The Pattern of Microbial Skin Flora in Children with Cancer

A thesis

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By

Thafir Tawfiq Naeem

M.B.Ch.B

Supervisor

Dr. Jenan Ghalib Hasan

C.A.B.P

Assistant Professor

Department of Pediatrics

College of Medicine

University of Basra

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Certificate

I certify that this thesis was prepared under my supervision at the scientific council of pediatrics in partial fulfillment of the requirements for the degree of fellowship of the Iraqi board for medical specialization in pediatrics.

Dr. Jenan Ghalib Hasan

C.A.B.P

Assistant Professor

Department of Pediatrics

College of Medicine

University of Basra

We, the examining committee, after reading this thesis and examining the candidate Thafir Tawfiq Naeem, in its content, find that it meets standards and requirements as a thesis in partial fulfillment for the degree of the Iraqi board for medical specializations in pediatrics.

Professor

Dr. Mea’ad K. Hassan

C.A.B.P

(Chief)

Assistant professor
Dr.Sawsan Issa Habib
F.I.C.M.S

(Member)

Assistant professor
Dr. Aida A. Munther
C.A.B.P

(Member)

I, the chairman of Scientific Council of Pediatrics, certify that this thesis was prepared by the candidate, **Dr.Thafir Tawfiq Naeem** and submitted to our council.

Assistant Professor

Dr. Lamia Abd-Alkareem Hamoodi

M.B.Ch.B. C.A.B.P

Chairman of Scientific Council of Pediatrics

Approved by the Iraqi Board for Medical Specializations.

Professor

Dr. Faleh A.Fadhil Al-Bayati

MRCP(UK)

Acting President of Iraqi Board for Medical Specializations

Dedication

I dedicate this research to my lovely parents whose words of encouragement and push tenacity ring in my ears

To my beloved sisters and brother

Without their love and support none of this would have been possible

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Summary

A cross sectional study was carried out to determine the pattern of microbial skin flora in children with malignant diseases.

Fifty patients who were newly diagnosed with malignancies and have been admitted to Oncology Center in Basra Specialty Hospital and were recruited in the study after a parental consent was obtained. The study took place from the first of April 2014 till the end of December 2014.

The patients included 24males (48%) and 26 females (52%). Within the male group, 15 & 9 were diagnosed with hematological & solid malignancies successively. Within the female group, 13 & 13 were diagnosed with hematological & solid malignancies successively.

A special questionnaire was designed for the purpose of the study, information included: age, sex, type of malignancy and the results of skin flora isolated from different body sites including the palms of the hands, soles of the feet, both axillary regions, the back between the scapulae and the groin area.

Skin swabs were taken from the cases within forty eight hours of admission before starting treatment with chemotherapeutic agents from the previously mentioned sites on their bodies. The specimens were examined in the microbiological unit.

A second set of skin swabs were taken from the patients from the same body sites after one month of treatment with chemotherapy. It was found that there is a significant change in the pattern of skin flora isolates obtained from the patients, especially isolates taken from the palms of the hands, soles of the feet and the axillary regions. Where it was found that only 28 (56%) patients showed no growth in specimens taken from the palms after commencing chemotherapy compared to

49 (98%) patients before starting treatment with p value < 0.05. Similar results were obtained from isolates taken from the soles of the feet, as 13 (26%) and 9 (18%) and 3 (6%) patients showed growth of *Proteus*, *Pseudomonas* and *Klebsiella* successively. (P <0.05)

Also specimens taken from the axillary region showed that 19 (38%), 13 (26%) and 7 (14%) showed growth of *Proteus*, *Pseudomonas* and *Klebsiella* successively. (P <0.05). Specimens taken from the groin and the back area between the scapulae did not give significant growth of pathological microflora as compared to the first culture results. (P>0.05)

These isolated floras showed no significant predilection to the type of malignancy or to the age group of patients they were isolated from. (p>0.05)

So, skin swabs taken from such patients should be considered as part of the initial laboratory workup along with follow up of these investigations and repeating them after commencing chemotherapy for any significant change in their pattern for proper management in preventing subsequent serious infectious complications.

Chapter One

Introduction

1. Introduction

An estimated 175,000 cases of cancer are diagnosed annually in children younger than 15 years of age worldwide, and fewer than 40% of patients (mostly in high-income countries) are adequately diagnosed and treated[(1)]. A child's probability of surviving cancer is poor in less developed countries, and extreme discomfort is likely in the absence of palliative care. Many childhood cancers are highly curable if diagnosed at an early stage, and some treatment regimens are relatively simple, inexpensive, and well-established[(2)].

