Hemophilus influenzae, streptococci, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, etc[11]. Pneumatocele formation is unusual in pneumococcal pneumonia, there are only a few reports present in the literature demonstrating pneumatoceles following pneumococcal pneumonia[3-5]. The present case developed pneumatoceles following pneumococcal pneumonia.

The majority of pneumatoceles are self-limited and disappear spontaneously within a few days or months. Rarely, they may attain such size as to severely affect respiration and require treatment[10]. In the present case, the pneumatoceles were aspirated via needle under fluoroscopic guidance. Complete resolution of pneumatoceles and recovery of a normal respiratory pattern were achieved.

We conclude that streptococcus pneumonia may cause pneumatocele formation, albeit rarely, and that giant pneumatoceles may be easily treated by needle aspiration.

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CONGENITAL SIALIDOSIS*

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SUMMARY: Ovalı F, Samancı N,Güray A, Akdoğan Z, Akdeniz C, Dağoğlu T, Petorak İ. (Neonatology Unit, Department of Gynecology and Obstetrics, İstanbul University İstanbul Faculty of Medicine, İstanbul, Turkey). Congenital sialidosis. Turk J Pediatr 1998; 40: 447-451.

Congenital sialidosis is a rare disease resulting from the absence of neurominidase and presenting with hydrops fetalis, hepatosplenomegaly, dysmorphic features, vacuolated lymphocytes and extensive vacuolation of the connective tissue. Elevated levels of sialooligosaccharides in the urine is characteristic. We describe a newborn baby with congenital sialidosis and discuss the difficulties in reaching the diagnosis.
Key words: sialidosis, neurominidase, hydrops fetalis.

Sialidosis in hereditary lysosomal storage disease which results from the absence of neurominidase. The absence of the enzyme leads to the accumulation of sialic acid in tissues and in urine. However, more than one clinical subtype is associated with abnormalities of neurominidase deficiency. The inheritance pattern is autosomal recessive.

The congenital form of this disease is extremely rare and presents with hydrops fetalis during pregnancy. We herewith present a case of congenital sialidosis who died in the early neonatal period.

Case Report

The baby boy was the first product of non-consanguineous parents delivered vaginally at 36 weeks of gestational age after an uncomplicated pregnancy. During prenatal follow-up, hydrops fetalis was diagnosed at 26 weeks of gestational age and an amniocentesis was performed. There was no blood group incompatibility and syphilis, toxoplasmosis, rubella, cytomegalovirus, herpesvirus and parvovirus serology were negative. The chromosomal analysis revealed 46XY genotype without any numeric or major structural abnormality. The fetus was evaluated as non-immune hydrops fetalis of unknown etiology.

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A first cousin of the mother had delivered a similar baby two months previously. That baby was also hydropic, had coarse facies and hepatosplenomegaly. A clinical diagnosis of congenital nephrotic syndrome was made and the baby was hospitalized for a month at the Pediatric Nephrology Department. The kidney biopsy suggested a storage disease but further investigations could not be made because the baby died suddenly due to septic shock.

This baby's appearance was hydropic with coarse facies with depressed nasal bridge and frontal bossing (Fig. 1). His weight was 3160 g (75^{th} percentile), length 47 cm (50^{th} centile) and head circumference 33 cm (50^{th} percentile). The abdomen was distended with massive ascites, hepatomegaly of four cm and splenomegaly of five cm. He had tachypnea and respiratory distress due to abdominal distention. There were a few crackles over both lung fields. The heart rate was 140/min with no murmurs. The peripheral pulses were palpable. He had bilateral inguinal herniae with communicating hydrocele. The corneal and funduscopic examinations of the eyes were unremarkable.

Fig. 1: Hydropic baby. Note the protuberant abdomen and swollen testes.

Complete blood count revealed a hematocrit of 41 percent, hemoglobin: 13.1 g/dl, mean corpuscular volume: 106 fl, red cell distribution width: 33 percent, platelets: 121,000/ml, and white blood cells: 10,400/ml. In the peripheal blood smear, most of the lymphocytes contained cytoplasmic vacuoles (Fig. 2). Routine blood chemistry was unremarkable. Slight proteinuria was observed in the urine examination. At the thin layer chromatography of the urine, a sialooligosaccharide band was observed. The amount of sialic acid in the 24-hour urine was 12.3 nmol/mg creatinine, about 12 times the normal amount. Abdominal ultrasonography revealed hepatosplenomegaly, ascites, nephromegaly and an increase in the kidney echogenity (Grade III). Echocardiographic examination was within normal limits. The roentgenographic examination of the bones was normal.

There were multiple foamy cells and lymphocytes with cytoplasmic vacuoles at the bone marrow aspiration. The electronmicroscopic examination of the tissue obtained at the skin biopsy showed extensive "empty" vacuolation at the capillary endothelium and pericytes among the collagen fibers of the subcutaneous connective tissue (Fig. 3). The baby thrived for 27 days during which his condition changed very little. His cardiorespiratory condition then suddenly deteriorated and he died on the 27th day of life.

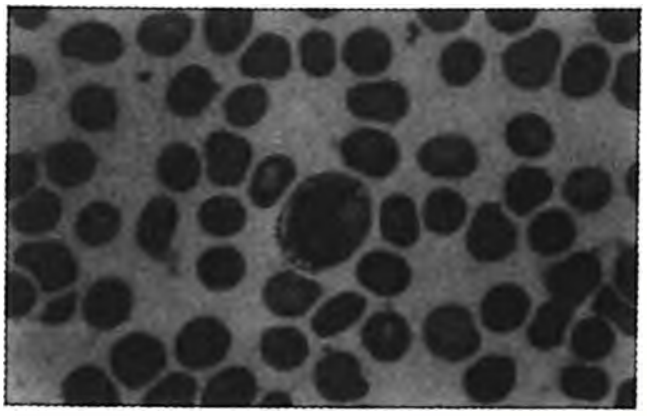

Fig. 2: Peripheral lymphocytes with multiple vacuoles.

