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The Iranian Childhood Cancer Biobank

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Abstract

Background: Biological resources, along with patient-related clinical data, are basically required for personalized medicine and translational research. In this regard, pediatric cancer biobanks have considerable significance due to their special challenges, which include the need for long-term sample collection (due to high diversity and rare tumors), the difficulty of working with children, as well as the limited volume of samples available in children.

Methods: After obtaining all necessary approvals (from the ethics committee, scientific board, and financial support), standard operating procedures (SOPs) were defined for all aspects of the biobank procedures, including equipping the lab, sample collection, processing, storage, as well as clinical data recording.

Results: Until July 2022, approximately 8,000 samples from 720 patients have been collected in the biobank. In summary, the samples in the biobank are classified into three categories: leukemia (40.7%), solid tumors (39.44%), and central nervous system tumors (15.56%). The unique activities of the biobank include the collection of various biological samples from patients and their parents, inter-university cooperation, the use of a vacuum system to preserve tissue, the launching of an online database for recording patients' medical data, and the setting up of a bilingual website for announcements at the national and international levels.

Conclusion: Iranian Childhood Cancer Biobank (ICCBB) is the first pediatric biobank center in Iran that collects various samples and associated clinical data from patients with a wide range of childhood cancers. The ICCBB aims to advance clinical research in the field of pediatric cancer by providing both the required quantity and quality of biological samples.

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1. Introduction

Cancer biorepositories, as disease-based biobanks, store a collection of biological samples along with associated clinical data from individuals for research purposes and translational medicine [1]. Biological samples are valuable and non-renewable resources, especially in combination with clinical and follow-up information [2]. Each biological sample should be considered a small genetic and molecular resource that can be used not only in research programs but also as an information database for the advanced treatment of the patient based on personalized medicine [3]. Since molecular changes occur before any clinical and pathological changes, providing targeted treatments based on the patient's individual genome can definitely be beneficial to the patient's outcome [4]. To achieve this goal, having access to a fresh tumor sample before starting the treatment is necessary. Additionally, a liquid biopsy (patient's blood) can be utilized for molecular investigations related to the patient's prognosis during the treatment process. Biobanks can be a useful tool for collecting and storing samples before and during the treatment process, for studies related to personalized medicine by researchers and advanced treatment for patients. Therefore, guidelines need to be prepared for collecting and storing patient samples at different stages of treatment in hospital-based biobanks. An organized and targeted biobank collects biological samples based on predetermined guidelines, along with obtaining the patient's consent, while maintaining the privacy of their information [5]. This provides a comprehensive database including biological samples and valuable clinical information, relieving researchers from scattered and sometimes incomplete efforts to collect their required samples.

Due to the lack of experience in the field of setting up and managing centralized and dedicated children's biobanks in Iran, and the importance of these valuable resources for developing researchers' clinical vision, it is essential to establish such biobanks in parallel with other countries. The Iranian Childhood Cancer Biobank (ICCBB) was officially launched in 2017 as the first pediatric biobank center in Iran to collect biological samples and relevant clinical data from children suffering from a wide range of cancers. The overall goal of the ICCBB is to facilitate basic and advanced clinical research in the field of pediatric cancer and provide qualified biological samples, which could potentially advance the personalized treatment of patients.

In ICCBB, fresh tissue samples, blood derivatives such as plasma, serum, and buffy coat, urine, and hair, as well as blood samples from parents (plasma and buffy coat), are collected, processed, and stored. After processing, the samples undergo preservation by being kept in a -80°C freezer for plasma, tissue coat, serum, and urine, or in a liquid nitrogen tank (-196°C) for tissue and PBMC.

The collection of blood and tissue samples can be utilized in molecular tests, DNA, RNA, and protein extraction, as well as in cell culture. Urine can be utilized for investigating electrolytes, exposure to environmental chemicals, as well as metabolic products. Additionally, hair can be used for measuring heavy metal content.