1.1 Childhood cancer and its management

Hematopoietic tumors (leukemia, lymphoma) are the most common childhood cancers, followed by brain/CNS tumors and sarcomas of soft tissue and bone[(3)]. Lymphohematopoietic cancers (i.e., ALL, lymphomas) account for ~40%, nervous system cancers for ~30%, and embryonic tumors and sarcomas for ~10% each among the broad categories of childhood cancers[(4)]. During the first year of life, embryonic tumors such as neuroblastoma, nephroblastoma, retinoblastoma, rhabdomyosarcoma, hepatoblastoma, and medulloblastoma are the most common tumors. Embryonal tumors, acute leukemias, non-Hodgkin lymphomas and gliomas peak in incidence from 2-5 yr of age. As children age, bone malignancies, Hodgkin disease, gonad germ cell malignancies (testicular and ovarian carcinomas), and other carcinomas increase in incidence[(4)]. Adolescence is a transitional period between the common early childhood malignancies and characteristic carcinomas of adulthood[(4)].

Clinical features of cancer are different and depend on type of cancer. Patients with leukemia or a tumor that has infiltrated the bone marrow typically have one or more of the following: fever, pallor, bruising, petechiae, and bleeding.

Lymphadenopathy and organomegaly also are common in leukemia, particularly with T cell ALL or non-Hodgkin lymphoma[1]. Patients with solid tumors usually have a palpable or measurable mass. Other signs and symptoms include pain, limp, cough, dyspnea, headache, vomiting, cranial nerve palsies, and papilledema[1].

Children with cancer are typically treated according to international protocols. ALL is usually treated with chemotherapy alone where as solid tumors are treated with surgery combined with chemotherapy and/ or radiotherapy. Hence, the type and length of treatment depends on a number of factors such as the type of cancer, location and stage of disease[5].

Adverse treatment effects are of two types, either acute or late adverse effects. Acute adverse effects that occur early in therapy can result in oncologic emergencies. These include metabolic disorders (hyperuricemia, hyperkalemia and hyperphosphatemia, hyponatremia, hypercalcemia), hematologic disorders (anemia, thrombocytopenia, disseminated intravascular coagulation, neutropenia, hyperleukocytosis, graft versus host disease), and space-occupying lesions (spinal cord compression, increased intracranial pressure, superior vena cava syndrome, tracheal compression)[5]. Late adverse effects occur months to years after the end of treatment[6]. These include any physical, psychological or social consequence of the disease or treatment.

1.2 Infection in cancer patients

Infectious complications are a serious cause of morbidity and mortality in cancer patients, especially those with underlying hematological malignancies where autopsy studies demonstrate that approximately 60 % of deaths are infection related[7-11].

Factors that predispose to infection are divided into those that are host associated and those that are treatment associated. Host-associated factors include underlying

immune deficiencies, medical comorbidities, past infections, poor nutritional status, and psychological stress. Treatment-associated factors include surgery, radiation, immunosuppressant therapies, antimicrobial use, and invasive procedures[12]. Clearly, more than one predisposing factor may exist in a given patient, and their cumulative burden more accurately reflects the risk of infection.

1.3 The Skin

The skin is an organ that contributes to several different epithelial systems as it represents the largest interface between the internal environment of humans and the external world. The skin is also the largest organ of the human body. The skin serves a number of key functions, with its most basic purposes surrounding its role as a physical barrier protecting the host's internal environment from external pathogens, as well as osmo- and thermoregulation. [13]

1.3.1 Skin layers

Skin is composed of three primary layers:

- The epidermis, which provides waterproofing and serves as a barrier to infection;
- The dermis, which serves as a location for the appendages of skin; and
- The hypodermis (subcutaneous adipose layer). [13]

1.3.2 Skin functions

Skin performs the following functions:

1. **Protection**: by the following mechanisms:
    - acting as a physical barrier against microbe penetration to tissues underneath

- secreting mucus layer so that microbes can not permanently attach to the epithelial cells beneath
- Shedding or keratinization of the outermost skin cells so microbes are removed from the body
- Secreting antimicrobial peptides and proteins to kill off microbes or at least prevent their growth[14]

2. **Sensation**: contains a variety of nerve endings that react to heat and cold, touch, pressure, vibration, and tissue injury.
3. **Heat regulation**: the skin contains a blood supply far greater than its requirements which allows precise control of energy loss by radiation, convection and conduction. Dilated blood vessels increase perfusion and heat loss, while constricted vessels greatly reduce cutaneous blood flow and conserve heat.
4. **Control of evaporation**: the skin provides a relatively dry and semi-impermeable barrier to fluid loss[15]. Loss of this function contributes to the massive fluid loss in burns.
5. **Storage and synthesis**: acts as a storage center for lipids and water, as well as a means of synthesis of vitamin D by action of UV on certain parts of the skin.
6. **Excretion**: sweat contains urea, however its concentration is 1/130th that of urine, hence excretion by sweating is at most a secondary function to temperature regulation.
7. **Absorption**: the cells comprising the outermost 0.25–0.40 mm of the skin are "almost exclusively supplied by external oxygen", although the "contribution to total respiration is negligible".[16] In addition, medicine can be administered through the skin, by ointments or by means of

adhesive patch, such as the nicotine patch oriontophoresis. The skin is an important site of transport in many other organisms.

