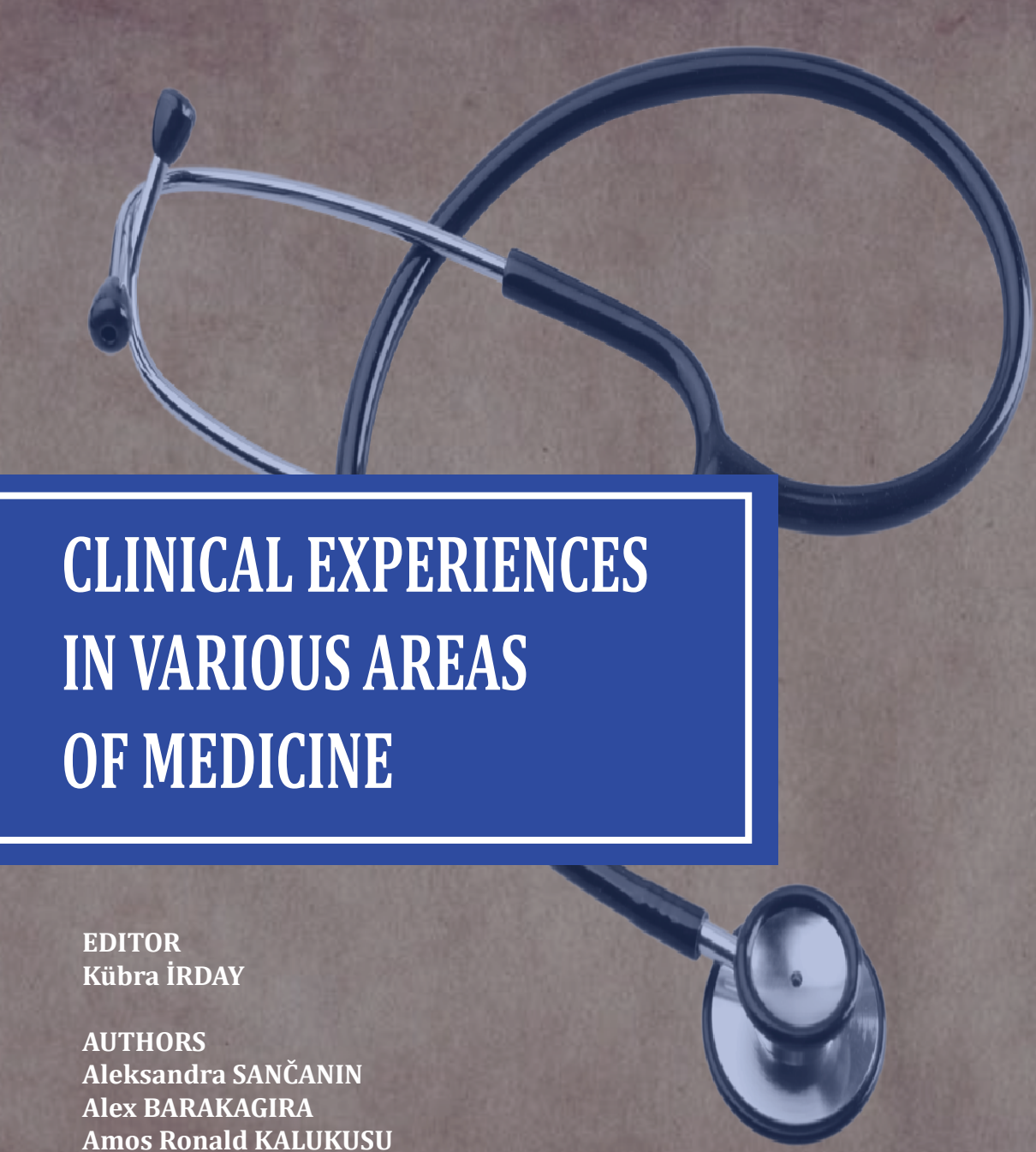

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EDITOR
Kübra İRDAY

AUTHORS
Aleksandra SANČANIN
Alex BARAKAGIRA
Amos Ronald KALUKUSU
Branislav SANČANIN
Çiğdem KARADAĞ
Dragomira NIKOLOVA
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Sofija SANČANIN

(The Licence Number of Publicator: 2014/31220)
TURKEY TR: +90 342 606 06 75
USA: +1 631 685 0 853
E mail: iksadyayinevi@gmail.com
www.iksadyayinevi.com

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PREFACE

This .book is designed to bridge the space in postgraduate medical education and support continuing medical education in various areas of medicine. Its content provides coverage of topics including:, newborns' screening, children immunization, stem cell application, artificial intelligence in otolaryngology, prostate laser enucleation, urinary tract infections and cancer radiotherapy.

Varying from traditional medical books, the organization of this resource is driven by clinical experience. The sections in the book are not intended to be across-the-board, but rather focuses on the content delivery. The text is meant to offer the necessary information in a concise form allowing the reader to understand and perform the details in an actual clinical experience. There is abundant literature in medicine science freely accessible on the internet and readers can find themselves struggling and devoting a great part of their precious time trying to find the right tools that are mostly unclear in providing the required information. This book serves as a source of information and reference for clinicians, other health professionals and clinician candidates with an interest in the topics included.

Hope all find the book interesting and useful.

Kübra İRDAY

October 2022

CHAPTER 1

A NECESSITY FOR EXPANDING THE

NEWBORNS' SCREENING PANEL IN BULGARIA

Assist. Prof. Dragomira NIKOLOVA[1, 2]
Acad. Prof. Draga TONCHEVA[2]

[1] Department of Medical genetics, Medical Faculty, Medical University – Sofia, 1431 Sofia, Bulgaria.ORCİD: 0000-0002-8929-8522, dnikolova@medfac.mu-sofia.bg, +359 895 78 40 96,
[2] Bulgarian Academy of Sciences, Bulgaria, 1040, Sofia, Noemvri st., 1, 15, Sofia, Bulgaria.ORCİD: 0000-0002-7451-6363,dragatoncheva@gmail.com

INTRODUCTION

The neonatal screening involves testing all newborns for certain hereditary diseases, the early diagnosis of which is key to the treatment process. To be included in a screening program, the disease should meet certain conditions, such as 1. to be curable;2. to be relatively common in the population and 3. to have fast, accessible, cheap and effective methods for early diagnosis. Neonatal screening is the best way to detect dangerous conditions for the child's health, which, in the first months of its life, do not give concrete manifestations. The earlier a health problem is diagnosed, the more likely it is that the child will receive timely and adequate care that will "suppress" the manifestations of the disease.

In Bulgaria, neonatal screening includes only three diseases - phenylketonuria, congenital hypothyroidism and congenital adrenal hyperplasia. In all three cases, the screening is done by taking a blood sample between the third and fifth day after the child's birth. The national screening program for hypothyroidism was introduced in Bulgaria in 2000, and for adrenocortical hyperplasia in 2010.

Phenylketonuria (PKU) is most often caused by a defect in the gene for the enzyme that converts the amino acid phenylalanine to tyrosine. The inability of the defective enzyme to metabolize phenylalanine leads not only to its accumulation, but also to the insufficiency of other substances that cannot be synthesized (melanin, dopamine, thyroxine). The early diagnosis of phenylketonuria and the introduction of a lifelong special diet poor in phenylalanine prevents the clinical development of the disease. Thus, screening for PKU is important for the prevention of the clinical manifestation of the disease.

Congenital hypothyroidism (CH) is the most common congenital endocrine disorder and the most common preventable cause of childhood mental retardation. The genetic causes of this condition are variable. It leads to developmental delay and mental retardation.

The treatment of CH is performed on a daily basis by an oral thyroxine intake.

Congenital adrenal hyperplasia (CAH) is caused by genetic defects in genes for enzymes involved in corticosteroid synthesis. Blood cortisol levels are low. Sometimes this is combined with a reduction in aldosterone concentration. In all cases, however, there is an increase in testosterone level and virilization. Treatment is performed by application of corticosteroids, which are taken for life, and their levels are adjusted depending on the condition.

Each year, over 6.5 million babies worldwide are born with congenital genetic abnormalities. In our country, the screening is extremely insufficient to provide precise and comprehensive information about the health status of the baby. Unlike Bulgaria, in most of the other countries of the EU, the screening covers from 5 to 30 different genetic conditions due to the specifics of individual populations and the financial capabilities of individual member countries. A large part of them belong to the so-called IEM (inborn errors of metabolism) due to the absence or a deficiency of an enzyme catalyzing a certain metabolic process in the body. A significant percentage of the IEMs are rare diseases, with an incidence of 1 in 100,000 births or less. Currently, the number of rare diseases is over 7,000, and for 6-7% of them, therapies have been developed.

The purpose of this study is to compare the screening programs in Bulgaria and other countries in EU, as well as in different states of USA, in order to emphasize the need to expand the panel of diseases included in the national screening program. In addition, we would like to present the capabilities of the next-generation sequencing technology to be used as a main screening method.