2. Materials and methods

2.1. ICCBB protocol

ICCBB was initially launched as a three-and-a-half-year research project and was later extended to five years due to the COVID-19 pandemic crisis. The ICCBB protocol and standard operating instructions (SOPs) were defined based on international standards. Instructions were provided for various operational aspects, including setting up the laboratory (required equipment and consumables), what types of samples to collect, how to collect samples, how to process samples, how to store samples, how to transfer samples, and finally, how researchers can access the samples.

2.2. Organizational structure

The financial support for the ICCBB project was provided by the National Institute of Medical Sciences and Research Development (NIMAD) and was approved by the National Ethical Committee of the Ministry of Health and Medical Education of Iran (IR NIMAD REC 1396 061) in 2017. The ICCBB's site is located at Mofid Children's Hospital in Tehran, Iran, serving as a referral center for children diagnosed with cancer. This multicenter project began its operation in 2016 in cooperation with surgeons, oncologists, and pathologists of the main children's oncology collaborating centers in three cities in Iran. The second phase, which involved recruiting patients and collecting samples, began in January 2017. Two other centers outside of Tehran, namely, Mashhad and

Uremia, collaborated on the project. An organizational chart for the plan was defined, including the scientific committee and the collaborating personnel in the hospital. The scientific committee was composed of professors from the departments of clinical pathology, surgery, and pediatric oncology. In addition, personnel from each department of the hospital cooperated in different parts of the plan as needed.

2.3. Setting up the ICCBB laboratory

The laboratory was set up in an independent space near the surgery/pathology theater at Mofid Children's Hospital. Once a suitable location was chosen, the room was renovated and equipped to meet the standards obtained from reputable biobanks. This included installing suitable cooling facilities and power failure warnings, laboratory cabinets, an internet network, and appropriate computers. In the next stage, all required equipment was purchased and installed, including two (-80°C) freezers, a refrigerator, four liquid nitrogen tanks, four small portable transfer LN tanks, a class II biosafety cabinet, a centrifuge machine, a barcode scanner and label printer, a freezer temperature alarm system, and other consumable items.The freezers are connected to both a temperature alarm system and an emergency electrical system.

2.4. Collection of samples

All children suspected of cancer, newly diagnosed patients, and relapsed patients aged between 0 to 18 were enrolled in this project. Blood and bone marrow samples were collected from patients along with their diagnostic samples. There are two commonly used anticoagulants in research which are citrate and EDTA. We have collected patients' blood samples in both anticoagulants to provide a wide range of research uses. In total, three milliliters of blood samples were collected in a citrate tube, three milliliters in a K2EDTA tube, and three milliliters in a serum tube (Greiner Bio-One®, Austria). Urine and hair samples were collected in accordance with the relevant SOP and with patient cooperation. In addition, if available, three ml of citrated blood were collected from each parent. For the collection of tissue samples, the daily surgical list was checked to identify eligible cases, which were presented to the pathologist. Any fresh cancerous tissue was immediately sent to pathology after excision. The pathologist then referred extra tissue samples to the biobank for processing and storage.

2.5. Processing of samples

Blood samples were processed according to a SOP within a maximum of four hours after sampling, to separate them into serum, plasma, and buffy coat (cryopreserved cells). All procedures were performed in a sterile environment, within a laminar flow cabinet, using autoclaved tips. EDTA and citrated blood tubes were then centrifuged at 1200 g for 10 minutes at room temperature. Separated plasma was further centrifuged at 1800 g for 10 minutes at room temperature. A density gradient centrifugation technique was implemented to isolate peripheral blood mononuclear cells (PBMC) from other blood components. Serum and urine were centrifuged at 1500 g for 15 minutes at 4°C and were aliquoted. Tissue samples were dissected using surgical blades into 1 cm^3 or smaller fragments. Finally, they were transferred into barcoded cryovials and registered in an electronic database system."