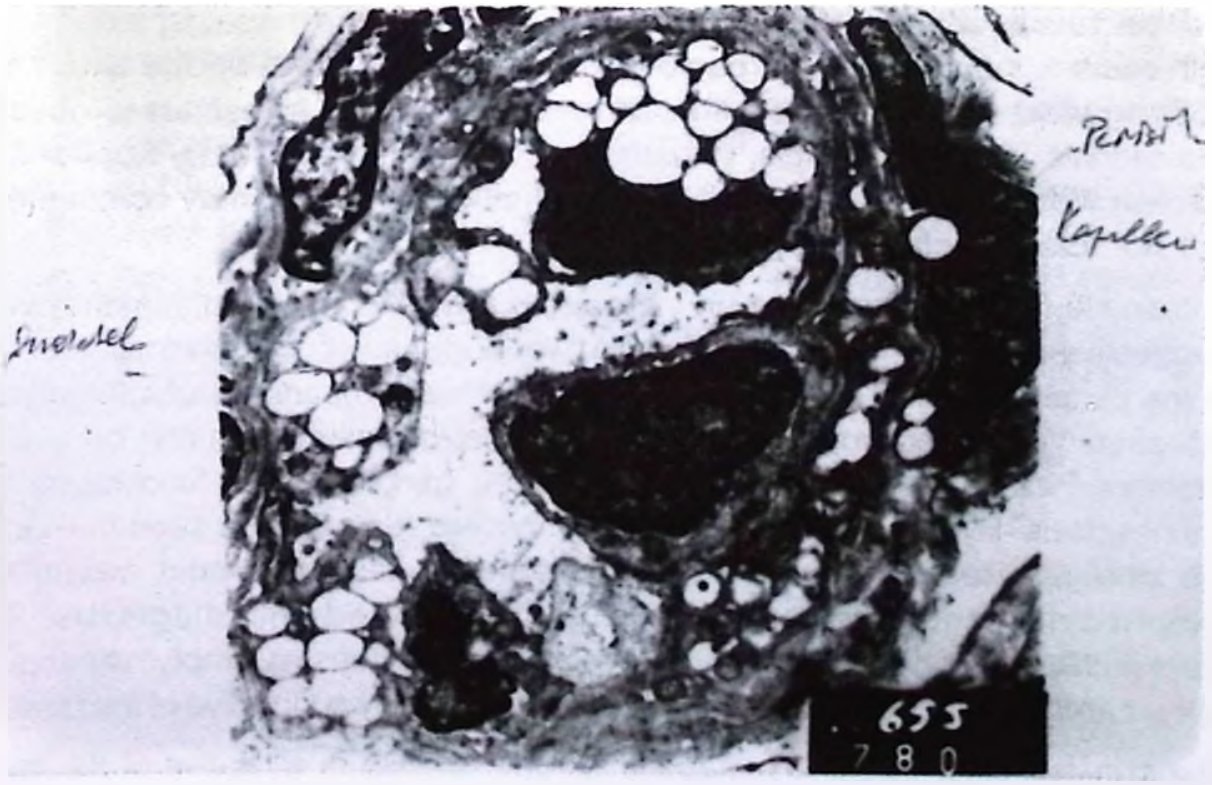

Fig. 3: Electron microscopic appearance of the skin. The "empty" vacuoles in the capillary wall are caharacteristic.

Discussion

Neurominidase is a lysosomal enzyme the absence of which leads to an accumulatilon of sialic acid in the tissues. According to Lowden and O'Brien[1] there are two types. Type I sialidosis is also called the normosomatic type or cherry-red spot myoclonus syndrome. Typical symptoms begin at eight to 25 years of age and the red degeneration of the macula with subsequent vision loss is characteristic. Lenticular opacities may be seen in some cases. Myoclonus is generalized and responds poorly to medical therapy. Most patients die at young ages[2].

Type II sialidosis is also called the dysmorphic type and there are three forms of this type. Coarse facies and dysostosis multiplex are common to all of them. The juvenile form is seen predominantly in Japan, at two to 20 years of age. Hepatosplenomegaly and renal involvement are not common. Most patients live until the fourth or fifth decade. The infantile form is symptomatic within the first 12 months of age and hepatosplenomegaly and renal involvement are common. Most patients die in the first or second decade. The congenital form is the rarest form and presents with hydrops fetalis in the perinatal period. Widespread edema, ascites, hepatosplenomegaly, inguinal hernia, renal involvement, epiphyseal stippling and corneal clouding are common features of the disease. Survival is limited in these infants[2-4].

In our patient, hydrops fetalis was diagnosed antenatally but the amniocentesis did not reveal any positive finding. Hydrops fetalis and hepatosplenomegaly with ascites, inguinal hernia, coarse facies, depressed nasal bridge and frontal bossing were all compatible with sialidosis[5]. The cause of ascites is obscure in sialidosis. Hypoproteinemia, sinusoidal obstruction in the liver by Kupffer cells swollen with storage material and diaphragmatic dysfunction may contribute to, but not totally explain, ascites.

Proteinuria, nephromegaly and an increase in the echogenity of the kidney were suggestive of renal involvement. Multiple lymphocytes with cytoplasmic vacuoles in the peripheral blood smear and bone marrow were important clues for storage diseases. Vacuolated lymphocytes and bone marrow foamy cells can be seen in Niemann-Pick disease, Wolman's disease, GM_1 gangliosidosis, fucosidosis and nannosidosis. Hydrops fetalis and neonatal ascites may also be seen in most of the storage diseases[2]. However, high levels of urinary sialic acid, vacuolated lymphocytes and dysmorphic features supported the diagnosis. The electronmicroscopic findings of the skin biopsy with extensive "empty" vacuolation of the capillary endothelium and pericytes were also highly suggestive of the disease.

The previous diagnosis of a metabolic storage disease in a relative of the family stimulated us to search for storage diseases in this patient also. The diagnosis of sialidosis was highly suspected after the observance of the sialooligosaccharide

band in the urinary thin layer chromatography and was confirmed by the more than 12-fold increase in the levels of sialic acid in the 24-hour urine. A definite diagnosis of sialidosis requires showing the deficiency of neurominidase activity in the fibroblast culture[2,5,6]. This investigation could not be performed due to technical difficulties. However, since the elevated levels of sialic acid in the urine is also definitive for diagnosis, the patient was evaluated as having congenital sialidosis[2].

Since a relative of the mother had died with similar findings, a prenatal diagnosis of this patient might have been made[7,8]. However, it was only after the baby was born that a detailed history was taken from the family which revealed this connection. Sialidosis and other storage diseases are rare causes of nonimmune hydrops fetalis; however, they should be kept in mind when evaluating a baby with hydrops fetalis. Detailed health histories are of paramount importance in this regard.

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The Turkish Journal of Pediatrics 1998; 40: 453-459

CHERUBISM: A RADIOLOGICAL AND CLINICAL PRESENTATION*

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SUMMARY: Yücel ÖT, Genç E, Kaya S. (Department of Otorhinolaryngology, Hacettepe University Faculty of Medicine, Ankara, Turkey). Cherubism: a radiological and clinical presentation. Turk J Pediatr 1998; 40: 453-459.

Cherubism is the hereditary form of the fibrous dysplasia of the jaws, but it may be seen sporadically as well. The disease has a self-limited nature and is rarely apparent before the age of two. There is no need to interfere surgically with these lesions of the mandible or the maxilla unless the child is severely affected, i.e. the disease deteriorates respiration, deglutition, vision, or the psychiatric makeup of the child due to cosmetic reasons. The clinical presentation and radiological evaluation of these children are so typical that the pediatrician and pediatric otolaryngologist need to be informed about this rarely seen disease. A case of a cherubic child, with his clinical appearance as well as his radiological evaluation, and discussion about the clinical outcome are presented. *Key words: familial fibrous dysplasia, cherubism, mandible, maxilla.*

Cherubism, the hereditary form of fibrous dysplasia, was first described by Jones[1] for in 1933, four out of five siblings of a Jewish family.

There is a proliferation of fibrous tissue and giant cells to the extent that the "bone-forming" mesenchyme is disturbed at the jaws, causing painless, symmetric swellings. Cherubism histologically appears as a giant cell reparative granuloma and is accepted to have an autosomal dominant inheritance[2].