8. **Water resistance**: The skin acts as a water resistant barrier so essential nutrients aren't washed out of the body[16] .

1.3.3 Skin Defenses

(1) Antimicrobial peptides

The skin creates antimicrobial peptides such as cathelicidins that control the proliferation of skin microbes. Cathelicidins not only reduce microbe numbers directly but also cause the secretion of cytokine release which induces inflammation, angiogenesis, and re-epithelialization. [17]

(2) Acidity

The superficial layers of the skin are naturally acidic (pH 4-4.5) due to lactic acid in sweat produced by skin bacteria. [18] At this pH, mutualistic flora such as *Staphylococci*,*Micrococci*, *Corynebacterium* and *Propionibacteria* grow but not transient bacteria as Gram negative as *Escherichia* and *Pseudomonas* or Gram positive ones such as *Staphylococcus aureus* or *Candida albicans*.[18]Another factor affecting the growth of pathological bacteria is that the antimicrobial substances secreted by the skin are enhanced in acidic conditions.

(3) Immune system

If activated, the immune system in the skin produces cell-mediated immunity against microbes such as dermatophytes (skin fungi). One reaction is to increase stratum corneum turnover and so shed the fungus from the skin surface. Skin fungi such as *Trichophyton rubrum* have evolved to create substances that

limit the immune response to them. [19] The shedding of skin is a general means to control the buildup of flora upon the skin surface.

1.3.4 Skin Flora

The skin flora, more properly referred to as the skin microbiota, are the microorganisms which reside on the skin. Many of them are bacteria of which there are around 1000 species upon human skin. Most are found in the superficial layers of the epidermis and the upper parts of hair follicles.

The normal flora of humans is exceedingly complex. The makeup of the normal flora may be influenced by various factors, including genetics, age, sex, stress, and diet of the individual[20] .

Human first becomes colonized by a normal flora at the moment of birth and passage through the birth canal. In utero, the fetus is sterile, but when the birth process begins, so does colonization of the body surfaces. Handling and feeding of the infant after birth leads to establishment of a stable normal flora on the skin, oral cavity and intestinal tract in about 48 hours[20] .

Skin flora is usually non-pathogenic, and either commensals (are not harmful to their host) or mutualistic (offer a benefit). The benefits bacteria can offer include preventing transient pathogenic organisms from colonizing the skin surface, either by competing for nutrients, secreting chemicals against them, or stimulating the skin's immune system. [15] However, resident microbes can cause skin diseases and enter the blood system creating life-threatening diseases particularly in immunosuppressed people.[15] Hygiene to control such flora is important in preventing the transmission of antibiotic resistant hospital-acquired infections.

1.3.5 Species variety

1.3.5.1 Bacteria

The estimate of the number of species present on skin bacteria has been radically changed by the use of 16S ribosomal RNA to identify bacterial species present on skin samples direct from their genetic material. Previously such identification had depended upon microbiological culture upon which many varieties of bacteria did not grow and so were hidden to science. [21]

Staphylococcus epidermidis and *Staphylococcus aureus* were thought from cultural based research to be dominant. However 16S ribosomal RNA research finds that while common, these species make up only 5% of skin bacteria. However, skin variety provides a rich and diverse habitat for bacteria. Most come from four phyla: Actinobacteria(51.8%), Firmicutis(24.4%), Protoebacteria(16.5%) and Bacteroids(6.3%) [21].

1.3.5.2 Fungi

A recent study found 14 different genera of fungi. These include yeasts such as *Candida albicans, Rhodotorularubra, Torulopsis* and *Trichoeporoncutaneum* dermatophytes (skin living fungi) such as *Microsporumgypseum,* and *Trichophytonrubrum;* and nondermatophyte fungi (opportunistic fungi that can live in the skin) such as *Rhizopusstolonifer, Trichosporoncutaneum, Fusarium, Scopulariopsis brevicaulis, Curvalaria, Alternaria alternate, Paecilomyces, Aspergillus flavus* and *Penicillium* species. [22]

1.4 Infections in cancer patients

Patients with underlying malignancies are at risk for a wide array of infectious diseases. Bacterial infections predominate, followed by fungal infections. Viral infections occur not infrequently, often as a result of reactivation of latent disease,

primarily in patients with hematological malignancies. Parasitic and other unusual infections are encountered less frequently but should be considered in individuals with appropriate exposure history [23-27].