RESULTS AND DISCUSSION

In Germany, for example, in October 2021, two new diseases were added to the newborn screening panel - spinal muscular atrophy (SMA) and sickle cell anemia (SCA). Thus, the total number of diseases becomes 17 ("https://www.eurordis.org/our-priorities/diagnosis/newborn-screening/,"). Some countries such as Italy, Portugal and Austria screen more than 25 different conditions as a part of their national screening panel, and Italy is the leader in the field, diagnosing more than 45 diseases. England and France, on the other hand, carry out a basic testing package for 10 diseases, and Romania and Cyprus for just two. PKU and congenital hypothyroidism are universally screened for in all 30 countries (Loeber et al., 2021). These diseases have a relatively high frequency compared to others (frequency higher than 1:10000) which have frequencies less than 1:250000. Diseases such as SMA (spinal muscle atrophy) and SCA (sickle cell anemia) have similarly high frequency and display large differences in terms of screening, being included in less than 30% of European countries ("https://www.orpha.net/orphacom/cahiers/docs/GB/Prevalence_of_rare_diseases_by_alphabetical_list.pdf,").

Some differences in Europe also concern autonomous regions in individual countries – for example, the autonomous region of Catalonia tests for over 20 different diseases, while tests at the national level recommend fewer than 10 diseases. The process of updating the lists also differs (in the UK this is done on an annual basis, and in Germany – if needed at any time), as well as the time for applying the changes (in Denmark and Belgium – less than a year, in Greece, Romania and Portugal – over 3 years) ("ttps://www.technologynetworks.com/diagnostics/blog/a-landscape-assessment-of-newborn-screening-in-europe-359964,").

The EU's aim is the governments of the least developed countries to commit to expanding the panel of diseases included in the newborn screening, but there are still a number of obstacles for this to happen

("ttps://www.technologynetworks.com/diagnostics/blog/a-landscape-assessment-of-newborn-screening-in-europe-359964,").

The newborn screening programs (NBS) in Southeastern Europe are not always synchronized with those in developed European countries (Koracin et al., 2021). According to a survey from 2013-2014 among 11 of these countries, none of them had an expanded NBS program (Koracin et al., 2021). The survey was repeated in 2020 with an aim to assess the current state, to evaluate the changes and main obstacles to implement the innovations. All of the surveyed countries (12 in the region), except Kosovo, were performing the screening test for CH, while PKU was not even screened in 4 out of 12 countries. The conclusion of the authors is that the NBS programs in Southeastern Europe are still underdeveloped or even non-existing in some of the countries.

In comparison, the US currently recommends screening for 61 conditions included in the so-called "Recommended Uniform Screening Panel (RUSP), with each state determining how many conditions to screen depending on their economic status, medical infrastructure, etc. (Figure 3).

Most states screen for at least 31 out of 35 genetic conditions that occur in children and newborns. These are known as the 'main'/'core' panel. Although these conditions are rare, more than 5,000 babies are diagnosed with one of them each year. The Newborn screening (NBS) is performed in three steps: 1. Taking a blood sample from the heel of the newborn; 2. Pulse oximetry to measure the oxygen level in the baby's blood and 3. Hearing test. The blood test is performed 24-48 hours after birth. Some of the conditions remain hidden if the sample is taken before the 24th hour, because an accumulation of the intermediate metabolite is necessary, whose amount is measured and detected.

In the most developed states of the US (Massachusetts and Connecticut), 66 congenital genetic conditions are tested, and the cost

of the tests is estimated at around $135 ("https://babysfirsttest.org/newborn-screening/states/connecticut," ; "https://babysfirsttest.org/newborn-screening/states/massachusetts,"). They include:

- Diseases of amino acid metabolism – 17
- Endocrine diseases - 2
- Diseases with defects in the oxidation of fatty acids - 13
- Hemoglobin defects – 4
- Lysosomal storage diseases – 2
- Defects of organic acids – 15
- Other diseases - 13

In New York State (NY), 60 genetic disorders are tested ("https://babysfirsttest.org/newborn-screening/states/new-york,"):

- Diseases of amino acid metabolism – 12
- Endocrine diseases - 2
- Diseases with defects in the oxidation of fatty acids - 13
- Hemoglobin defects – 4
- Lysosomal storage diseases – 3
- Defects of organic acids – 15
- Other diseases - 11

In one of the least developed states Alabama (Alabama, AL), Arkansas (Arkansas, AR) and West Virginia (West Virginia, WV) – 47, 32 and 39 diseases, respectively ("https://babysfirsttest.org/newborn-screening/states/alabama," ; "https://babysfirsttest.org/newborn-screening/states/arkansas," ; "https://babysfirsttest.org/newborn-screening/states/west-virginia,"). The cost of the screening is $150, $131, and $79. In addition, the screening programs include not only the tests, but also the logistics, the involvement of consultants and general practitioners, as well as postnatal monitoring of the child's

development. In most states, the screening is covered by health insurance and is available only to insured individuals.

The core screening panel includes 35 primary conditions (Figure 4) and 26 secondary conditions (Figure 5) ((ACMG), 2006; Sweetman et al., 2006) that are recommended for inclusion in every state's list. This panel is optional but recommended and serves as a guide and reference for each individual state ("https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp/index.html,").

A core condition is defined as follows:

- For which there is a specific and sensitive detection test;
- The disease development is well known;
- There is an available and effective treatment;
- Diagnosis of the condition will help the family's future reproductive decision("https://babysfirsttest.org/newborn-screening/the-recommended-uniform-screening-panel,").

Secondary conditions are those that are found when trying to identify a primary one ("https://babysfirsttest.org/newborn-screening/ the-recommended-uniform-screening-panel,"). If a condition is included in the screening list, it goes through mandatory procedures such as validation of the laboratory test, confirmatory testing with a sensitive and specific diagnostic test, and conducting a prospective pilot study.

The US system of including a broad range of diseases into the screening panels is often criticized for covering conditions that do not necessarily require therapy in the neonatal period or those for which there is no consensus regarding therapy. The fact is, however, that currently the screening panels of even the least developed states cover more diseases than those covered by the countries in Europe.

Canada does not have a national strategy for newborn screening, and there are large differences between provinces, including the

number of diseases for which a testing is offered, the information provided to parents, and carrier status determination policy (Howard et al., 2015).

Is the next generation screening (NGS) technique applicable to perform neonatal screening?

Advances in sequencing technology have led to a reduction in the cost and time to generate sequence data for the human genome. Both the European Ethics Committee, the European Society of Human Genetics (ESHG) and the International Pediatric Platform have focused on the issue of the potential use of sequencing technologies in newborn screening. The main goal is to use targeted analysis and to identify gene variants that carry a high risk of preventable diseases with an existing treatment. After the diagnosis of the disease, the therapy should start in the period of the newborn or in the early childhood.

Sequencing technologies have already made their way into identifying target genes for anti-cancer therapy as well as non-invasive prenatal testing during pregnancy. NBS (newborn screening) by sequencing would replace many other tests used at the same time, such as tandem mass spectrometry (MS), as well as other complementary genetic tests. Only for the congenital hypothyroidism (CH) sequencing cannot replace existing methods, because it is a non-genetic condition (Castellani & Massie, 2014). It is estimated that sequencing technologies in newborns will become routine in about 10 years, applied to the future generations. The cost of this method is still being determined, although currently it is around 43 Euros per newborn in the Netherlands, but it is known that the price will drop further as the number of screening reactions increases.

The best option would be to use the capabilities of modern genetic tests based on the next-generation sequencing - a technique that makes it possible to examine more than 200 genes responsible for more than 220 different diseases with a single sample. In addition to common

genetic diseases, these tests also include lysosomal storage diseases as well as primary immune deficiencies. Depending on the manufacturers, the tests may also include an option to identify pharmacogenetic variants of known clinical significance to determine the child's response to some of the most commonly used medications in clinical practice.

It would be possible, in the presence of consensus at the national level, to develop an own (handmade) test according to the frequency of genetic diseases in the Bulgarian population. The undoubted advantage of this test is the speed and very high accuracy (over 99%). In combination with high-tech bioinformatic analysis, it establishes with absolute accuracy the presence of potential mutations in the observed genes. In addition, the procedure can be non-invasive - it is enough to take a swab from the baby's buccal mucosa. The test makes it possible to screen from a very early stage for conditions that may remain hidden and/or be registered only when symptoms appear. It is also important to note that a more extensive panel can be used in children who have symptoms of metabolic diseases (convulsions, coma, lethargy, deafness, blindness, etc.), as well as those with a family history of such conditions.

CONCLUSION

Despite the efforts of various patient organizations and medical professionals specializing in rare diseases, the screening of only three diseases in Bulgaria is extremely insufficient. The register of rare diseases in Bulgaria was started as a part of a European project only in 2017 by the

National Center of Public Health and Analyses, and some rare diseases are not included in the list of rare diseases of the Ministry of Health. There is also a lack of care regarding rehabilitation programs and means of therapy for some diagnoses. Thus, in Bulgaria, transplants (lung, liver, etc.) are needed more often than in Europe.

This is a result of timely diagnosis and the existence of a wider panel of diseases included in the neonatal screening.

The expansion of the neonatal screening (NBS) panel is also important for another reason – very often the symptoms of various diseases in childhood are similar or overlap, and can be difficult to distinguish. For example, muscular dystrophies and SMA in infancy can be misdiagnosed as cerebral palsy. With early diagnosis, the child will receive adequate and timely medical care. In order to make possible the expansion of neonatal screening in Bulgaria, state commitment, a close relationship with patient organizations, as well as simplification of the procedure for registration of rare diseases is required.