Cherubism has been described as a hereditary illness, but we peresent a sporadic case with typical clinical and radiological presentation to inform physicians regarding this rarely seen disease.

Case Report

A seven-year-old boy was admitted to our hospital because of the symmetrical swelling of his malar areas and lower jaw. His father stated that the swelling on the face had begun at the age of four, simultaneously with that at the chin. Family history inquired of discretely was negative for such a disease in other siblings of the family and for the relatives as well.

The patient's eyes were rolled with an upward gaze, disclosing narrow bands of sclera beneath the iris. The palatal vault was found to have a "V" shape and there was a considerable bulging of the roof, mostly on the right side. (Figs. 1 and 2).

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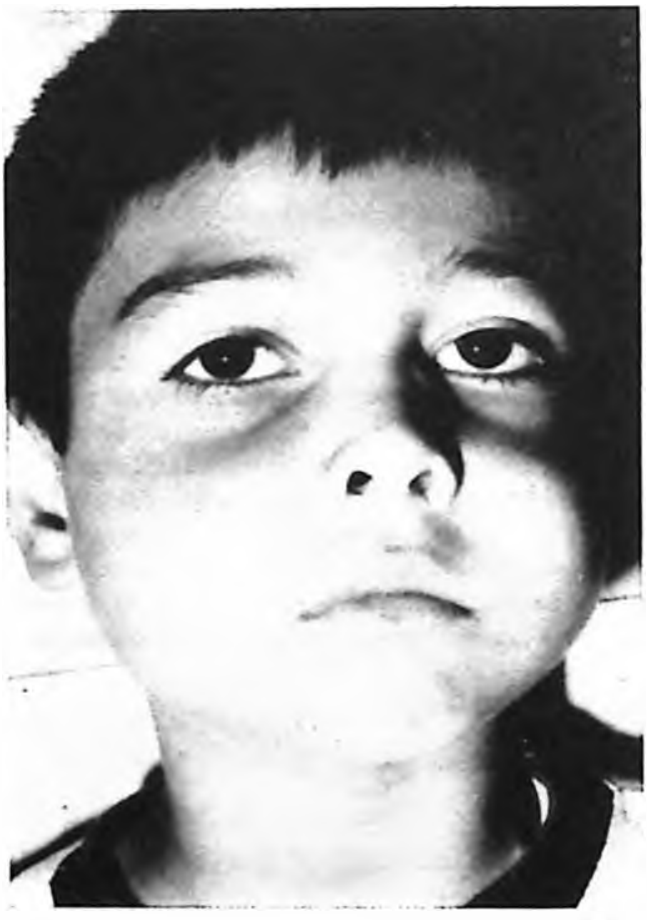

Fig. 1: General appearance of the cherubic child with upward gaze and porminent malar areas.

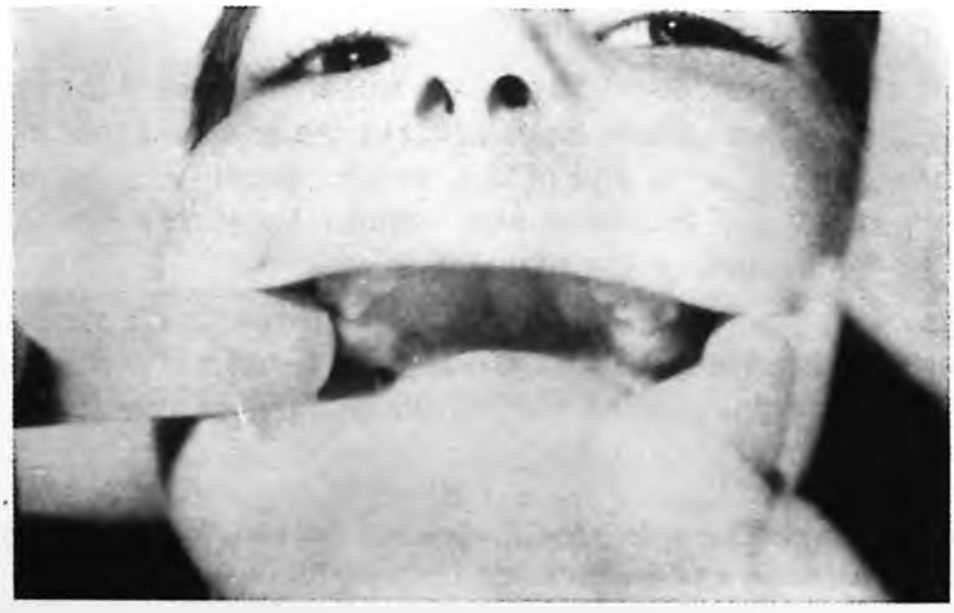

Fig. 2: The "V"-shaped palate.

X-ray studies showed bilateral symmetric, well-defined, multilocular areas of radiolucency, mostly at the molar areas. The thinning of the cortical bone is very typical with the expansion of the medullary part of the bone (Figs. 3 and 4). Computed tomography (CT) scan of the mandible and maxillary region showed these lesions more clearly as symmetric thinning of the cortical bone; multilocular hypodense areas in the core can be noted (Figs. 5 and 6).

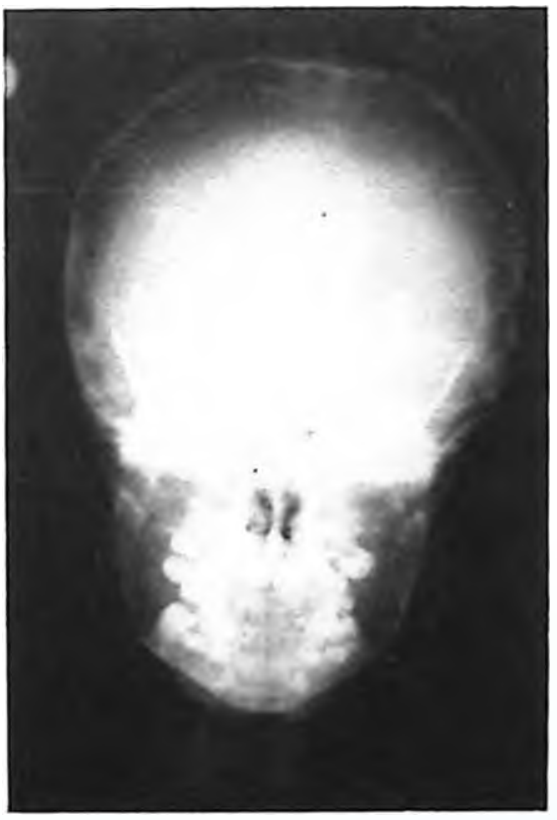

Fig. 3: The antero-posterior mandibular x-ray of the patient showing cortical thinning.