2.6. Storage of samples

The storage stage for samples was completed when they were labeled and their barcode was recorded in the online database. All plasma, serum, and urine samples were stored in -80°C ultra-freezers located in the biobank room, while PBMC samples were stored at -80°C for 24 hours and then transferred to liquid nitrogen tanks (-196°C) for long-term storage. Tissue samples were snap-frozen in liquid nitrogen and later transferred to -80°C freezers.

2.7. Online database of clinical data and another database for tracking the location of each sample in freezers

All clinical information of the patients, including demographic information, pathology reports, relevant laboratory tests (complete blood count (CBC), biochemical, hematology, BMA/B, flow cytometry, etc.), imaging reports, and surgical reports, has been registered in the biobank's online database. Additionally, a database has been designed to register the location of the vials, such as the freezer rack and box, accurately tracking each sample vial in the biobank.

3. Results

3.1. ICCBB establishment

The ICCBB was established in October 2016 and began officially collecting data and samples from December 2017. Tissue and non-tissue samples (such as blood,

bone marrow, urine, and hair) were collected from patients prior to treatment at the time of diagnosis for primary or relapsed cancer. Demographic and clinical information of patients were recorded in the biobank's online database. More information, such as follow-up and outcomes of patients, can be accessed through linkage to the hospital's registry system.

Totally, from January 2017 to July 2022 (three and a half years), 8,000 aliquoted samples from 720 patients and their parents have been processed and stored. For the biobanking consent process, we have dedicated a trained interviewer for this purpose. It should be noted that the patients' parents are very sensitive and vulnerable at the beginning of the diagnosis. Therefore, the interviewer should explain all aspects of the project to them in a simple and trustworthy manner and patiently answer all their questions. In the following, we have categorized patient samples based on three criteria: the type of cancer, gender, and primary diagnosis/recurrence.

3.2. Classification of collected samples

3.2.1. Classification based on the type of cancer

Based on the tumor type, the samples were classified into three categories: leukemia tumors, solid tumors, and central nervous system (CNS) tumors. Approximately 40.8% of patients were diagnosed with leukemia tumors, 39.44% with solid tumors, and 15.56% with tumors related to the central nervous system (CNS). 4.17% of all the collected samples were excluded due to non-tumoral diagnosis **(Figure 1).**

Leukemia-diagnosed samples were classified into four subgroups: acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), and mixed phenotype acute leukemia (MPAL). Solid tumors were classified into eight tumor subtypes, including neuroblastoma, kidney tumors, germ cell tumors, lymphoma tumors, liver tumors, soft tissue sarcoma, primitive neuroectodermal tumors (PNETs), and other rare solid tumors. Tumors of the CNS were classified into six subgroups, including glioma, glioblastoma, ependymoma, medulloblastoma, craniopharyngioma, atypical teratoid/rhabdoid tumor (AT/RT), and other rare CNS tumors. (**Figure 1** and **Table 1** show the classification of these tumors.)

3.2.2. Classification based on the gender of patients

A total of 720 patients, comprising 372 boys and 348 girls, were enrolled in the study. Majority of the cases belonged to the age group of 0-4 years, with 301 cases (169 boys and 132 girls). For more information, refer to **Figure 2.**

3.2.3. Classification based on primary diagnosis/ recurrence

Approximately 82% of collected samples belong to newly diagnosed cases and about 18% belong to recurrent ones **(Figure 3)**.

4. Discussion

Childhood cancer is the leading cause of death among children in developing countries. Therefore, understanding the molecular and genetic mechanisms of childhood cancer is very important in improving diagnosis, prognosis, and treatment outcomes. Additionally, investing in the collection and storage of biological materials enables large-scale clinical research. Biobanks are sources of collection and storage of biological samples, along with associated clinical data.