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CHAPTER 2

IMMUNIZATION COVERAGE AGAINST DISEASES AND DROPOUT RATE AMONG CHILDREN AGED FIVE YEARS AND BELOW IN MAWOGOLA HEALTH SUB-DISTRICT, SEMBABULE DISTRICT, UGANDA.

Herbert TUMUSIIME[1]

Alex BARAKAGIRA[2]

Amos Ronald KALUKUSU[3]

[1] Herbert Tumusiime; King Ceasor University ,P O Box 88,Kampala,Uganda
Corresponding Author: htumusiime@kcu.ac.ug
[2] School of Sciences, Nkumba University, P.O Box 237, Entebbe, Uganda
[3] School of Sciences, Nkumba University, P.O Box 237, Entebbe, Uganda

INTRODUCTION

Immunization is the protection of susceptible individuals from acquiring diseases by administering a suspension of killed organism, a living modified agent or an inactivated toxin (WHO, 2005). It is done with the aim of attaining herd immunity which is a situation where susceptible individuals are protected from disease because a significant proportion of the community is immune as a result of either natural disease or immunization. Immunization assessments in terms of delivery and impact of immunization services can be monitored through immunization coverage.

Immunization coverage can be defined as the proportion of individuals in the target population who are vaccinated (WHO, 2005). Immunization coverage can be crude or valid. Crude coverage is the proportion of children surveyed who received an antigen or a set of antigens as recorded on the child health card and or by history. It includes all antigens reported to have been given irrespective of interval and age at which they were given. Valid coverage on the other hand is the number of children who receive vaccinations in accordance with the national immunization schedule with respect to the age and interval at which they are given as recorded on the child health card.

In Uganda, routine immunization is provided on a daily basis in most health centers although some provide it on weekly basis. Most health centers provide an average of four out reaches in hard to reach areas and once a week within their catchment areas. At these health centers, Parish Mobilizers and Village Health Teams (VHTs) work with health workers from the nearest health centers in planning and mobilizing their communities for the immunization out reaches.

Despite significant increase in routine immunization coverage since the launch of the Expanded Program on Immunization (EPI) in 1974, over 27 million children worldwide remain overlooked by routine immunization services. The lowest coverage persists in Sub-Saharan Africa where it is estimated that only about 50% of the

children are fully vaccinated within their first year of life (AFENET Newsletter, 2007).In Uganda, EPI services were revitalized during Health sector strategic Investment plan (HSSPI, 2001/02-2005/06). However, only 71% of the estimated one million children under one year are fully immunized and DPT I-III dropout has remained high at 16% (MoH, 2005).

The disparity between the high coverage of first vaccines (DPT1 and polio vaccine: 93 percent) and low coverage of follow-up on vaccines (DPT3: 63%) reflects a high dropout rate which remains a challenge to strengthening routine immunization services in Uganda (UBOS 2011). Sembabule is one of the poorly performing districts with un-acceptably high dropout rate of 22.53% for DPT and 18.0% for Measles (Sembabule district report, 2010). The coverage for DPT1 is 109.7%, DPT3 87.3%and Measles is at 90.0%.Mawogola Health Sub-District (HSD) stands at DPT1 87.77%, DPT3 67.09%, Measles 60.4% with a dropout rate of 23.5% for DPT and 22.14% for measles.

Low immunization coverage and high dropout rates put the lives of children in the sub district at risk of acquiring Vaccine Preventable Diseases. In view of the prevailing situation, a study was required to assess the factors affecting immunization coverage and dropout rates in the health sub-district. The results of this study will be of use to various stakeholders in their efforts to improve the immunization program in the sub-district.

METHODOLOGY

Study area

The study was carried out in Mawogola HSD in Sembabule district. The population included households with children below 5 years, and health workers in Mawogola HSD within the study area.

Study population

The study population included caretakers for children 1 to 5 years of age. This age group was chosen because it represents the most recent

birth cohort to go through the immunization system and therefore can be used to assess recent performance of the program. The younger age range (less than 12 months) contains some children who are not yet eligible for vaccination and therefore resulting survey results would be lower than they actually are. Caretakers of these children were interviewed to provide information on immunization of their children. These were the mothers of the children or the person who looks after the child.

Inclusion criteria

A child who was 1 to 5 years of age and whose care taker consented for interview

Exclusion criteria

A child who was not a permanent resident of a sampled village and any other children above 5 years of age in a household.

Study design

A cross sectional-survey design was used to gather information on the factors affecting immunization dropout rates in Mawogola health sub- district. This design was appropriate because it enabled the collection of detailed information that described the existing situation in study area. Cluster sampling was used to select study participants for interview while retrospective review of immunization records was done to calculate coverage and dropouts.

Sample size determination

The sample was calculated using the Bennet formula (Bennet *et al* 1991).

C =

Where:

C = Number of clusters needed (cluster=LC1)

P = Average immunization dropout rate in the district

D = Design effect

B = Number of households per cluster (LC1)

S = Standard error (= C.I +_5%)/C alpha

C alpha = Value from normal curve corresponding to alpha value,

D = 1+ (b-1) roh

Roh = Rate of homogeneity (intra and inter cluster variability)

Substituting,

P = 20% Current Sembabule District dropout rate

CI = -+ 5% therefore CI width = 0.05

Roh = 0.02, got from c-size on computer

B = Number of households in cluster, say 5 which can be selected in one cluster.

Therefore,

D = 1+ (5-1) x 0.02 = 1.08

S = 0.05/1.96 = 0.0255

C = 0.20 x 0.8 x 1.08(0.0255) x (0.0255) x 5

= 53.15, Approximately 53 clusters.

53.15 x 5 = 266 households, since each house hold has at least one care taker therefore 266 caretakers were used in this study. The care takers were the unit of analysis.

Sampling procedure

Multi stage sampling was used. All the sub counties in the HSD were listed. One half of the sub counties were selected by simple random sampling. Mawogola has 4 sub counties therefore 2 were selected.

All the villages (LC I) in the sampled sub counties were listed using data from the district population office to make a sampling frame. Then using systematic sampling, 14 villages were selected from the 2 sub counties. 20 households with children 1 to 5 years of age were visited per village, therefore to make 266 households. The first household to be visited in each cluster was selected randomly using a table of random numbers from an ad hoc village household listings made with the LC I chairman who also acted as the guide. After the first household, systematic sampling was then used until 20 households with eligible children had been studied in that village/cluster. In families with more than one eligible child, the youngest child 1 to 5 years age-range was sampled.

Study variables

Dependent variables

- Immunization dropout
- Full or complete immunization

Independent variables

- Socio-demographic factors of caretakers
- Socio- demographic factors of children
- Child sicknesses
- Immunization side effects
- Presence of child health card
- Knowledge of immunization schedule
- Knowledge of immunizable diseases
- Missed opportunities for immunization

- Distance to immunization post

Data collection methods

The aim of the study was to establish the factors affecting complete immunization coverage and dropout rates in the sub district. Semi structured questionnaires were administered to caretakers of children 1 to 5 years of age and the immunization status of their children was noted.

Data on immunization history was collected either from vaccination cards or caretakers' verbal report. After identifying a child eligible for the study from the household through house-to-house visits, caretakers of the child were asked for the presence of child's immunization card. In case there were two or more children aged 1-5 years the youngest child was selected. For the child with immunization card, the information on the doses and types of vaccines was copied from the card. In the absence of vaccination card, caretakers were asked for immunization history of the child. The number of doses the child took and its route of administration was the way of collecting immunization history of the child. Information on other variables was asked directly from the child's caretakers. Caretakers of children were also interviewed about their knowledge on immunization and vaccine preventable disease.

RESULTS

Sample description

A total of 266 caretakers of children aged 1-5 years from 12 villages in 2 sub-counties were interviewed and provided information regarding immunization status of their children. The immunization status was verified using child health cards. Administrative immunization coverage and dropout rates were obtained by reviewing the district Health Management Information System (HMIS) data.

Socio-demographic characteristics of the caretakers interviewed

Table 1 shows the distribution of study participants by socio-demographic characteristics. A total of 266caretakers of children aged between 1-5 years old were interviewed, with a response rate of 100%. Of the total 266caretakers, 77.4% were female. The majority (53.0%) of the caretakers was aged 20-29 years. Only 14.7% and 7.1% of the caretakers attended secondary education and tertiary, respectively. The main occupation of the study participants was subsistence farming (82.0%) and most of them (77.8%) were married.

Table 1: Socio-demographic characteristics of the caretakers interviewed

Characteristic	***Frequency (N=266)***	***Percentage (%)***
Sex		
Male	60	22.6
Female	206	77.4
Marital Status		
Single	25	9.4
Married	207	77.8
Widowed	8	3.0
Divorced/Separated	26	9.8
Occupation		
Subsistence farming	218	82.0
Petty business	19	7.1
Civil servant	17	6.4
Casual laborer	3	1.1
Others	9	3.4
Age group		
Less Than 19 Years	3	1.1
20-29years	141	53.0
30-39years	73	27.4
40 Years And Above	49	18.4
Education Level		
None	30	11.3
Primary	178	66.9
Secondary	39	14.7
Tertiary	19	7.1

Coverage by survey

As shown in Table 2, crude Immunization coverage (by card and history) in Mawogola HSD for BCG, DPT I, DPT III, OPV III and Measles were 92.7%, 90.9%, 72.7%, 70.9% and 65.7% respectively.