Factors predisposing to infections in cancer patients can be illustrated in the following table:

Table (1-1) Risk factors for infection in cancer patients

Host factors	Treatment-associated factors
Disrupted anatomical barriers	Surgery Radiation therapy
Humoral immunodeficiencies	Chemotherapeutic agents
Cell-mediated immunodeficiencies	Antimicrobial use
Organ dysfunction	Diagnostic and invasive procedures, which includes: Central venous catheters Urinary catheters Tracheostomy & Blood transfusions
Concurrent illnesses and past infections	
Nutritional status	
Psychological stress	

A patient's intact normal flora protects the surfaces of the skin and mucous membranes by competing with non-indigenous organisms for binding sites and by producing substances that inhibit or kill these microorganisms. The use of antimicrobial or chemotherapeutic agents can radically alter host flora, predisposing to infections. To understand the changing microbial flora, it is important to understand a concept known as "colonization resistance". Individuals are colonized with non-invasive flora that, in a sense, can be considered ''protective.'' This normal flora prevents colonization and subsequent infection

with more invasive, pathogenic bacteria. Patients who have lost their normal flora, are at greater risk of colonization and infection with these more invasive organisms [28].

Dramatic changes in microbial flora can also occur in debilitated patients; ie, severity of illness and medications given, not hospitalization per se, is associated with changes in endogenous flora[29].

The skin is the body's largest organ and its most important barrier against infections. It's the first line of defense in protecting internal tissues from harmful germs. It also keeps body tissues from drying out (dehydrating). When there's a break in the skin, it's easier for germs to enter the body and cause infection.

Cancer treatments (such as chemotherapy, radiation therapy, or surgery) and certain procedures (like putting in catheters or IVs, or getting shots) can damage the skin or mucous membranes. This makes it easier for germs to get in. [30]

Some types of cancer can damage the immune and blood systems or change the way they work. For instance, lymphomas (Hodgkin and non-Hodgkin) and certain types of leukemia start in immune system cells. They change the immune system cells so that cells that once protected the body begin to interfere with the normal way the immune system works. Many other types of cancer can also affect the immune system.

In most cases it's not the cancer itself, but the cancer treatment that changes the immune system. Treatments can cause short- or long-term damage. For example, long-term damage happens when immune system organs such as the spleen are removed. A splenectomy is sometimes done to remove cancer or learn how much it has spread. On the other hand, chemotherapy, radiation therapy, immunotherapy, either alone or in combination can lead to short term immune system damage. Bone marrow or stem cell transplant uses very strong treatments to kill cancer cells. [30] This treatment also kills immune system cells, which can worsen and

prolong the risk of infection. Sometimes this damage can last for months after treatment ends. Some people with cancer have a higher risk of infection because of the changes in their body's defense systems. Cancer and cancer treatments can affect these systems in different ways.

The increase in hospital infection rates caused by gram-negative bacteria in recent years, particularly in the compromised host, has raised the question of the role of the skin as a source of the infectious material. Whereas the microbial flora of skin is predominantly gram-positive in healthy individuals, gram-negative bacteria have been found to be the predominant organisms isolated from the skin of critically ill patients, which suggests that with certain systemic disease processes gram-negative bacteria may more readily colonize the skin[31] .

1.5 Aims of the study

The purpose of this study is to:

(1) Study the pattern of microbial skin flora in patients with malignant diseases.

(2) Study the change in the pattern of microbial skin flora in relation to chemotherapy.

Chapter Two

Subjects and Methods

2. Subjects and Methods

2.1 Subjects

2.1.1 Study Design

This is a cross sectional study which was carried out in the period from the first of April 2014 till the end of December 2014. It includes children and adolescents with ages ranged from 1 to18 years.

2.1.2 Study population

The study population included children and adolescents aged from 1 to 18 years of age who were newly diagnosed with malignancy and admitted to oncology center in Basra Specialty hospital.

A total of 50 patients with newly diagnosed malignancies were included in the study. Of them, 28 patients were newly diagnosed cases of hematological malignancies; the remaining patients had different types of solid tumors.

2.1.3 Exclusion criteria:

Exclusion criteria included:

- Patients younger than 1 year or older than 18 years of age.
- Patients with skin disease or lesion of any type, e.g. impetigo, eczema… etc.

2.2Data collection

A special Questionnaire was designed for the purpose of the study. The following information were taken: serial number, name, age, sex, date of birth, type of malignancy and recording the results of skin swabs taken from 8 sites of the body which are both palms of the hands, soles of the feet, over the back

between the scapulae, both axillary regions and the groin. Recording both results taken first at the time of diagnosing their malignancy and a second sample was taken after one month of commencing chemotherapy.

2.3 Sampling procedure

- All patients were sampled within 48hrs of admission to the hospital and prior to receiving treatment of any kind, including antibiotics. The skin swabs were taken by the study researcher. Patients and their parents were told to rinse the selected areas with normal saline only and not to apply any disinfectant of any kind. Then all patients were resampled after one month of starting chemotherapeutic drugs.
- The sites cultured were palms of hands, soles of feet, and back over the scapulae, axillae, and groin.
- Samples were taken from both right and left sides of the body. Specimens were collected with a sterile CultureSwab™ Plus Amies Gel and then sent to a laboratory for analysis.