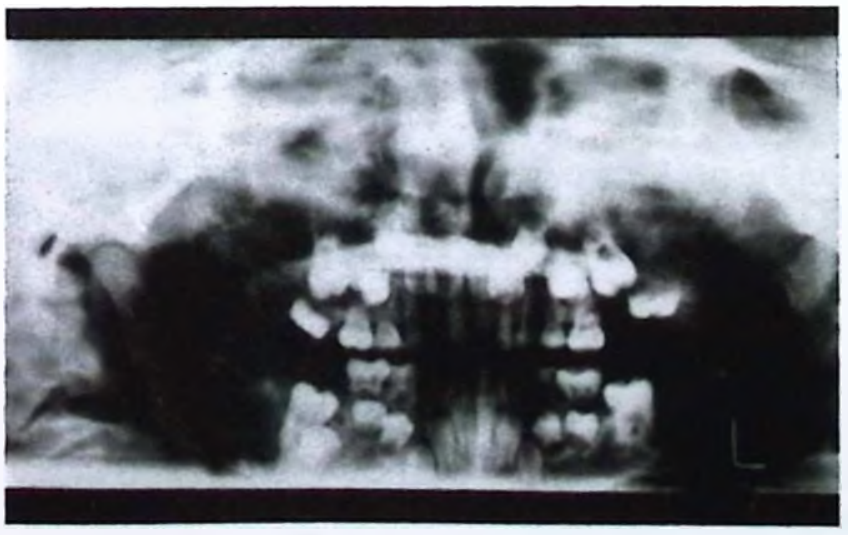

Fig. 4: The panoramic mandibular x-ray of the patient. The status of the teeth is typical.

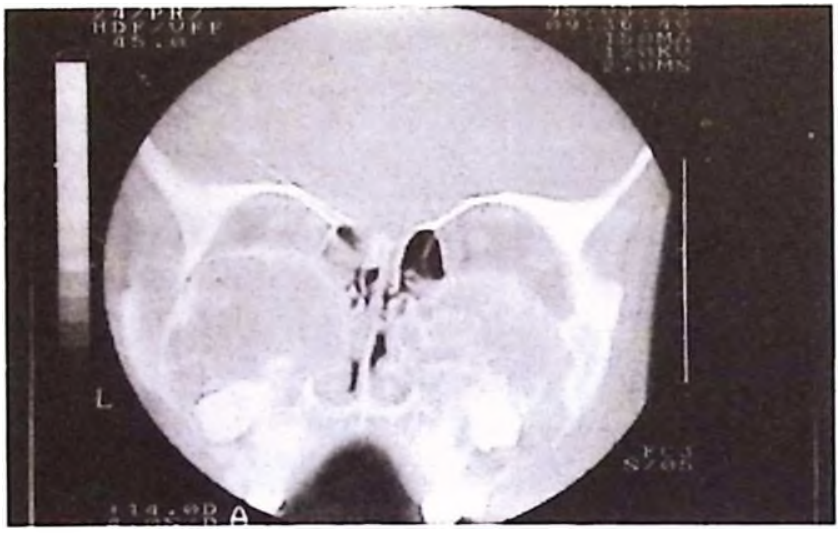

Fig. 5 The computed tomography (CT) scan of the maxilla showing the cortical thinning and medullary ballooning.

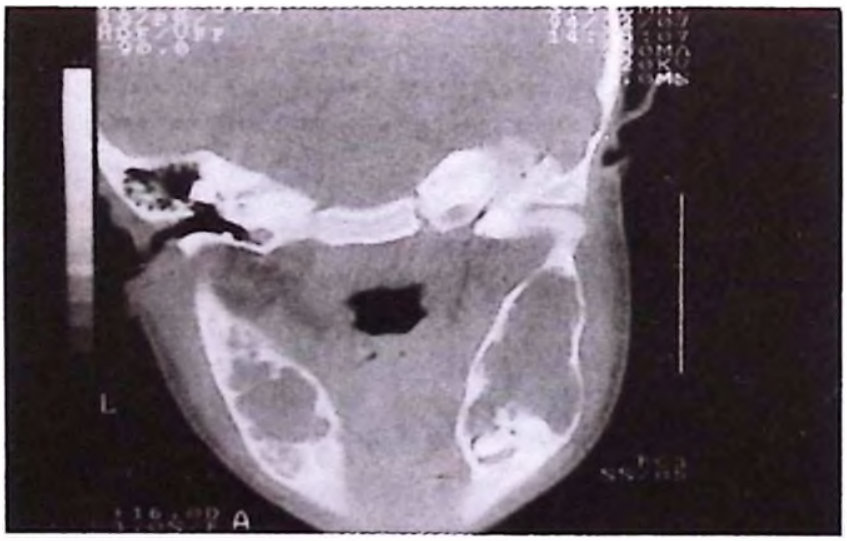

Fig. 6: The CT of the mandible with extreme cortical thinning and multilocularity in the medullary part.

On both sides of the neck there were enlarged lymph nodes, multiple in number, with the largest being two cm in diameter. A lymph node biopsy done previously in another center and confirmed in our pathology department revealed that the nodes had chronic inflammatory nonspecific changes. The bone marrow aspirate taken by the same center showed the hypercellularity of the bone marrow.

The systemic examination and laboratory work-up, except for a slight elevation of alkaline phosphatase, were normal.

In view of the history, the physical examination and the radiological evaluation of the patient, a diagnosis of "cherubism" was made.

Discussion

Since 1933 when cherubism was first reported many synonyms have been used to define the condition. Some of them are: the original name given by Jones of "familial multilocular cystic disease"[1], "bilateral giant cell tumors"[3], "familial fibrous swelling"[4], "familial intraosseous fibrous swelling"[5], disseminated juvenile fibrous dysplasia"[6], "familial osseous dysplasia"[7], and "hereditary fibrous dysplasia"[8].

The disease is generally not realized by the family at birth but rather by the age of two; it becomes overt especially at the region of the angulus of the mandible, the molar, retromolar regions and sometimes may involve the maxilla as well[8, 9]. In addition, a "V"-shaped palatal vault is frequently observed in these patients.

The maxilla, which can be affected up to 67 percent, showed the same morphological changes as seen in the mandible. The initial maxillary lesions occur in the region of the maxillary tuberosity and at the alveolar bone adjacent to it[8]. The maxillary sinuses are small or totally obliterated. According to Burland et al.[7], the cherubic look is seen in the patients in whom the disease involved the maxilla, especially the infraorbital ridge, the anterior antral wall and the floor of the orbita. The presented case is one in which the floor of the orbita and the infraorbital ridge were severely involved (Fig. 5).

The radiological appearance of cherubism is characterized by multilocular cysts that are separated by a thin bone and irregular shapes, with bilateral involvement, affecting the mandible and the maxilla. These cysts cause the expansion of the bone, but cortical bone is not involved and no periosteal bone formation is observed[4, 8, 9].

The laboratory work-up of the patients contain normal values except for the alkaline phosphatase levels[8]. Our case's laboratory work-up was consistent with these findings.

Cervical lymphadenopathy, which is characteristic of the disease, regresses synchronously with the facial lesions[8]. Our patient also had bilateral cervical lumps, and the histologic examination of these lumps revealed lymphatic hyperplasia. This reactive state of the lymph nodes is attributed to various things, including an inflammatory event secondary to stasis or possible dental sepsis[9].