Table 2: Immunization Coverage by Community Survey in Mawogola HSD

Antigen/Vaccine N=266	*Valid Coverage (By card)*	*By history*	*Crude Coverage (by history and card)*
BCG	70.5%	22.2%	92.7%
DPTI	68.9%	22.0%	90.9%
DPTII	62.1%	20.6%	82.7%
DPTIII	53.0%	19.7%	72.7%
OPVI	67.2%	22.2%	89.4%
OPVII	61.9%	19.9%	81.8%
OPVIII	52.7%	18.2%	70.9%
Measles	48.5%	17.2%	65.7%
DPT1-III dropout rate	23.1%	10.5%	20.0%
BCG-Measles dropout rate	31.2%	22.5%	29.1%

Immunization Coverage and dropout rates by records review

The coverage of the different antigens is shown in Table 3. Immunization reports for the year 2012 were reviewed to estimate coverage. From the records review, BCG had the highest coverage at 91.5% while measles had lowest coverage at 85.7%.DPTI-III and BCG-Measles dropout rate were 14.3% and 6.3% respectively. Contrary to what is expected, the later drop out is lower than the former because of the high measles coverage.

Table 3: Mawogola HSD Immunization Coverage and dropout rates by records review

Antigen	*Coverage (%)*
BCG	91.5
DPT I	86.7
DPT III	74.3
OPV III	70.2
Measles	85.7
DPT I -III dropout	14.3
BCG to Measles dropout	6.3

Comparing coverage obtained by survey and records

Table 4 shows a comparison of coverage and dropout rates by records review and survey. The highest difference is for measles antigen at 20%, while the least is for BCG at 1.2%.Coverage rates calculated by records review were generally lower than those obtained by survey except DPT III and Measles. The dropout rates obtained by record review were lower than those obtained by community survey.

Table 4: Comparison of coverage and dropout rates obtained by survey and records

Antigen	*Coverage by survey*	*Coverage by records*	*Difference*
BCG	92.70%	91.50%	1.20%
DPTI	90.90%	86.70%	4.20%
DPTII	82.70%	78.9%	3.8%
DPTIII	72.70%	74.30%	-1.60%
OPVI	89.40%	80.5%	8.9%
OPVII	81.80%	70.20%	11.60%
OPVIII	70.90%	80.5%	9.6%
Measles	65.70%	85.70%	-20.00%
DPT1-III dropout	20.0%	14.3%	5.7%
BCG-Measles Dropout	29.1%	6.3%	22.8%

Factors associated with complete immunization coverage

Caretakers' knowledge about immunization

As shown in table 5, of the total respondents, about 92.5% knew what immunization is. The majority of respondents (86.1%) knew that the objective of immunizing children was to protect them from diseases. About 77% of the respondents cited less than three types of vaccine preventable diseases, while 23.3% mentioned four or more types of vaccine preventable disease. With regard to respondent's knowledge about the age at which the child begins and finishes immunization, 45% of them knew the age at which child immunization begins and 67.5% knew the age at which the child finishes immunization. In addition to these, only one fourth (25.0%) of the respondents knew the session needed to complete the child immunization.

Table 5: Knowledge about immunization

Caretaker's knowledge	*N= 266*	*Percent (%)*
Knows what immunization is		
Yes	246	92.5
No	20	7.5
Immunization objectives		
Protects child from some diseases	229	86.1
Protects child from severe forms of diseases	37	13.9
Knew four or more types of vaccine preventable diseases		
Yes	62	23.0
No	204	77.0
Knew the age at which child immunization begins		
Yes	120	45.0
No	146	55.0
Knew the age at which child completes immunization		
Yes	180	67.5
No	86	32.5
Knew the total number of sessions needed for child immunization		
Yes	67	25.0
No	199	75.0

Association between caretakers' knowledge of immunization in relation to coverage

Table 6 shows the association between caretakers' knowledge about immunization in relation to complete immunization coverage among children aged 1-5 years of age. Bivariate analysis shows that children of caretakers who knew age at which the child begins (5.9, 95% CI: 3.9-8.7) and completes (10, 95% CI: 5.7, 17.7) immunization were more likely to be fully vaccinated. But knowledge of what immunization is and its benefits had no association with the complete immunization status of children. In addition, caretakers who knew the total number of sessions needed for child immunization were also less likely to have fully vaccinated children.

Table 6: Association between caretakers' knowledge of immunization in relation to coverage

Characteristics	***Child vaccinated, n (%)****		***Odds Ratio (95% C.I)***	***P-value***
	Yes	**No**		
Caretaker knows what immunization is				
Yes	206 (83.7)	40 (16.3)	0.736	0.634**
No	16 (8.0)	4 (20.0)	(0.208 – 2.603)	
Caretaker knows immunization benefits				
Yes	217 (83.5)	43 (16.5)	1.260	0.850**
No	5 (83.3)	1 (16.7)	(0.115-13.786)	
Caretakers knew the age at which child immunization begins				
Yes	68 (56.7)	52 (43.3)	5.9 (3.9, 8.7)	0.000*
No	26 (17.8)	120 (82.1)		
Caretaker knew the age at which child immunization completes				
Yes	87 (48.3)	93 (51.7)	10 (5.7, 17.7)	0.000*
No	7 (8.1)	79 (91.9)		
Caretaker knew the total number of sessions needed for child immunization				
Yes	30 (44.8)	37 (55.2)	1.7 (1.1, 2.5)	0.077**
No	65 (32.7)	133 (66.8)		

**Significant, **Not significant*

Service related factors associated with immunization coverage

Table 7 shows service related factors associated with immunization coverage. The majority of the respondents (97.4%) were found to have immunization posts in their parishes which were located at least 5km from their homes (54.5% less than 1 km and 43.2% 1-5 km). About 61.7% of the respondents mentioned that they did not have an immunization mobilizer in their parishes. Also, 75.6% mentioned that they usually spend 1-2 hours at their respective immunization post.

Table 7: Service related factors

Service factor	*N= 266*	*Percent (%)*
Immunization post present in parish		
Yes	259	97.4
No	7	2.6
Distance of immunization post		
Less than 1 km	145	54.5
1-5 km	115	43.2
More than 5 km	6	2.3
Immunization mobilizer present in parish		
Yes	102	38.3
No	164	61.7
Time spent waiting at immunization post		
Less than 1 hour	65	24.4
1-2 hours	201	75.6
Is treated well by health worker		
Yes	256	96.2
No	10	3.8
Has ever taken child and found service postponed		
Yes	181	68.0
No	85	32.0

Association between service related factors of immunization in relation to coverage

Table 8 shows bivariate and multivariate logistic regression analyses of service related factors associated with complete immunization coverage. Bivariate analysis showed that distance of immunization post (0.131, 95% CI: 0.049-0.351), and the time spent

waiting at the immunization post before being worked on (0.108, 95% CI: 0.049, 0.240) are significantly associated with immunization status of children.

Table 8: Association between service related factors of immunization in relation to coverage

	Child vaccinated, n (%)		***Odds Ratio***	
Service factor	**Yes**	**No**	***(95% CI)***	***P-value***
Immunization post present in parish				
Yes	215 (96.8)	44 (100.0)	0.000	0.999**
No	7 (3.2)	0 (0.0)		
Distance of immunization post				
Less than 1 km	109 (49.1)	36 (81.8)	0.131	0.000*
1-5 km	107 (48.2)	8 (18.2)	(0.049-0.351)	
More than 5 km	6 (2.7)	0 (0.0)		
Immunization mobilizer present in parish				
Yes	81 (36.5)	21 (47.7)	0.945 (0.447,	0.883**
No	141 (63.5)	23 (52.3)	1.998)	
Time spent waiting at immunization post				
Less than 1 hour	42 (18.9)	23 (52.3)	0.108 (0.049,	0.000*
1-2 hours	180 (81.1)	21 (47.7)	0.240)	
Session postponed				
Yes	151 (68.0)	30 (68.2)	1.560 (0.689,	0.286**
No	71 (32)	14 (31.8)	3.532)	
Is treated well by health worker				
Yes	213 (95.9)	43 (97.7)	2.423 (0.217,	0.472**
No	9 (4.1)	1 (2.3)	26.993)	

**Significant, **Not significant*

Dropout

The survey dropout rate calculated from DPT I and III coverage was 20% while the BCG to Measles dropout was 29.1%. However caretakers were asked if they ever started immunizing their children but for some reason didn't complete the schedule. This was cross checked on the child's card if it was available. Of the 266 caretakers interviewed in this study, 44 (16.5%) admitted to not immunizing their children at all. Of the remaining 222 (83.5%) who had immunized their children, only 109 (41.0%) had fully immunized (completed schedules), while 113 (42.5%) had dropped out.