2.3.1 Microbiological procedures

Swabs were cultured on the following media:

1. Blood agar: to show the hemolytic properties of micro-organisms.
   The plates were incubated under 5-10 % CO2.
2. Chocolate agar for growth of *Haemophilus influenzae*. The plates were incubated under 5-10 % CO2.
3. MacConkey agar for isolation of Enterobacteriacea .The plates were incubated aerobically.
4. Sabouroud's agar: to allow the growth of fungus.

The swabs cultured on these four media were then incubated overnight at 35 – 37°C. The microorganisms recovered from plates were fully identified by standard microbiological methods [32, 33] which involve:

Colony morphology

This includes type of hemolysis (if any), pigment (if present), size, and texture (opaque, transparent, translucent) and many other characteristics.

- **Gram's stain**

  The organism can be either Gram positive or Gram negative according to bacterial cell wall characters and Gram reaction.

- **Biochemical tests**

  These various tests were designed to identify various metabolic properties of different bacterial species. These tests were performed either manually or by using commercial identification systems (e.g. : Api staph , Api strept , Api 20E), or by using automated microbiology system (Phoenix BD and VITEK 2 compact).

- **Catalase test**

  It is the key in differentiation between many Gram positive organisms. Staphylococcus is catalase positive while streptococcus and enterococci are catalase negative.

- **Coagulase test**

  Coagulase test differentiates *Staphylococcus aureus* (COPS) from coagulase negative staphylococci (CONS), such as *S. epidermidis, S. saprophyticus.*

2.4 Statistical analysis:

Statistical analysis was done using SPSS program V. 20, data were expressed and comparisons of proportions was performed, P-value of<0.05 was considered statistically significant.

Chapter Three

Results

3. Results

3.1. Age and sex distribution in cancer patients

A total of 50 patients who were newly diagnosed with malignancy were included in the study. Their ages ranged from one to eighteen years (mean 9yrs± 3 months) as shown in the following table:

Table (3-1) Distribution of cancer patients according to the age and sex

Age (years)	Male		Female		Total		P value *
	No.	%	No.	%	No.	%	
1-6	5	20.8	9	34.6	14	28	>0.05
7-12	14	58.4	14	53.8	28	56	
13-18	5	20.8	3	11.6	8	16	
Total	24	48	26	52	50	100	

*Chi-square test

This Table shows that within the 50 patients included in the study, 24 of them are males and 22 are females. The majority are between 7 and 12 years of age, 58.4% and 53.8% for males and females respectively. There is no statistically significant difference regarding sex and age distribution between cancer patients (P>0.05).

3.2 Types of malignancy according to sex of the patients

Malignancy type distribution in relation to sex of the patients is shown in table (3-2)

Table (3-2) distribution of malignancy type according to sex of the patients

Sex	Type of malignancy				P value *
	Hematological		Solid		
	No.	%	No.	%	>0.05
Male	15	53.6	9	40.9	
Female	13	46.4	13	59.1	
Total	28	100	22	100	

*T-test

This Table shows that among the fifty patients with cancer, 28 are diagnosed with hematological and 22 are diagnosed with solid tumors. There is no significant difference in terms of distribution of malignancy types according to patients' sex. (P >0.05).

3.3 Frequency of malignancy types in cancer patients

The following table shows the frequency of malignancy types in patients included in the study.

Table (3-3) The malignancy type frequency in patients of the study

Type of malignancy		No.	%
Hematological	**ALL**	17	34
	AML	2	4
	HL	6	12
	NHL	1	2
Solid	**Neuroblastoma**	12	24
	Wilm's tumor	5	10
	Rhabdomyosarcoma	4	8
	Retinoblastoma	1	2
Total		50	100

This table shows that among patients with hematological malignancies, 17 (34%) are diagnosed with ALL followed by 6 (12%) with Hodgkin lymphoma. Whereas among those with solid tumors, 12 (24%) have Neuroblastoma and 5 (10%) with Wilm's tumor.

3.4 Comparison between the first and second samples of skin flora cultured from the palms of the hands of the patients

The following table shows the results of cultured skin flora taken from the patients' palms both initially and one month after commencing chemotherapy.

Table (3-4) The results of skin swabs taken from the patients palms initially & 1 month after chemotherapy

Culture results	Swabs from palms of the hands				P value *
	Specimen 1		Specimen 2		
	No.	%	No.	%	<0.05
No growth	49	98	28	56	
Klebsiella	0	0	18	36	
Nonpathogenic flora	1	2	4	8	
Total	50	100	50	100	

*Chi-square test

This table shows that there is a significant increase in presence of pathological micro-organisms over the palms of the hands in cancer patients following chemotherapy, where only 56% of patients showed no growth after chemotherapy compared to 98% before starting chemotherapy.(P <0.05).