Involution usually begins by the age of 10 for girls; boys reach that stage at about the age of 14[10]. With the regression of the disease, new bony septa are formed with an increase in bone density. The mandibular margins are more easily defined and the multilocular pattern is observed to have a sclerotic bony shape in the regressed patient[11].

Cherubism is considered a mutation of a non-sex linked gene affecting the bone development of the jaws. Although Quan et al.[12] related the genetic changes to the spontaneous deletion in the FMR1 gene, this still needs to be proven. Although the disease was accepted as a familial condition for many years, it is clear that our case is a sporadic one like some others reported in the literature[13, 14].

In the differential diagnosis of this disease, fibrous dysplasia, bilateral parotid swellings, masseter hypertrophy, gingival fibromatosis, infantile cortical hyperostosis, giant cell reparative granuloma, benign giant cell tumor, hyperparathyroidism, odontogenic cysts, histiocytosis X, and ameloblastoma should be considered[8, 9].

In the case of fibrous dysplasia, since the histopathological appearance is very similar to that of cherubism, a difficulty arises. As a definitive criteria, fibrous dysplasia is not hereditary, and more commonly affects the female. Sexual precocity is usually present[15]. When fibrous dysplasia is considered, maxillary involvement is more frequent than mandibular involvement and the lesions are not symmetrical. It has an onset in older ages as well. In fact, the two diseases are very close but not similar entities. As our case is a sporadic one, we had to depend on the clinical presentation and radiological evaluation in the diagnosis of our case.

The giant cell tumors are seen at an older age with no bilateral symmetric involvement and are "extremely rare in the jaw bones"[16]. Infantile hyperostosis is seen as early as six months of age, but the lesions appear as cortical thickening instead of thinning and the cystic changes are in the medullary part of the bone.

There are many controversies about the treatment modality of the disease. Jones[1] states that each patient should be evaluated individually according to the manifestations of the tumor. His guidelines, which still have a place among treatment modalities, include: a) no active treatment, b) extraction of the teeth in the involved area, c) contouring of the involved area, and finally d) complete curettage. But Lawrence et al.[9] points out that the extraction of the teeth makes no difference. The role of surgery in this disease is limited and sometimes may aggravate the disease[17]. Since spontaneous regression is reported for many of the cases, surgery is planned only when cosmetic, respiratory, vision or speech problems exist[15, 18].

The use of radiation therapy is universally condemned for the treatment of cherubism since its use may lead to sarcoma formation, osteoradionecrosis or interference in the bone growth and facial development of the child[8, 15].

Our main reason to operate on this patient would be for cosmetic purposes. But after two years of follow-up we did not see any progression of the lesions and, as the patient did not have any concern about his appearance, we did not perform any surgery. We think that if the child has problems about his appearance in the future, we can trim the mandible after regression of the lesions at the age of fourteen.

In conclusion, cherubism is a rarely seen disease of the maxilla and mandible with a clinical presentation of firm and painless, symmetric swelling of the jaws. The radiological appearance of these lesions is quite typical and must be distinguished from other lesions of the jaws. As this disease has a self-limiting nature, there is no need to interfere surgically unless a complication develops such as vision or psychiatric problems, or difficulty in breathing or swallowing.

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The Turkish Journal of Pediatrics 1998; 40: 461-466

A CASE OF A FOUR – DAY – OLD MALE WITH CARPENTER'S SYNDROME WITH TRANSPOSITION OF GREAT ARTERIES*

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SUMMARY: Balcı S, Haytoğlu T, Özer S. (Genetics Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). A case of a four-day-old male with Carpenter's syndrome with transposition of great arteries. Turk J Pediatr 1998; 40: 461-466.

Carpenter's syndrome (acrocephalopolysyndactyly type II) is an autosomal recessive syndrome characterized by peculiar facies, synbrachydactyly on fingers and preaxial polysyndactyly on feet. To our knowledge there are about 40 reported cases of Carpenter's syndrome in the literature. Congenital heart disease is an uncommon entity in Carpenter's syndrome. In the case we present, transposition of great arteries, subpulmonic ventricular septal defect (VSD) and secundum atrial septal defect (ASD) were diagnosed with echocardiographic examination. Therefore, a cardiologic examination should be done in every newly diagnosed case of Carpenter's syndrome for possible heart defect. Early fatality is seen in Carpenter's syndrome cases associated with congenital heart disease. This is particularly important from the genetic counselling point of view. *Key words: acrocephalopolysyndactyly type II, Carpenter's syndrome, transposition of great arteries.*

Carpenter's syndrome (acrocephalopolysyndactyly type II) was first described by Carpenter[1,2] in two sisters with peculiar facies, synbrachydactyly and polysyndactyly on feet. Temtamy[3] reviewed the findings of 12 similar cases in the literature together with her case and stated that the cardinal features of Carpenter's syndrome are different from those of Laurence-Moon Biedl and Apert's syndromes. To date there have been about 40 reported cases of Carpenter's syndrome[4]. Balcı et al.[5] reported the first prenatal diagnosis of Carpenter's syndrome in a 20-week-old male fetus with clinical and post mortem findings. Although very rare, congenital heart defects such as patent ductus arteriosus (PDA), ventricular septal defect (VSD), atrial septal defect (ASD), pulmonary stenosis and transposition of great arteries could be associated with Carpenter's syndrome[6].

We present a case of Carpenter's syndrome diagnosed with clinical and radiological findings in the neonatal period in association with transposition of great arteries, subpulmonic VSD and secundum ASD according to echocardiographic evaluation. As far as we know, this is the first reported case of Carpenter's syndrome with congenital heart disease from Turkey.

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Case Report

M.B., a four-day-old boy, was admitted to our clinic because of deformities on his hands, feet and head. The child was the first product of a full-term gestation to a 20-year-old mother and a 26-year-old father who were first-degree relatives. He was 3500 g at birth. There was no known exposure to infection, drug or irradiation during the pregnancy. No similar malformations were known in either the mother's or the father's family.

On physical examination, weight was 3500 g (50-75th percentile), length 48 cm (10-25th percentile) and head circumference 32.5 cm (10th percentile). Body temperature was 37 °C and heart rate was 148 beat/min. He was cyanotic and icteric. The patient was found to have a tower skull (acrocephaly) and a peculiar facies characterized by low-set ears, epichanthal folds, frontal bossing, and a relatively flat nasal bridge (Fig. 1). The anterior fontanel was closed and the sutures were overriding. Nipples were hypoplastic. Preaxial polydactyly on the hands and feet were present. Also, syndactyly between the second and fifth fingers and toes were noted. In addition, bilateral pes equinovarus deformity was present (Fig. 2). The cardiac examination revealed a grade 2/6 pansystolic murmur on the left third intercostal space. Both femoral pulses were palpable.