Reasons for dropout

Table 9 shows the common reasons given by care takers for dropout. Caretaker factors as well as service delivery factors were assessed to determine what contributed most to dropout. Most respondents cited caretaker factors as the reasons why they did not complete their immunization schedules or immunize their children at all. The commonest reasons given were family problems, caretakers being busy, child illness, postponing of immunization sessions, and immunization posts being far.

Caretaker reasons were the major causes of drop out in Mawogola HSD compared to service related factors. The commonest five reasons reported by caretakers for not completing their children's immunization schedules were: child being ill and not brought to facility on due date (23.6%), caretaker had family problems (21.6%), caretaker was too busy to come to facility (21.0%). Service related factors cited were: that place of immunization being too far away 14.6% and vaccinator not being available even after caretaker has gone to either facility or immunization outreach post 10.8%.

Table 9: Reasons for dropping out

	Reason for dropout	***Frequency***	***Percent (%)***
Caretaker factors	Child ill, not brought	37	23.6
	Family problems	34	21.6
	Caretaker too busy	33	21.0
	Unaware to return for 2nd or 3rd dose	22	14.0
	Fear of side effects	14	8.9
	Unaware of need for immunization	6	3.8
	No trust in immunization	5	3.2
	Wrong ideas about contraindications	2	1.3
	Lack of information	2	1.3
	Other	2	1.3
	Total	**157**	**100.0%**
Service factors	Place of immunization too far	23	14.6
	Vaccinator absent	17	10.8
	Session postponed	13	8.3

	Vaccine not available	11	7.0
	Place/time of immunization unknown	7	4.5
	Time of immunization inconvenient	6	3.8
	Child ill not immunized	6	3.8
	Other	3	1.9
	Not stated	71	45.2
	Total	**157**	**100.0%**

Factors affecting dropout

The study assessed possible factors that lead to dropout. Both caretaker factors as well as service delivery factors were assessed. Any child who did not complete their scheduled immunizations was considered a dropout. These were compared to those who completed all their scheduled immunizations. A total of 157 children were reported to have dropped out while 109 children completed all their immunizations.

Comparison of caretakers' socio-demographics and drop out

There was a statistically significant association between the education level of a caretaker and dropout status, as shown in Table 10. Children of uneducated caretakers were far more likely to dropout than those of educated caretakers. However, there was no statistically significant association between the other socio-demographic factors and drop out.

Table 10: Caretaker Socio-demographics of dropouts and non-dropouts

Characteristics N=222	***Dropout n=157 (%)***	***Non dropout n=109 (%)***	***Total (%)***	***Odds Ratio (95% CI)***	***p-value***
Education level					
None	15 (13.3)	11 (10.1)	26 (11.7)	16.377 (4.15 – 64.56)	0.001
Primary	74 (65.5)	73 (67.0)	147 (66.2)		
Secondary	21 (18.6)	15 (13.8)	36 (16.2)		
Tertiary	3 (2.7)	10 (9.2)	13 (5.9)		
Occupation					

Subsistence farming Petty business Civil servant Others	87 (77.0) 16 (14.2) 4 (3.5) 6 (5.3)	96 (88.1) 0 (0.0) 10 (9.2) 3 (2.8)	183 (82.4) 16 (7.2) 14 (6.3) 9 (4.1)	0.065 (0.010 – 0.414)	0.037
Marital status					
Single Married Widowed Divorced/Separated	17 (15.0) 78 (69.0) 3 (2.7) 15 (13.3)	2 (1.8) 95 (87.2) 1 (0.9) 11 (10.1)	19 (8.6) 173 (77.9) 4 (1.8) 26 (11.7)	0.000	0.163

DISCUSSION

Immunization coverage

This study found BCG, DPT I, DPT III, OPV III and Measles coverage by card and history at 92.7%, 90.9%, 72.7%, 70.9% and 65.7% respectively. This coverage is similar to findings of the national EPI community survey (UNEPI, 2000). However these rates are lower than the national figures. The national immunization averages are higher for all the antigens except measles (UBOS, 2006). This shows that immunization services in Mawogola HSD have declined in the last 6 years.

Immunization coverage obtained by community survey was minimally higher than that that obtained by record review except measles which was higher by 14.5 percentage points. This is similar to findings from other studies which showed a discrepancy between administrative coverage and that obtained by community survey (Bakhuti*et al* 2003; Khan *et al* 2005). However administrative coverage tends to be higher. The differences in coverage in this study could be due to poor integration of child registers as some children are immunized from different posts depending on the convenience of the caretaker. In addition, coverage from records could be low due to the low rate of health facility reporting to the district. In 2012 only 76% of all the units in Sembabule district submitted their immunization reports.

The coverage obtained from records could therefore be underestimated by 14%.

Immunization dropout

In the study, the DPTI-III and BCG-Measles dropout rates were 20% and 29.1% respectively. Although the goal of the immunization program is to have all children below 1 year fully immunized, some children will drop out because of inevitable circumstances such as migration and death. That is why drop outs of <10% and <18% for DPTI-III and BCG-Measles respectively are generally considered acceptable in Uganda. However the dropout rates obtained in this survey are much higher than even the national average of 14%. The proportion of children aged 1-5 years who were reported to be fully immunized were 41.0% which is lower than the national average of 46% reported in the UBOS, 2006. However the proportion of children who were not immunized at all by the time of the survey was 16.5% which is higher than the rate of 6.6% reported in the UBOS, 2006. The decrease in the number of fully immunized children could probably be due to lack of mobilization.

The common caretaker reasons cited for dropping out included caretaker illiteracy, family problems, caretaker too busy, not aware of the need to return for the next doses, fear of side effects and lack of knowledge on immunization while the major service delivery reasons given included immunization post very far, vaccinator absent, postponed session, vaccine out of stock and lack of knowledge of place or time of immunization. The EPI coverage survey (2005) also reported similar reasons for not getting the vaccinations. However the extent to which these factors influence dropout rates varies from place to place and therefore there is need to design interventions appropriate to a given place.

Low income levels could cause immunization defaulting as some costs like transport are involved in bringing a child for immunization

(Tugumisirize*et al*. 2002). In this study a small number of the caretakers were formally employed, the rest (82.0%) being peasant farmers. However this study found out that the majority of the caretakers (77.8%) of the caretakers were married. It is not surprising therefore that there was no association between marital status and immunization dropout. Married couples are more likely to seek better health care for their children including immunization (UBOS, 2006).

In conclusion, full immunization coverage among children aged 1-5 years remains very low in Mawogola HSD. This is due to the low awareness among caretakers about age at which child immunization begins and completes it, as well as total sessions needed to complete immunization. Also, service delivery related factors like longer waiting hours by the caretakers at the immunization posts and postponement of immunization schedules discourage the care takers and eventually they drop out of the whole program. Furthermore, inadequate and in some villages, lack of mobilizers has greatly affected immunization and contributed to the high immunization dropout rates in Mawogola HSD and Sembabule district at large. It is also observed that there is a high DPTI-III and BCG-measles dropout rate in Mawogola HSD. The significant risk factor for immunization drop out in the health sub district was illiteracy among the caretakers.

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CHAPTER 3

ARTIFICIAL INTELLIGENCE IN OTORHINOLARYNGOLOGY

Assist. Prof. Dr. Meltem TULĞAR[1]

[1] **Meltem Tulğar** Ankara Yıldırım Beyazıt Univertsity Yenimahalle Training and ResearchHospital Orcid No: 0000-0002-2537-4573 tulgarmeltem@gmail.com

INTRODUCTION

Artificial Intelligence (AI) is a set of data-oriented technologies and algorithms designed to predict or infer new data based on past data [Crowson, 2019]. The application of AI technology in medicine is not new. Nevertheless, health data is big data that cannot be quickly removed with current statistical methods. In the past few years, high-performance computing has made significant progress, leading to substantial scientific breakthroughs in machine learning (artificial intelligence), especially in image processing and data analysis [Karlıdağ, 2019]. Therefore, collecting high-quality data on patients and diseases is essential for developing AI technology. The purpose of AI in medicine is not to replace doctors but to provide them with advice and suggestions based on patient data. AI and humans work together like in the human-in-the-loop model (HITL), purchasing the machine's power and human intelligence to create machine learning-based AI models.

Although AI's application in healthcare services appears revolutionary, its application in otorhinolaryngology is currently constrained in practice and patient care. Otorhinolaryngologists are essential participants in creating and applying AI in medical technologies. Consequently, gathering high-caliber information on individuals and illnesses is crucial for improvements in AI technology [Karlıdağ, 2019; Freeman, 1992]. The initial instances of machine learning, a branch of AI otorhinolaryngology, are used to recognize the automatic waveforms from the auditory brainstem, and acoustic sound is categorized [Freeman, 1992, Whipple 2004].

In otorhinolaryngology, the use of AI is relatively new. The earliest reports of machine learning applied to otolaryngology include automatic recognition of auditory brainstem response waveforms, genomic prediction of oral squamous cell carcinoma, and acoustic voice feature classification [Freeman, 1992, Whipple 2004, Schönweiler, 2000]. Since then, the amount of otolaryngology literature

has dramatically increased, describing new machine learning applications [Crowson, 2019]. [Promising applications include computer vision algorithms that can quickly identify radiological abnormalities, describe surgical anatomy, and classify malignant tissues in pathologic specimens (such as intraoperative frozen sections and acceptable needle aspiration samples) at a speed comparable to humans [Crowson, 2019].