3.5 Comparison between the first and second samples of skin flora cultured from the soles of the feet of the patients

The following table shows the results of cultured skin flora taken from the patients' soles both initially and one month after commencing chemotherapy.

Table (3-5) The results of skin swabs taken from the patients soles initially & 1 month after chemotherapy

Culture results	Swabs from soles of the feet				P value *
	Specimen 1		Specimen 2		
	No.	%	No.	%	<0.05
No growth	45	90	20	40	
Proteus	0	0	13	26	
Peudomonas	0	0	9	18	
Klebsiella	0	0	3	6	
Nonpathogenic flora	5	10	5	10	
Total	50	100	50	100	

*Chi-square test

This table shows that there is a significant increase in presence of pathological micro-organisms on soles of the feet in cancer patients following chemotherapy, P <0.05.

3.6 Comparison between the first and second samples of skin flora cultured from the axillary region of the patients

The following table shows the results of cultured skin flora taken from the patients' axillary region both initially and one month after commencing chemotherapy.

Table (3-6) The results of skin swabs taken from the patients axillary region initially & 1 month after chemotherapy

Culture results	Swabs from the axilla				P value *
	Specimen 1		Specimen 2		
	No.	%	No.	%	<0.05
No growth	33	66	7	14	
Proteus	1	2	19	38	
Peudomonas	1	2	13	26	
Klebsiella	1	2	7	14	
Nonpathogenic flora	14	28	4	8	
Total	50	100	50	100	

*Chi-square test

This table shows that there is a significant increase in presence of pathological skin flora cultured from the axillary region of the cancer patients one month after commencing chemotherapy, where only 14% showed no growth after chemotherapy compared to 66% before chemotherapy, this gives substantially significant result. ($P < 0.05$)

3.7 Comparison between the first and second samples of skin flora cultured from the back of the patients between the scapulae

The following table shows the results of cultured skin flora taken from the patients' back area between the scapulae both initially and one month after commencing chemotherapy.

Table (3-7) The results of skin swabs taken from the patients' back area between the scapulae initially & 1 month after chemotherapy

Culture results	Swabs from the back				P value *
	Specimen 1		Specimen 2		
	No.	%	No.	%	>0.05
No growth	32	64	10	20	
Proteus	1	2	15	30	
Peudomonas	2	4	12	24	
Klebsiella	1	2	7	14	
Nonpathogenic flora	14	28	6	12	
Total	50	100	50	100	

*Chi-square test

This Table didn't reveal a significant increase in the presence of pathological microflora over the patients' backs before and after therapy of cancer patients. (P>0.05)

3.8 Comparison between the first and second samples of skin flora cultured from the groin region of the cancer patients

The following table shows the results of cultured skin flora taken from the patients' groin region both initially and one month after commencing chemotherapy.

Table (3-8) The results of skin swabs taken from the patients' groin region initially & 1 month after chemotherapy

Culture results	Swabs from the groin				P value*
	Specimen 1		Specimen 2		
	No.	%	No.	%	>0.05
No growth	11	22	3	6	
Proteus	3	6	20	40	
Peudomonas	2	4	11	22	
Klebsiella	1	2	7	14	
Nonpathogenic flora	33	66	9	18	
Total	50	100	50	100	

*Chi-square test

This Table didn't reveal a significant increase in the presence of pathological microflora in the groin region of the cancer patients before and after therapy of cancer patients. (P>0.05)

3.9 The isolation of pathological microflora in relation to patients' age and type of malignancy

The isolation of pathological skin flora cultured from cancer patients in relation to type of malignancy and patients' age is showed in the following table.

Table (3-9) pathological microflora in relation to patients' age and malignancy type

Type of malignancy	Results of pathological flora			
	Positive		Negative	
	No.	%	No.	%
Hematological	24	60	4	40
Solid	16	40	6	60
Total	40	100	10	100
P>0.05 *				
Age (years)				
1-6	9	23.1	5	45.4
7-12	22	56.4	6	54.6
13-18	8	20.5	0	0
total	39	100	11	100

*chi-square test

This table shows that there is no substantial significant difference regarding the isolation of pathological microflora from the skin of patients with cancer treatment in terms of type of malignancy or the patients' age group. (P>0.05)

Chapter Four

Discussion

Conclusions

Recommendations

References

4.1 Discussion

Childhood cancer occurs regularly, randomly and spares no ethnic group, socioeconomic class or geographic region. Childhood cancer is not just one disease; it is made up of a dozen types and countless subtypes. The cause of most childhood cancers is unknown and is not strongly linked to lifestyle or environmental risk factors, unlike many adult cancers. Two-thirds of childhood cancer patients will have long lasting chronic conditions from treatment. [34]

Infectious complications are still a major cause of morbidity and mortality in pediatric patients undergoing therapy for malignancy. Therapy-induced neutropenia is the most important risk factor for infectious risk in pediatric patients with cancer, but other factors, such as alterations in skin/mucosal barriers, and defects in cell-mediated or humoral immunity also contribute to the risk for infection. In most centers, about two thirds of bacteremic isolates are gram-positive pathogens, whereas gram-negative organisms are isolated less frequently, but are associated with considerably higher mortality rates[35] .