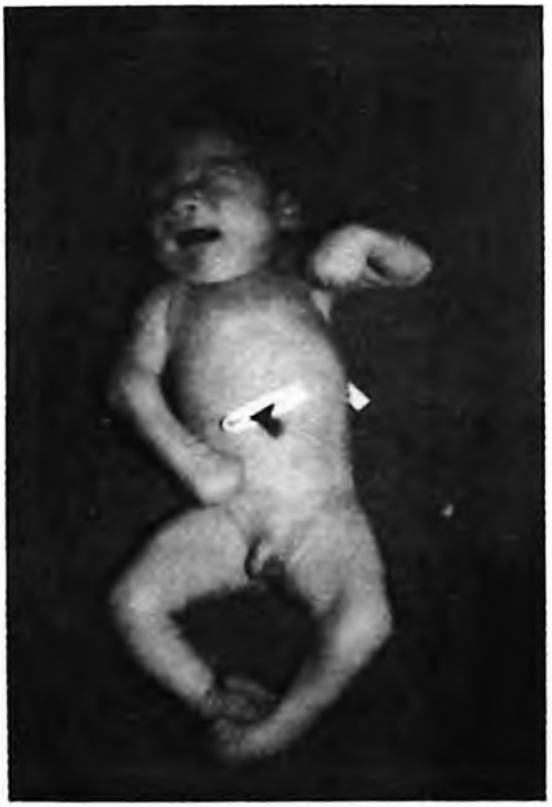

Fig. 1: Peculiar facies characterized by "tower skull" appearance (acrocephaly), low-set ears, epichantus, flat nasal bridge and frontal bossing. Note preaxial polysyndactyly of both hands and feet.

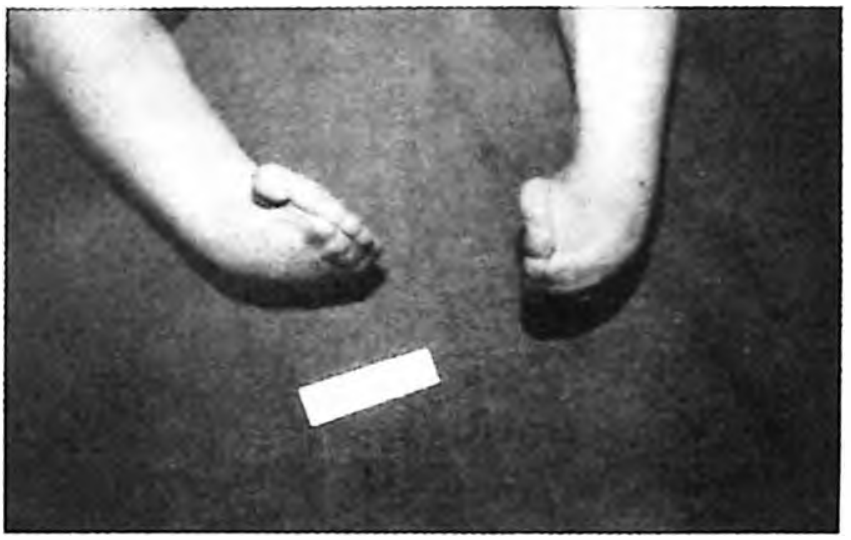

Fig. 2: Pes equinovarus deformity and preaxial polysyndactyly of feet.

The laboratory examination disclosed unconjugated hyperbilirubinemia with a total bilirubin level of 17.4 mg/dl and a conjugated bilirubin level of 0.3 mg/dl. Complete blood count, serum glucose-6-phosphate dehydrogenase level and urine-blood amino acids were within normal range. Routine skull x-ray revealed closure of the anterior fontanel. Radiological examination of the chest revealed cardiomegaly, bilateral thymic shadow and normal vasculature (Fig. 3). Right axis deviation with

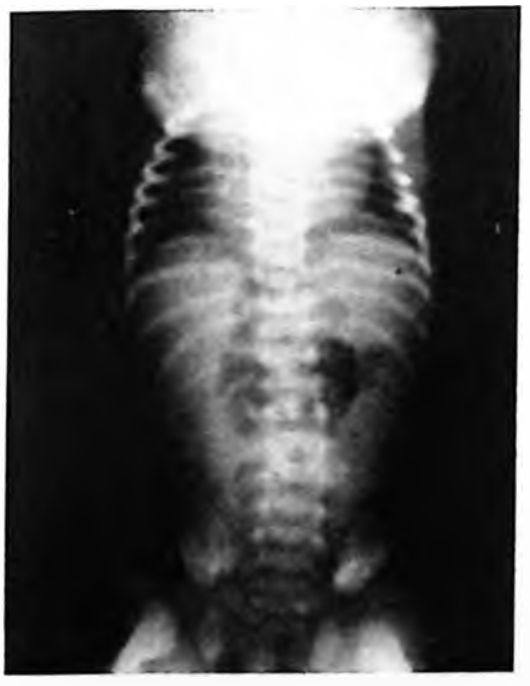

Fig. 3: Chest x-ray revealing cardiomegaly, bilateral thymic shadow and normal vasculature.

right ventricular hypertrophy was found on electrocardiogram. Echocardiogram demonstrated subpulmonic VSD with a diameter of five mm and secundum ASD with a diameter of six mm. As for the great vessels, the aorta was anteriorly located and originating from the right ventricle, and the pulmonary artery was posteriorly located and originating from the left ventricle. No pressure gradient was noted with Doppler echocardiographic examination. All the findings suggested possible diagnosis of transposition of great arteries, subpulmonic VSD and secundum ASD.

Cranial computed tomography demonstrated open coronal sutures but a closed anterior fontanel with normal intracerebral structures, although cavum septi pellicidi et vargae was noted.

The child was hospitalized on his fourth day of life and given phototherapy. After his discharge from the hospital on his 10th day of life he died at home. Postmortem examination could not be done.

Discussion

Carpenter[1] defined a new syndrome in two sisters in characterized by acrocephalopolysyndactyly, and defined the associated congenital malformations of the syndrome in 1909[2]. But this entity was accepted as a distinct syndrome only after Temtamy[3] reviewed 12 previously reported similar cases, added one case and suggested the term Carpenter's syndrome to the complex of associated malformations. Cardinal features of Carpenter's syndrome are acrocephaly, peculiar facies, brachysyndactyly on fingers, preaxial polysyndactyly on feet, hypogenitalism, obesity and mental retardation. The occurrence of this syndrome in siblings of consanguineous parents suggests an autosomal recessive inheritance. The case we present was the first child of the family of first-degree relative parents.

Other manifestations which might be present include genu valgum, coxa vara, pes varus, umbilical hernia, congenital heart defect and diabetes. To date more than 40 cases of Carpenter's syndrome have been reported in the literature[4]. Several authors reported Carpenter's syndrome in more than one sibling[4, 7-10]. However, there are also reported sporadic cases[11]. It is now certain that Carpenter's syndrome is inherited in an autosomal recessive pattern.

Cohen[12] reviewed the cases of this syndrome, and Cohen et al.[7] reported that Goodman's and Summit syndromes fall well within the clinical spectrum of Carpenter's syndrome.

In differential diagnosis of Carpenter's syndrome: 1) Apert's syndrome (acrocephalosyndactyly type I), 2) Bardet-Biedl syndrome, 3) Sakati-Nyhan syndrome, and 4) Summit syndrome should be considered.

Sakati et al.[13] reported a new syndrome with characteristic findings of acrocephalopolysyndactyly, brachydactyly of fingers, polysyndactyly of toes, bowing of femur, hypoplasia of tibia, cutaneous atrophy of scalp, and ear and cardiac abnormalities.