Computer vision applications using neural networks are being used to perform high-precision and high-throughput classification of radiological and pathological images to achieve faster diagnostic categories [Halicek, 2017].

Machine-learning techniques and recent applications in otolaryngology–head and neck surgery are used in neurotology, head and neck oncology, laryngology, and rhinology.

Otology-Neurotology

Similarly, AI technologies are used in otology and neurotology, determining the prognosis of sudden sensorineural hearing losses, imaging endolymphatic hydrops, identifying the phenotypes of hearing loss, and automatically classifying auditory brainstem responses [Liu, 2017]. Although the inner ear is inaccessible and otologic studies are challenging, AI algorithms can identify detailed temporal bone structures from computed tomography (CT) images, intracochlear anatomy, and individual cochlear implant electrodes [Heutink, 2020; Chi 2019]. AI is used to program cochlear implants (CI); studies have demonstrated that programming can affect standardization across centers [Crowson, 2019].

Laryngology

When AI programs are used with voice analysis and video stroboscopic examination images in laryngology, T1a glottic cancer and other vocal cord lesions can be accurately diagnosed with an accuracy rate of almost 100% [Unger, 2015]. Approximately two-thirds

of laryngeal malignancies start in the vocal cords. Cancerous vocal fold changes early detection, and premalignant lesions are differentiated, representing that precancerous vocal folds are a significant challenge in laryngology. Small carcinomas and lesions are difficult to determine simply by standard endoscopy. A computerized analysis of high-speed laryngeal movies can be utilized to measure the characteristics of vocal fold dynamics and distinguish between malignant and precancerous lesions.

Head and Neck Oncology

Head and neck malignancies are the most common cases in which AI technologies are used in otorhinolaryngology [Karlıdağ, 2019]. AI has been used in hyperspectral imaging for differentiating between average head and neck tissue and thyroid malignancies and is reported to function with 97% sensitivity, 96% specificity, and 96% accuracy [Liu, 2017]. Accordingly, it automatically segments anatomic structures to accelerate radiotherapy planning [Livingstone, 2020]. In addition, clinical pathology and genomic markers have been integrated into the system, and successful results have been obtained in predicting the prognosis of cancer patients. Another promising application of AI is automated radiation therapy planning [Zhu, 2020]. Especially the three-dimensional tumor volume can be calculated, and the dose can be planned more realistically [Karlıdağ, 2019].

Also, an automated machine learning approach was used to build a computer vision algorithm for the otoscopic diagnosis, and the algorithm made correct diagnoses with an accuracy of 88.7% [Livingstone, 2020]. Likewise, an automatic diagnostic algorithm facilitates diagnosing pediatric patients with otitis media as a public health problem [Tran, 2019]. Soon, otolaryngologists and audiologists will incorporate this technology into their clinical practices.

Rhinology

In rhinology, the use of AI is limited. Therefore studies in paranasal sinuses, diagnosis of sinusitis, and detection of sinus pathologies are continuing. AI is used to differentiate anterior ethmoidal artery locations on sinus computed tomography scans [Huang, 2020]. On coronal CT images, several methods are utilized to help locate the anterior ethmoidal artery. The identification of the "nipple" is among the most common techniques. The indication is the lamina papyraceous triangular evagination between the superior oblique and the anterior ethmoidal foramen, the medial rectus extraocular muscles, and the entryway of the orbital artery into the nasal cavity. With the help of convolutional neural networks, a deep learning technique, computers can learn from massive amounts of data by identifying patterns and generating predictions. The structure of a convolutional neural network is similar. The location of each convolutional layer in the visual cortex's biological organization is input and transmitted to the following layer. Convolutional neural networks, as a result, are ideal for and have been employed chiefly in visual image recognition algorithms.

Similarly, machine learning is helpful in sinonasal squamous cell carcinomas to predict treatment outcomes [Fujima, 2019].

Probabilistic networks for medical diagnosis have been used for a while; helping physicians to diagnose diseases, e.g., liver diseases [Onisko,1999], predicting the progression of ALS disease [Ahangaran, 2020], for early detection and diagnosis of Ebola virus infection [Aror ve Rahmana,2016], diagnosis and treatment of typhoid fever [Oguntimilehin,2014] and detect vitamin and mineral deficiencies [Sevani ve Chandra,2016].

CONCLUSION

While machine learning has some unique advantages as an analytic and modeling tool, it also exemplifies a broader trend; the convergence of health and data sciences. Machine learning cannot turn data into magically helpful information. Instead, it is a logical progression from classical statistical methods. The use of machine learning in the current health care system is beneficial and becoming more and more essential. A doctor may need to assess a significant quantity of data, including the patient's personal history, familial illnesses, genetic sequences, drugs, activity on social media, and admissions to other institutions. By taking all of this information into account, finding insights might be challenging. It is crucial to remember that these new algorithmic decision-making tools do not guarantee justice, equitability, or even truthfulness as more power is turned over to algorithms. Even with the most robust machine learning algorithms, the adage "garbage in, garbage out" still holds, despite our reluctance to use it. It may be too much to ask of any individual to lead healthcare decisions. This review aims to introduce machine learning technology and its application prospects in the clinical challenges of otolaryngology head and neck surgery. Using AI will provide significant otorhinolaryngology guidance under challenging decisions, complex diagnoses, and treatments. Given the rapid application and advancement of healthcare data sets in machine learning, it is prudent for otolaryngologists to have a thorough understanding of the functions and limitations of these analysis tools.

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CHAPTER 4

CURRENT AESTHETIC APPLICATIONS OF ADIPOSE-DERIVED STEM CELLS

Assist. Prof. MD. Çiğdem KARADAĞ[1]

[1] Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Plastic, Reconstructive and Aesthetic Surgery, Istanbul, Turkey. e.cigdem.karadag@acibadem.edu.tr, Orcid ID: 0000-0002-8363-5858

INTRODUCTION

Stem cells are cells with the potential to develop into many different types of cells in the body. They are able to differentiate into any cell of an organism and have the ability of self-renewal. Due to these properties, stem cells have unlimited potential in advanced cell therapies, regenerative medicine, and tissue engineering. The major characteristics of stem cells are:

(a) self-renewal (the ability to extensively proliferate),
(b) clonality (usually arising from a single cell),
(c) potency (the ability to differentiate into different cell types).

Compared with differentiated cells, the application of stem cells has proved to be advantageous because of the following unique stem cell characteristics:

1. Ease of harvesting in larger numbers,
2. Much higher proliferation capacity,
3. Ability to undergo a larger number of passages,
4. Ability to undergo senescence and subsequent differentiation into a wide range of desired cell phenotypes,
5. Ability to support the vascularization of scaffolds.

Application of regenerative medicine and cellular therapy is rapidly growing. For regenerative medicinal purposes, stem cells should meet these criteria's:

I. They could be provided in abundant quantities (millions to billions of stem cells).
II. They could be differentiated into many cell lineages in a regulatable and reproducible way.
III. They could be harvested by minimally invasive techniques.
IV. They could safely and effectively be used in either an autologous or allogeneic host.
V. They could be produced according to current Good Manufacturing Practice guidelines (Gimble JM. Adipose tissue-derived therapeutics. *Expert Opin Biol Ther* 2003; 3:705–713.).

Stem cell candidates include embryonic stem cells obtained from embryonic tissue, induced pluripotent stem cells, genetically

reprogrammed differentiated somatic cells, and postnatal adult stem cells obtained from specific locations in the adult organism, such as fat, skin, bone marrow, blood, or skeletal muscle.

1. MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs), originally identified in bone marrow, are adult stem cells (Vining and Mooney 2017; Wei et al. 2017). It has been demonstrated that MSCs can be isolated from almost every tissue of the body (Kasoju et al. 2017; Klein et al. 2015; Packer 2018) including adipose tissue, trabecular bone, skin, skeletal muscle, pericytes, umbilical cord blood, periosteum, peripheral blood, synovial membrane, dermis, dental pulp, periodontal ligament, and even tumors (Bianco 2014; Agha et al 2017). However, not all sources provide clinically useful MSCs (Maqsood et al 2020). Bone marrow yields the most enriched and characterized MSCs for clinical use (Mazini et al. 2019). BMC was the most common procedure to prepare MSCs; however, bone marrow aspiration is highly invasive and results in low stem cell numbers. In literature, the variations in the differentiation potential of MSCs from different tissue sources were highlighted (Mushahary et al. 2018). Adipose-derived stem cells (ADSCs) have become one of the most promising stem cell populations identified so far because they are ubiquitous and can be relatively easily harvested in larger quantities with less donor-site morbidity. Subcutaneous adipose tissue is the most clinically relevant source of ADSCs among the several adipose tissue types and can be isolated from subcutaneous adipose tissue of the abdomen, thigh, and arm. Liposuction has been performed extensively for patients worldwide with increasing popularity as an option for fat removal and body contouring.