Pediatric biobanks are important facilitators of research in the field of pediatric diseases, but they have unique challenges. Since childhood cancers have high diversity and are rare compared to those found in adults, collecting large groups of biological samples from children poses a challenge. Hence, biobanks dedicated to children need to operate with a long-term perspective. Additionally, the vulnerability of the participating population and the limited volume of biological samples in children pose potential challenges in all pediatric biobanks. Thus, pediatric patient populations often remain under-researched in medical research due to ethical issues and difficulties faced during sample collection. Regarding the particular challenges faced by pediatric biobanks, only a few biobanks, primarily focused on pediatric cancers, are present globally.

According to the articles review, the Westmead Biobank, with a 20-year history, is a tumor bank located at the children's hospital in Westmead, Australia. As a single institutional biobank, it has collected approximately 20,340 specimens from 3,788 patients over the course of two decades, including tissue, blood, and bone marrow [6].

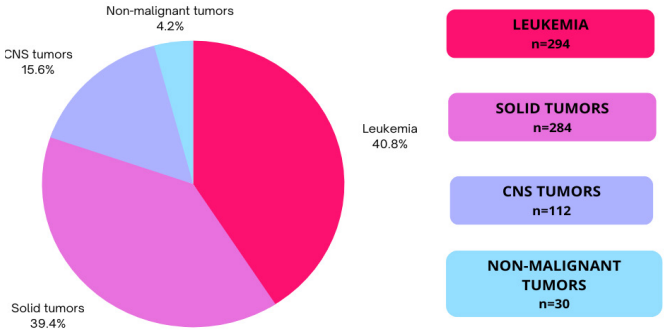

Figure 1. The number and percentage of collected samples

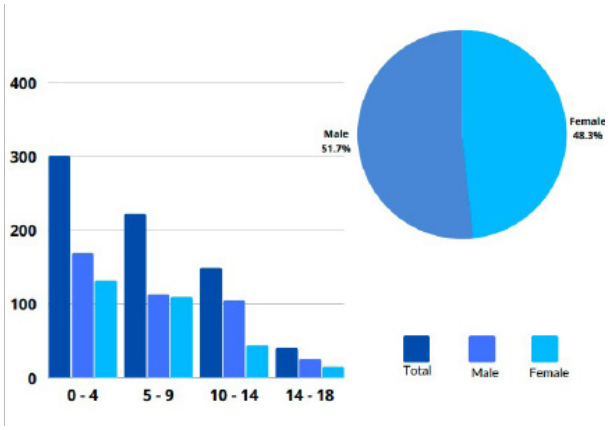

Figure 2 The umber and age-related percentages of collected samples

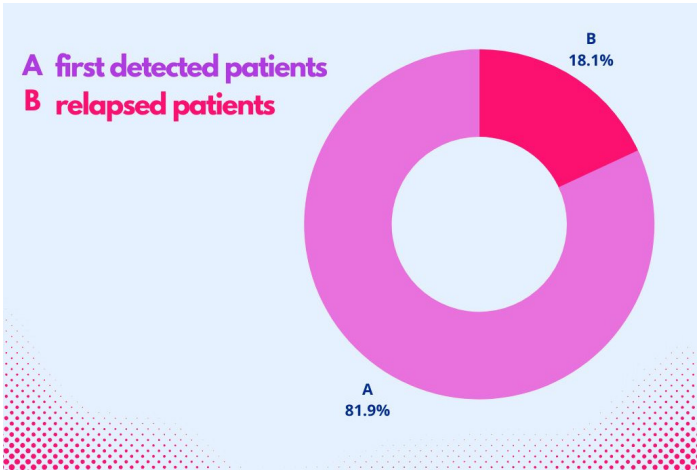

Figure 3. Percentages of new case patients and relapsed patients

Table 1. The number and percentage of collected samples

Diagnosis		*Number of patients*	*Percentage of patients*
Leukemia		**294**	**40.83%**
	ALL	262	36.39%
	AML	28	3.89%
	CML	3	0.42%
	MPAL	1	0.14%
Solid tumors		**284**	**39.44%**
	Neuroblastoma tumors	88	12.22%
	Kidney tumors	77	10.69%
	Germ cell tumors	30	4.17%
	Liver tumors	23	3.19%
	Lymphoma tumors	22	3.06%
	Soft tissue tumors	22	3.06%
	PNET	7	0.97%
	Rare tumors	15	2.08%
CNS		**112**	**15.56%**
	Glioma	51	7.08%
	Ependymoma	17	2.36%
	Medulloblastoma	14	1.94%
	Craniopharyngioma	10	1.39%
	ATRT	9	1.25%
	Rare tumors	11	1.53 %
Other		**30**	**4.17%**
	Non-malignant tumors	30	4.17%

In addition, a hospital biobank in Egypt collects, processes, and stores samples from pediatric cancer patients and their parents. All samples are recorded, along with epidemiological and clinical data, through an electronic healthcare system. This biobank has stored approximately 40,000 human samples of various pediatric cancer types since 2012, including blood derivatives, malignant and normal tissues, and cerebrospinal fluid (CSF) at different clinical time points like initial diagnosis, relapse, and remission [7]. Furthermore, the Norwegian childhood cancer biobank, as a multi-center biobank, has collected 12,000 samples from 510 patients since 2019, including tumor tissue, germline tissue (blood samples, or buccal swabs), microbiota tissue, and hair samples collected at the time of diagnosis or relapse [5].

Based on our review, only a few childhood cancer biobanks gather parent samples. However, ICCBB sets itself apart by collecting samples from parents, which could prove essential in genetic studies, specifically in the context of childhood cancers. Furthermore, patient samples are divided into multiple aliquots, providing the biobank with the advantage of being able to use these samples in several studies.