Current data indicate that gram-positive organisms cause 45%–70% of documented infections in patients with malignancies. However, many epidemiological surveys focus only on blood stream infections.[36,37]This may result in an incomplete and/or inaccurate picture, because only 15%–25% of patients with neutropenia develop a blood stream infection (other common sites of infection include the respiratory tract, the urinary tract, skin and the gastrointestinal tract), and because blood stream infections are caused predominantly by gram-positive organisms, whereas infections at most other sites are predominantly gram-negative or polymicrobial [38] .

The current study included 50 patients who were newly diagnosed with cancer and were assigned for the survey. As explained previously, specimens of skin swabs were taken from the patients from five body sites which include both palms of the hands, both soles of the feet, axillary regions, the groin and the back area between the scapulae. The samples were sent for microbiological study. Then a second set of specimens were taken again from the same sites one month after commencing chemotherapy to be compared with the results of the first isolates. It was found that, by the second specimens, the patients showed significant growth of Gram negative bacteria (*Proteus*, *pseudomonas* and *Klebsiella*) in samples taken from the palms of the hands, soles of the feet and from the axillary regions as compared to the first culture results. This observation was in agreement with a study performed in Texas by Mollie E et al[39] , although their study showed similar growth in groin and the back area. This can be attributed to our small sample size. Our results were also in agreement with those obtained from Abdulaziz Zorgani et al[40] in Libya and Ramadan Al domain et al[41] in Egypt.

In the current study, it was also found that the isolation of pathological skin flora was not directly related to the type of malignancy whether hematological or solid type of tumors, this is in contrast to a study performed by Teresa R in Chicago where they found that such isolates are seen more in leukemic patients than in patients with solid tumors, probably this is largely because the standard chemotherapeutic regimens used to treat these malignancies do not usually result in either long-term or profound neutropenia.[42]

On the other hand, isolating such pathological skin flora from patients with cancers did not show any predilection to a specific age group in the study population ($P > 0.05$).

However, there are some limitations in this study, the time during which the study was performed was only eight months during which only a small number of newly diagnosed patients with malignancies were assigned to the survey, and this small population of patients included small number of both types of malignancies; also the patients included in the study were residents of one or two city districts and not from different regions in Iraq which should be considered to clarify whether such geographical differences can alter the results of the isolated skin flora in such patients.

4.2 Conclusions

The current study has demonstrated that:

- Normal skin flora is altered in patients with malignancies and in those receiving chemotherapy.
- Pathological skin flora can be isolated from certain sites of the patients' bodies such as the axillary region, palms of the hands and soles of the feet after around one month of commencing chemotherapy.
- There is no specific predilection for the age group of the patients with malignancies regarding isolating such pathological skin flora.
- There is no correlation between the type of malignancy and the isolation of pathological skin flora.

4.3 Recommendations

- Considering performing skin swabs from different body sites from the patients with malignancies as one of the other initial laboratory workup aspects.
- Performing skin swabs to the patients after commencing chemotherapy to establish any significant change in the pattern of the flora.
- Identifying the specific species of the isolates and their antibiotic sensitivity for proper management.
- The use of media and local hospital programs for education of the patients and their care givers about skin health and care.
- Further studies are needed to evaluate the skin as a source of significant infectious complications in patients with malignancies or those receiving related chemotherapy, covering more geographical areas with larger sample size and more types of oncological disease.

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Serial no. ☐

Name: ☐

Age / Date of birth: ☐

Type of malignancy

Hematological: Leukemia... ALL ☐ AML ☐ CML ☐

Lymphoma... HL ☐ NHL ☐

Solid tumors: Wilm's ☐ RMS ☐ NB ☐

Bone tumor ☐ Brain tumor ☐

Sites of skin swabs...

1st specimen within 48 hours of admission...

Palms of hands: RT ☐ LT ☐

Soles of feet: RT ☐ LT ☐

Back over scapulae: ☐

Axilla: RT ☐ LT ☐

Groin: ☐

2nd specimen after one month of starting chemotherapy...

Palms of hands: RT ☐ LT ☐

Soles of feet: RT ☐ LT ☐

Back between scapulae: ☐

Axilla: RT ☐ LT ☐

Groin: ☐

الخلاصة

اجريت الدراسة لتحديد النمط البكتيري المستوطن لبشرة الاطفال المصابين بالامراض السرطانية و الذين يتعاطون العلاجات المتعلقة بهذه الامراض.