Frank mental retardation or low-to-normal intelligence is present in more than three-fourths of reported Carpenter's syndrome patients[7]; however, there have been reported cases with normal intelligence despite a tower skull appearance[8, 14, 15]. Although surgical correction of skull defects has been advocated as a method of facilitating normal brain development and preventing mental retardation, patients with early surgical intervention who were subsequently found to be intellectually subnormal have been described[16].

The case that Balci et al.[5] diagnosed prenatally at 20-weeks gestation had obvious craniosynostosis and severe central nervous system malformations, including corpus callosum agenesis, agyria, cerebellar hypoplasia, dilatation of lateral ventricles, broadened cerebral aquaduct and absence of third ventricle. The presence of severe central nervous system malformations in this prenatally diagnosed 20-week-old fetus may explain why mental retardation occurs in cases who had an early craniectomy[5]. Thirty-three percent of the Carpenter's syndrome cases are associated with congenital heart defects like VSD, ASD, PDA, pulmonary stenosis and tetralogy of Fallot[4]. Transposition of great arteries as in our case and superior vena-cava duplication have also been reported in Carpenter's syndrome[12, 17].

The case we present is interesting because of the presence of transposition of great arteries, a very rare congenital heart defect, in Carpenter's syndrome. Unfortunately, a post-mortem examination could not be done since the patient died at home. Therefore, every newly diagnosed Carpenter's syndrome case should be evaluated for congenital heart disease which could be associated with early fatality. Since the case was the first child of the family, genetic counselling was given to the parents and availability of prenatal diagnosis in the next pregnnacy by early ultrasonography at the 12^{th}-14^{th} week of gestation was explained.

In summary, we presented a case with radiologic and clinical findings of acrocephalopolysyndactyly in association with a very rare congenital heart defect transposition of great arteries. We suggest prenatal diagnosis of Carpenter's syndrome because of its autosomal recessive inheritance which has a 25 percent risk of recurrence.

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LUPUS VULGARIS SECONDARY TO SINGLE BCG VACCINATION*
A Case Report

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*Muammer Parlak MD*****, Mukaddes Eşrefoğlu******

SUMMARY: Selimoğlu MA, Erdem T, Parlak M, Eşrefoğlu M. (Department of Pediatrics, Dermatology, and Histology and Embryology, Atatürk University Faculty of Medicine, Erzurum, Turkey). Lupus vulgaris secondary to single BCG vaccination. Turk J Pediatr 1998; 40: 467-471.

A 10-year-old girl with lupus vulgaris following single BCG vaccination is reported. She had a 15 x 20 cm painless lesion covering her left shoulder, axilla, triceps and biceps region. PPD test was positive. Histopathological picture was identical to lupus vulgaris. *Key words: lupus vulgaris, BCG.*

Lupus vulgaris, a form of cutaneous tuberculosis, is a common and progressive disorder. It usually occurs as a result of hematogenous or lymphogenous dissemination or of direct contact with an internal tuberculosis focus[1,2]. It rarely occurs following BCG (Bacillus Calmette-Guérin) vaccination. Lupus vulgaris following a single BCG vaccination is very rare; multiple BCG vaccinations increase the incidence[2,3].

We present here a lupus vulgaris case following a single BCG vaccination.

Case Report

A 10-year-old girl presented with a painless lesion on her left shoulder. Her parents reported that she had had the BCG vaccination at the age of four months. The lesion which occurred at the vaccination site had never healed, despite several treatments with unknown drugs, and had progressively enlarged, particularly in the last three months. On physical examination, no pathological sign was detected except the 15 x 20 cm painless lesion covering her left shoulder, axilla, triceps and biceps region, with irregular margins and geographic pattern. The center of the lesion was atrophic and hypopigmented and surrounded by a border consisting of 0.5-1 cm papules with crusts. New active papules were seen on the atrophic central region (Fig. 1).

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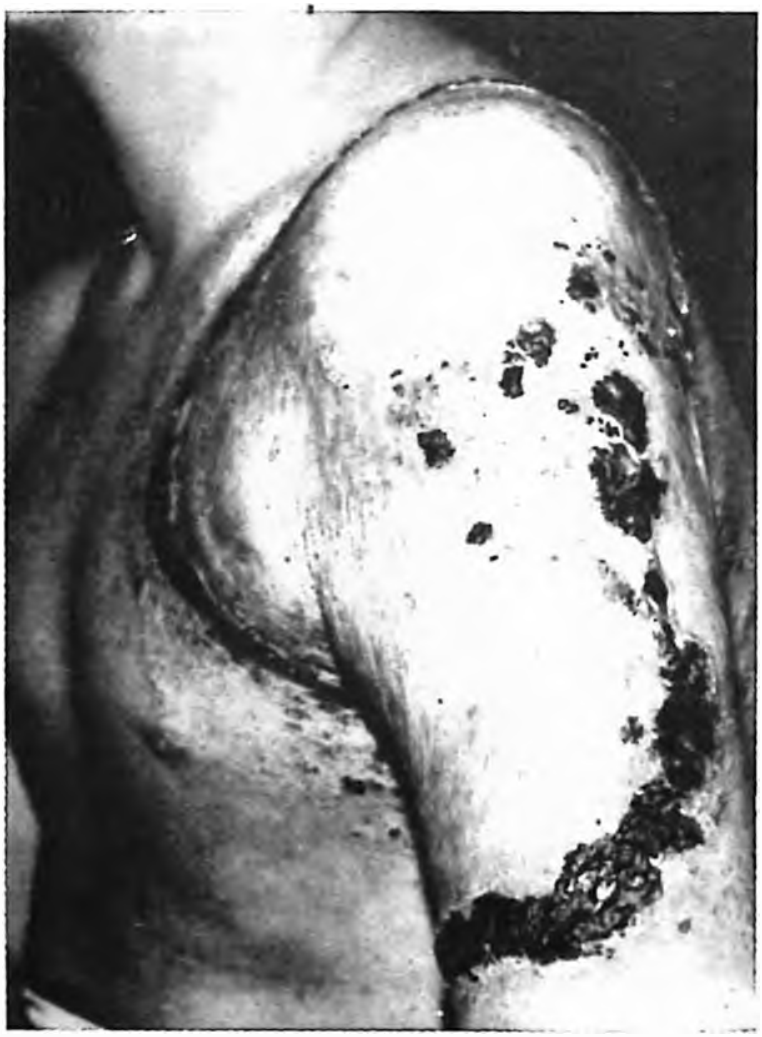

Fig. 1: Lupus vulgaris lesion covering left shoulder, axilla, triceps and biceps region. Irregular margins and geographic pattern and new active papules were seen on the atrophic central region.

Since the lesion was covered with crusts, apple jelly appearance was not observed, when pressed with a diascope. Regional lymphadenopathy was not detected.

Erythrocyte sedimentation rate was 38 and 72 mm at one and two hours, respectively. The other hematological parameters were within normal limits. Chest and shoulder roentgenograms were normal. PPD (purfied protein derivative) test was considered to be positive with 12 mm of induration and a 1 cm zone of surrounding erythema at 48 hours, and was positive with ulceration at 72 hours.