The purpose of ICCBB is to collect a diverse range of biological samples from newly diagnosed or relapsed children with various types of cancer to meet the needs of researchers in different research fields. This is a new perspective compared to other cancer biobanks established in Iran, which focus on a specific cancer or a specific biological sample. While ICCBB collects all types of common and rare cancers in children and all kinds of biological samples, including fresh tissues (malignant/normal), PBMC, plasma, serum, urine, and hair.

Another gained experience in this procedure involves cooperation with other pediatric centers in far cities, including Mashhad and Urmia, with different protocols. The cooperation in Mashhad was done in such a way that collection, processing, and storage took place in the same city. To assess and solve the problem of the probable absence of a pathologist at the time of surgery in Mashhad, we used a vacuum-based method by providing the TissueSAFE® device. In this method, the tissue is vacuumed in special bags and stored fresh in a refrigerator at 2-4°C for 24-72 hours. This method was applied for the first time in Iran, and the results were satisfactory.

In Urmia, the samples were sent by airmail immediately after sampling. Based on our experience, it is possible to send blood samples by airmail if there are regular daily flights from the city of origin and personnel present during the evening and night shifts at the destination. The limitations of sending samples via airmail include the possibility of flight delays or cancellations, as well as the samples arriving outside of working hours for the receiving staff. In general, the experience gained from working with various centers has shown that trained and motivated personnel play a crucial role in the sample collection process, which is the most essential aspect of any biobank's executive part. Moreover, close collaboration between surgeons, oncologists, pathologists, laboratory technicians, and nurses with biobank staff is essential. In fact, the success of a biobank is a result of the teamwork of various hospital departments.

5. Conclusion

In conclusion, given that the biobank project is of national and multi-center nature and is being launched for the first time in Iran, the lessons learned should be implemented to expand and continue this activity. We should strive to remove the existing limitations and advocate for sufficient financial and human resources. In addition, the following idea can be developed in the future for maximum productivity, including establishing a living biobank (organoid biobanks) for the collection and storage of viable and functional tissues for use over long periods of time.

Detailed information and an introduction of ICCBB would be available for researchers and those interested for more communication on httpp://iccbb.ir/EN.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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