The main advantages of harvesting ADSCs rather than bone marrow are ease of access, safe and abundant harvesting, and low mortality risk. Unlike the bone marrow, adipose tissue (AT) is very

easy to collate and use for tissue engineering in large quantities without requiring an ex vivo expansion step (Taghavi-Farahabadi et al. 2020).

The innovative idea of using AT as a grafting tool was introduced by plastic surgeons. AT transfer is used for various purposes, including breast reconstruction, repairing surface contour deformities, enhancing the aesthetic appearance, and enhancing tissue regeneration (Karadag Sari and Ovali 2022). The stromal vascular fraction (SVF) content is composed of multipotent stem and/or stromal cells (Dos-Anjos et al. 2014). The SVF is isolated from AT and has tremendous regenerative potential for proliferation and differentiation due to so-called adipose-derived stem cells (ADSCs) (Alaaeddine et al. 2018).

2. ISOLATION, IDENTIFICATION, AND LOCATION OF ADIPOSE-DERIVED STEM CELLS (ADSCs)

2.1. Harvesting And Collection of Adipose Tissue for The Isolation of ADSCs

ADSCs harvested from different anatomical areas exhibit different characteristics. For example, ADSCs harvested from superficial abdominal regions undergo significantly less apoptosis than do ADSCs harvested from the upper arm, medial thigh, trochanteric, or superficial deep abdominal regions. ADSCs isolated from different locations, cell types, and species have different density (Fraser et al. 2007; Zuk et al. 2001) and different procedures may affect ADSCs quality, functionality, and plasticity (Argentati et al. 2018; Palumbo et al.2018). The highest concentrations of ADSCs are found in arm adipose tissue area (Si et al. 2019). The ADSC specimens obtained via liposuction are the gold standard. Lipoaspirates from the subcutaneous adipose tissue are harvested usually with using tumescent technique.

A tumescent solution infiltrated through the 3-mm incisions contains ringer lactate with 1:500.000 units of epinephrine and 20 mg bupivacaine hydrochloride, following the infiltration resting is performed for 10 minutes to achieve hemostasis (Karadag Sari and

Ovali 2022, p. 250). Following the hemostasis, a blunt cannula (the "Fat Grater") with a 2-mm diameter, the tip of which has 10 holes measuring 1.5 mm in diameter, is used to harvest the adipose aspirate. The cannula attached to a 60-ml syringe and gently pulling pack on the syringe's plunger provides light negative pressure while the cannula is advanced and retracted through the harvest site. After filling the syringe with harvested tissue, the cannula is removed from the syringe, and the lipoaspirate is transferred into sterile 50-ml Falcon tubes for further enzymatic processing (Karadag Sari and Ovali 2022, p.250).

2.2. Isolation of the SVF

Karadag Sari and Ovali (2022) noted the isolation of the SVF and revealed that all production processes carried out following Good Manufacturing Practices. Fat tissue samples taken from the patient are placed in Falcon 50-ml Polypropylene Conical Tube (Avenida Industrial del Norte S/N, Parque Industry del Norte, Reynosa, Tamaulipas, Mexico). A sample is drawn from the tissue with an injector, and an input sample is given for microbiological quality control.

The AT samples in the tubes are arranged in Falcon tubes, and collagenase enzyme (SIGMA-ALDRICH, St. Louis, MO, USA) is placed in each Falcon tube. The collagenase enzyme has been sterility controlled, and the endotoxin level is approved as less than 10 IU/ml before use. The caps of the tubes are tightly closed, and the tubes are placed in the shaker. The shaker is adjusted to 100 rpm, and the tubes incubate for 60 to 90 minutes.

When the incubation is finished, the tubes are placed directly in the centrifuge device and centrifuged at 500 x g for 10 minutes. After centrifugation, the supernatant is removed using a pipette, and the pellet containing the cells is manually vortexed.

The pellet is removed and collected in a single tube via pipette. Filtering is done into a new tube with Cell Strainer, and the volume is

completed with Ringer Lactate Solution. The mix is spun in a centrifuge again for 10 minutes at 500 x g. Following the second round of spinning, the supernatant is removed, Ringer Lactate Solution is again added to the pellet in the desired amount, and the mixture is centrifuged for a third time at 500 x g for 10 minutes (Karadag Sari and Ovali 2022).

2.3. Quality Control Tests

To assure microbiological quality control after the final centrifugation, the sample has been drawn from the supernatant with an injector and run through the BD BACTEC Automated Blood Culture System (BD, East Rutherford, NJ, USA) for aerobic and anaerobic cultures, and the fungus culture sample is obtained. Additionally, endotoxin levels are analyzed. Once the Gram staining is negative in the sampling, the product is released for microbiological culture results. Culture results are monitored for two weeks (Karadag Sari and Ovali 2022, p.250).

2.4. Counting of SVFs, Assessment of Viability, Immunophenotyping

Red blood cells are lysed via lysis buffer (eBioscience, CAT No: 000-433357). Hemocytometer is used to perform the cell count. Using flow cytometry analysis for 7-aminoactinomycin D staining, cell viability is assessed.

Immunophenotyping is performed on SVF for flow cytometry analysis. Cell surface antigen expression of AT-derived MSCs is detected using flow cytometry. Using CD45, CD34, CD105, CD90 monoclonal antibodies, positive versus negative expression by flow cytometry using Cell Quest software (BD, East Rutherford, NJ, USA) is evaluated. It is calculated which cells were negative for CD45 and CD34 and which were positive for CD90 and CD105 as MSCs and then calculated the percentage in the total population (Karadag Sari and Ovali 2022, p.250).

3. AESTHETIC APPLICATION AREAS OF ADSCs

The interest in regenerative medicine is increasing, and it is a dynamically developing branch of aesthetic surgery. Biocompatible and autologous-derived products such as platelet-rich plasma or adult mesenchymal stem cells are often used for aesthetic purposes. Subsequently, cell-assisted lipotransfer (CAL) has been widely used in breast augmentation, for facial lipoatrophy and augmentation during face-lift and facial contouring surgeries, among other applications (Kolle et al. 2013; Lee et al. 2012; Rowan et al. 2014).

3.1. ADSCs for Facial Rejuvenation

In regenerative medicine, skin is an attractive model organ for the use of stem cells. ADSCs are a powerful agent in skin rejuvenation. They secrete growth factors and anti-inflammatory cytokines, stimulate tissue regeneration by promoting the secretion of extracellular proteins and secrete antioxidants that neutralize free radicals. In an office procedure, without cell incubation and counting, the obtained product is stromal vascular fraction, which consists of not only stem cells but also other numerous active cells such as pericytes, preadipocytes, immune cells, and extra-cellular matrix. ADSCs, when injected into dermis, improved skin density and overall skin appearance, and increased skin hydration and number of capillary vessels. The main limitation of mesenchymal stem cell transfers is the survival of the graft. The final outcomes are dependent on many factors, including the age of the patient, technique of fat tissue harvesting, technique of lipoaspirate preparation, and technique of fat graft injection (Surowiecka and Strużyna 2022, p.1).

In literature, increased expression of dermal collagen was shown following the intradermally injection of ADSCs (Park et al. 2008). ADSCs improve lesions caused by photo-aging. They secrete antioxidants and cytokines that neutralize the effects of the primary indicators of skin damage: UVB and reactive oxygen species (ROS)

(Xiong et al. 2020, Chen et al. 2021). These lesions are acquired pigmentary lesions that mainly affect women. These are areas of increased melanin concentration in the skin and epidermis. The accumulation of UV radiation causes inflammation while also stimulating melanogenesis and angiogenesis. ADSCs secrete TGF-β1, which is a suppressor of tyrosinase, an enzyme necessary for melanin synthesis (Chen et al. 2021).

The SVF gel can be transferred separately or with the fat transfer. SVF increases the survival of the fat graft by promoting neoangiogenesis. Adding SVF over the lipotransfer is one of the factors prolonging the result. Transferring SVFs alone improves skin density and thickness as well as dermis density (Gaur et al. 2017, Yao et al. 2018). The results are shown in Figure 1.

Figure 1: Results of skin improvements 6 months after SVF injection (Chen et al. 2021)

ADSC injections improve skin density and have anti-wrinkle properties. Skin hydration improves and there is a global improvement in appearance.

3.2. ADSCs for Breast Aesthetic

It has been reported that fat grafting may complicate breast imaging and breast cancer surveillance because of fat necrosis and calcification in breast tissue.

Whether the use of ADSCs in the form of CAL for breast augmentation can be used after breast cancer therapy remains unclear.

Several studies have demonstrated that MSCs can enhance the metastatic potential of breast cancer cells when mixed with them and reimplanted (Si et al. 2019). However, the interaction between ASCs and cancer cells still are not fully understood (Eterno et al. 2014; Karnoub et al. 2007).

Yoshimura et al. (2008) used adipose-derived SVF cells in augmentation of soft tissues by CAL in treatment of breast augmentation and facial lipoatrophy. In facial lipoatrophy, no complications or adverse side effects were noticed. In augmentation of the breast, the augmentation of breast was successful and had satisfactory clinical results without any major complications too.