تم دراسة خمسون طفلا تم تشخيصهم حديثا بامراض سرطانية و تم ادخالهم لمستشفى البصرة التتخصصي للأمراض السرطانية لدى الأطفال بعد ان تم اخذ موافقة ذوي الأطفال لغرض شملهم بالدراسة. الدراسة تم اجراءها في الفترة الممتدة من الاول من شهر نيسان لغاية شهر كانون الأول لسنة 2014.

الدرلسةشملت 24 (48%) و 26 (52%) من الاطفال الذكور و الاناث بالتتابع. من مجموعة الاطفال الذكور, 15 و 9 منهم كانوا مصابين بسرطان الدم و اورام صلبة تتابعا. و من بين مجموعة الاناث, 13 و 13 منهم كانو مصابين بسرطان الدم و اورام صلبة بالتتابع.

تم تصميم استمارة استبيان خاص للدراسة تتضمن معلومات العمر، الجنس ،نوع المرض السرطاني، بالاضافة الى نتائج المسحات الجلدية المأخوذة من بعض الأماكن على الأجسام و التي تشمل مناطق الابط، راحة اليدين، اخمص القدمين، منطقة الحوض بين الأفخاذ و منطقة الظهر بين الكتفين.

تم أخذ عينات لمسحات جلدية من المناطق المذكورة سابقا من المرضى خلال ثمانية واربعين ساعة من دخولهم للمستشفى قبل البدء بالعلاج الخاص بالامراض السرطانية وتم ارسالها لوحدة الاحياء المجهرية لغرض دراستها.

بعد فترة شهر من بدء العلاج بالادوية الكيمياوية الخاصة بالامراض السرطانية، تم اخذ عينات لمسحات جلدية ثانية من نفس المناطق الاولى للجسم لتتم مقارنتها بالنتائج المستحصلة من المسحات الأولى حيث تم ملاحظة ان هناك تغير ملحوظ للنمط البكتيري خصوصا تلك العينات المأخوذة من مناطق راحة اليدين و اخمص القدمين و منطقة تحت الابطين. حيث لوحظ انه فقط 28 (56%) من المرضى لم يظهروا اي نوع من البكتريا في منطقة راحة اليدين بعد العلاج الكيمياوي مقارنة ب 49 (98%) منهم قبل البدء بالعالاج حيث كانت نسبة الاحتمالية < 0.05 . تم الحصول على نتائج مماثلة من المسحات الماخوذة من اخمص القدمين حيث ان 13(26%) و9 (18%) و 3 (6%) من المرضى اظهروا نمو بكتريا من نوع بروتيوس و تسيدوموناس و كليبسيلا بالتتابع مع نسبة احتمالية < 0.05.

المسحات المأخوذة من منطقة تحت الابطين اظهرت ان 19 (38%) و 13 (26%) و 7 (14%) من الاطفال اظهروا نمو لبكتريا البروتيوس و التسيدوموناس و الكليبسيلا بالتتابع مع نسبة احتمالية < 0.05. بينما المسحات المأخوذة من منطقة بين الافخاذ و منطقة الظهر بين الاكتاف لم تظهر فرقا احصائيا كبيرا بين النتائج المستحصلة من العينات الثانية عن تلك المستحصلة من العينات الاولى من حيث نمو تلك الانواع من البكتريا مع نسبة احتمالية > 0.05.

هذه الأنواع من البكتريا المستوطنة لبشرة المرضى المصابين بالسرطان لم تظهرا رتباط معين لنوع الامراض السرطانية او الفئة العمرية للاطفال المصابين بهذه الامراض.

لهذا يمكن اعتبار اخذ مثل هذه العينات من المسحات الجلدية كجزء من التحاليل المختبرية الاولية التي تجرى للاطفال المشخصين بالامراض السرطانية واعادة اجراء هذه المسحات بعد فترة من العلاج الكيميائي لبيان اي اختلاف حاصل في نمط البكتريا المستوطنة لبشرة هؤلاء الاطفال و اخذ الأجراءات اللازمة لتفادي المضاعفات المحتملة من جراء مثل هذه البكتريا.

النمط البكتيري المستوطن لبشرة الاطفال المصابين بالأمراض السرطانية

أطروحة مقدمة الى المجلس العراقي للأختصاصات الطبية

وهي جزء من متطلبات نيل درجة زمالة الهيئة العراقية

للأختصاصات الطبية فرع طب الأطفال

من قبل

ظافر توفيق نعيم

بكالوريوس طب وجراحة عامة

اشراف

الأستاذ المساعد

د.جنان غالب حسن

فرع طب الأطفال

كلية الطب-جامعة البصرة

شباط 2015