Specimen taken from the margins of the lesion was negative for acid-fast bacilli and the culture was negative.

Biopsy specimen was taken from the margin of the active lesion and stained with hematoxylin-eosin. Epitheloid tubercles surrounded by lymphocytes and Langhans-type giant cells were observed. Neither caseation nor mycobacteria was seen (Fig. 2).

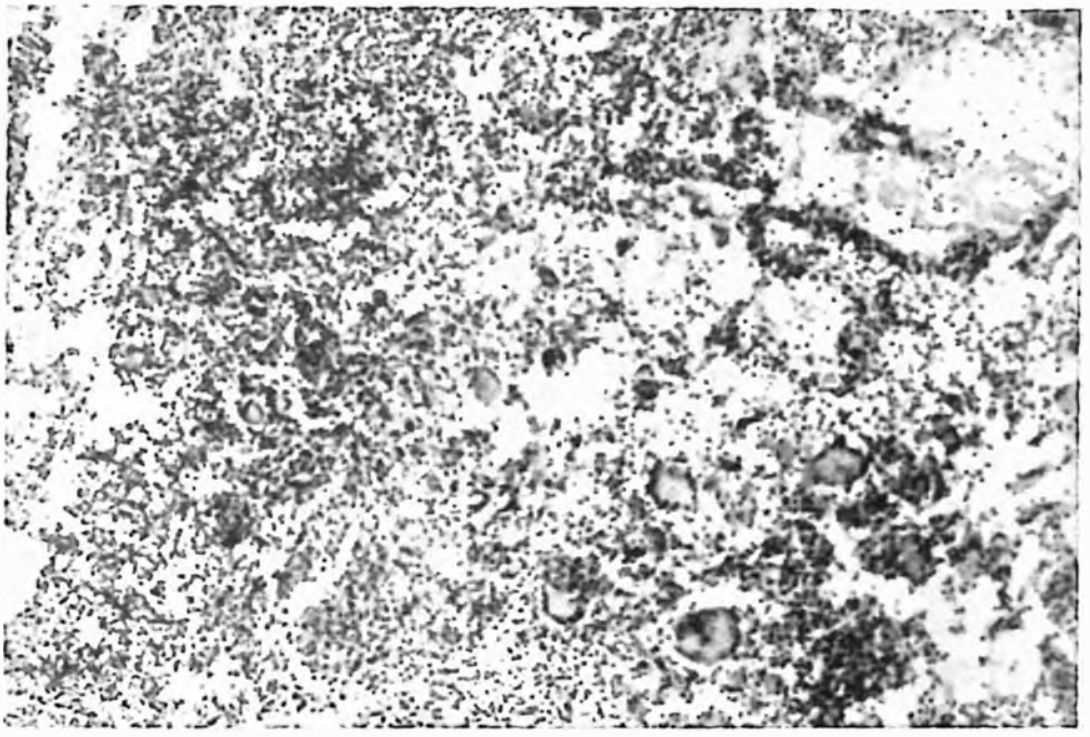

Fig. 2: Epitheloid tubercles surrounded by lymphocytes and Langhans' type giant cells, HE X 100.

Discussion

BCG vaccine is provided from attenuated bovine bacillus[5]. Severe complications following BCG vaccination are rare. A series of 17 cases of fatal disseminated BCG vaccine infection was reported; the majority of these patients were immunodeficient. In another reported series of 100 patients with complications of BCG vaccination, 71 had suppurative or perforating adenitis, 16 had simple adenitis, 11 had tuberculoids, 10 had lupus vulgaris, 10 had lupoid infection and 10 and Koch's phenomenon[4].

Among 33 patients who developed 43 lesions following BCG vaccination, two lupus vulgaris cases were also reported[4].

Complications following BCG vaccination were considered as specific and nonspecific in the literature. Keloids, erythema nodosum, simple macular eruptions, eczemas, toxicoderma hemorrhagica, urticaria, erythema multiforme, lichen scrofulosorum, epithelial cysts, and spinocellular epithelioma on scars are nonspecific; lupus vulgaris, Koch's phenomenon-like reaction, severe regional adenitis, slow scarring, local subcutaneous abscess, BCG osteitis, distant tissue tuberculosis, generalized adenitis and BCGitis are specific complications[2-4]. In 1946, the first case with lupus vulgaris following BCG vaccination was reported[4]. The incidence of lupus vulgaris following single BCG vaccination is 1/100,000-200,000 and it increases after multiple vaccinations[4,5]. In our patient, lupus

vulgaris had developed following single vaccination. There was no family history of tuberculosis or of exposure to patients with tuberculosis.

Lupus vulgaris may develop immediately after vaccination; however, it was also reported as developing on vaccine scars as long as three years after vaccination. Mean duration is approximately one year[1,5]. The interesting feature of our patient is that the non-healing lupus vulgaris lesion developed immediately after BCG vaccination.

The appearance of the lupus vulgaris lesion developed following BCG vaccination is the same as that of the classical lupus vulgaris lesion. If the vaccination is performed intradermally, the lesion is rounded. If it is performed by scarification, the lesion is band-like[3,5]. In our case, the vaccination was administered intradermally and the lesion shape was rounded. It is reported that the size of the lupus vulgaris lesion due to BCG vaccination may range from 1 mm to 10 cm[4]. The lesion of our patient was 15 x 20 cm.

Izumi and Matsunaga[4] reported a case with lupus vulgaris for 30 years due to BCG vaccination and the lesion was defined as 6.6 x 6.5 cm. In our case, although the lesion was present for only ten years, the diameter was greater than 10 cm.

Absence of pathological/radiological signs such as hilar lymphadenopathy confirmed our diagnosis. Like Izumi and Matsunaga[4], we also did not detect lymphadenopathy. In lupus vulgaris following BCG vaccination, mycobacteria generally cannot be shown on direct or histopathological examination[4,6]. Seven culture-positive cases among a series of 33 patients were reported[4]. But other series in literature had no culture-positive patients[4]. We also could not observe mycobacteriam on direct microscopic or histopathological examination, and the culture was negative.

PPD was positive in our patient. PPD contains immunologically active tuberculoproteins and it shows hypersensitivity against tuberculosis. In tuberculosis, a relation between hypersensitivity and immunity has not yet been established. Both develop at the same time. Although both are related to mononuclear cells, their antigens are different. For this reason, hypersensitivity can be used as an indicator of immunity[2,3]. Izumi and Matsunaga[4] also reported a strong reaction to PPD in one case.

Histopathological appearance of epitheloid tubercles surrounded by lymphocytes and Langhans-type giant cells was identical with lupus vulgaris[7,8].

Treatment of lupus vulgaris due to BCG vaccination is similar to that for classical lupus vulgaris[5]. Isoniazid (INH) (15 mg/kg/d) and rifampicin (20 mg/kg/d) were administered orally, and the treatment is continuing.

We presented this case with lupus vulgaris due to BCG vaccination in order to call to mind this rare entity, and we reviewed the literature.

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