Although CAL will not replace implants or reconstructive procedures (due to the shape and projection profiles they are able to achieve in the breast, for example), it has the potential to serve as an adjunct for small corrections or volume increases and may serve as a less invasive option for patients hoping to achieve subtle aesthetic enhancements (Arshad et al. 2018).

3.3. ADSCs For Scar Deformity

SVF can be successfully combined with lasers in a combined therapy of scars. Fat-grafting has been reported in burn scars in both adults and children. The indications for the lipo-filling of post-burn scars include hypotrophic scars.

After lipofilling or after application of ADSCs, improvement of scar appearance or reduction in scar-related pain has been reported in many case reports and clinical studies. Lipofilling and ADSCs seem promising to lessen the severity of developing as well as pre-existent fibrotic scarring (Spiekman et al. 2017).

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CHAPTER 5

URINARY TRACT INFECTIONS AND RISK FACTORS

Assist. Prof. Tutku TUNÇ[1]

Assist. Pharm. Selen ÖZDEMİR[2]

[1] Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Sivas, Türkiye. tutkutunc58@hotmail.com, ORCID ID https://orcid.org/0000-0002-8274-9386

[2] Güney Pharmacy, Temenni Mah. Şehit Cem Bul Street No: 7 Ürgüp Nevşehir, Türkiye. selenozdemir099@gmail.com, ORCID ID https://orcid.org/0000-0003-1562-9971

INTRODUCTION

Urinary system infection is defined as the infection of the normally sterile urinary system with microorganisms such as bacteria, fungi, viruses, and various clinical manifestations accompanying this (Stamm et al., 2001). Urinary tract infections (UTIs) are bacterial infections that affect approximately one hundred and fifty million people worldwide every year and are the most common bacterial infections encountered by doctors both in and outside the hospital today. It also includes very different clinical conditions ranging from asymptomatic bacteriuria to acute pyelonephritis with sepsis (Mamıkoğu and Inan, 2010). The non-specificity of its symptoms can often be overlooked, and in the absence of diagnosis and treatment, renal scarring, hypertension, and end-stage renal disease may result (Rubin et al., 1996). The risk factors can be listed as follows; having a previous urinary tract infection, presence of sexually transmitted pathogens in the lower genital tract, multiparity, low socioeconomic status and living in crowded environments, sexual activity, and urinary system anomalies (Tuzcular et al., 2000; Inci and Davarcı, 2011).

Escherichia coli is the most frequently isolated uropathogen in the first urinary tract infection and recurrent urinary tract infections. *E. coli* is followed by *Proteus* and *Klebsiella* species. In addition, many bacteria, fungi, and viruses can cause urinary tract infections (Neyzi and Ertuğrul., 2002).

Antibiotics, which have an important place in the treatment of infections, provide important benefits when used appropriately, but they cause the emergence of resistant strains rapidly when used inappropriately. In addition, super-infections with these resistant strains negatively affect morbidity and mortality. Since antibiotic susceptibilities vary between countries and regions, resistance studies should be carried out periodically in each region, and these studies should be taken into account during empirical antibiotic selection, especially while waiting for culture results (Cebe et al., 2008).

1. URINARY TRACT INFECTION (UTI)

Urinary tract infections are infections in any part of the urinary tract, from the kidneys and ureters to the bladder. It is the colonization of the urinary system, which is expected to be sterile in a healthy person, with microorganisms such as bacteria, fungi, viruses, and the detection of these microorganisms in the urine. The disease is defined by the appearance of clinical symptoms. These; burning in the urinary tract, pollakiuria (frequent urination), fever, and kidney pain. Urinary tract infection can cause serious mortality and morbidity. The localization of UTI affects the course of treatment and prognosis (Hansson and Jodal, 2004).

Bacteriuria seen in urinary tract infections describes the presence of bacteria in the urine (Table 1). There is a risk of contamination when taking a urine culture. Significant bacteriuria is also mentioned when the skin contains more and different microorganisms than the urethra, vagina, and external urinary system tissues. 100,000 or more colony-forming units in 1 ml of fresh urine are defined as CFU. It is a value used to distinguish between contamination and infection. A growth of 100,000 or more colonies/ml in the urine sample is considered significant bacteriuria (Schlager et al., 1995).

Table 1. Meaningful Definitions of Bacteriuria (Hansson and Jodal, 2004)

- Detection of 100 CFU of coliform bacteria per ml or 100,000 CFU of non-coliform bacteria per ml in a symptomatic female patient
- Detection of 1000 CFU bacteria per ml in a symptomatic male patient
- Detection of 100,000 CFU bacteria per ml in two consecutive urine samples in an asymptomatic patient
- Any bacterial growth in the suprapubic aspiration in the symptomatic patient
- Detection of 100 CFU bacteria per ml in the urine sample taken by the catheter

Symptomatic Urinary Tract Infection

Painful, frequent and forced urination in the patient with bacteriuria; is the presence of fever, abdominal pain, and systemic symptoms. According to the localization of the infection, the name of the disease and systemic findings also differ. The most common forms are cystitis and urethritis (lower urinary tract infections), voiding findings, and in pyelitis and pyelonephritis (upper urinary tract infections), fever and kidney pain are the main symptoms. According to the definitions of the "American Academy for Cerebral Palsy and Developmental Medicine", patients with symptoms of significant bacteriuria and changes in urine color and odor are defined as symptomatic UTI (Sobel 2000; Elder et al., 2004; Nina et al., 2019).

Complicated Urinary Tract Infection

A Complicated urinary system infection is an infection that occurs in the presence of anatomical and functional pathologies that will predispose to a urinary tract infection.

Uncomplicated Urinary Tract Infection

It is a urinary system infection that occurs without anatomical and neurological pathologies that may predispose to urinary tract infection (Chang et al., 2006).

1.1. Epidemiology

UTI is one of the most common health problems. It is the most common urinary tract infection among bacterial infections (Neyzi et al., 2002). 40% of women experience a UTI at least once in their lifetime (Foxman et al., 1990). On the other hand, 27% of this group show signs of UTI again within 6-12 months following the infection. The incidence of asymptomatic bacteriuria without UTI is 3.5%. At least 10% of men over the age of 65 and 20% of women experience asymptomatic bacteriuria (Nicolle, 2001). People of all age groups are affected by UTI. Its incidence and risk factors vary depending on age and gender.

Estimates from United States registries have shown that UTIs account for 0.7% (standard error of 0.1%) of outpatient care. The predominant group of patients with community-acquired urinary tract infections is women. According to US registry data, it is estimated that 7 million women will be admitted to outpatient care every year due to uncomplicated UTIs. Often, a UTI in men will be complicated by an underlying infection (Ellidokuz and Aksakoğlu, 2002; Hansson and Jodal., 2004; Elder 2004).

Recurrence of infection was observed in 12-30% of children who developed UTI. Recurrent UTI result; Irreversible conditions such as proteinuria, hypertension, renal scarring, and renal failure may occur. The prevalence of UTI, which is 1% in boys and 1-3% in girls, changes with age. While the male/female ratio is 2.8-5.4/1 in 0-24 month babies, the male/female ratio is 1/10 after the age of 1, and the probability of UTI in girls increases. Being uncircumcised in boys aged 0-6 months increases the risk of UTI 10-12 times (Bent et al., 2002).

Although the incidence of bacteriuria in pregnant and non-pregnant women is the same, the incidence of pyelonephritis is higher due to the anatomical and physiological differences brought about by pregnancy (Zor and Savaşcı, 2014; Nicolle et al., 2005). The risk of bacteriuria is doubled in women with sickle cell anemia compared to women without hemoglobin S (Inci and Davarcı, 2011; Tuzcular et al., 2000). Asymptomatic bacteriuria is seen in 2-7% of pregnant women. Observation of asymptomatic bacteriuria can be done in the second and third trimesters. If asymptomatic bacteriuria is not treated, 30-40% of pyelonephritis is seen. With appropriate treatment, this risk can be reduced by 70-80% (Kazemier et al., 2015). While acute cystitis is seen in 1-2% of pregnant women, the incidence of acute pyelonephritis is between 0.5-2%. Cases caused by pyelonephritis can be seen in the second and third trimesters (Gilstrap et al., 2005; Wing et al. 2005).

1.2. Etiology

Gram-negative bacteria and *Escherichia coli* included in this group are the most isolated microorganisms from uncomplicated urinary tract infections. *E. coli* is found in 75-95% of cases. While it is the most common bacterium in the first UTI, the incidence of *E. coli* has decreased in recurrent urinary tract infections. Due to its virulence characteristics, *E. coli* has a wide clinical picture ranging from asymptomatic bacteriuria to pyelonephritis (Cattel and Jones, 1998). *E. coli*, which is the most frequently reproducing microorganism in the formation of UTI, is isolated from women more frequently than men. The same comparison exists for other uropathogens. While the incidence of *E. coli* rises with increasing age, the opposite course was observed in other pathogens (Mazzulli, 2012; Topal, 2018).

The incidence of *Staphylococcus saprophyticus* in cases is 5-15%. Especially isolated in uncomplicated cystitis. The bacteria responsible for the remaining infections include *Klebsiella spp*., *Proteus spp., Enterecoccus spp., Staphylococcus aureus, Staphylococcus epidermidis, Haemophilus influenzae,* and *group B streptococci.* (Joseph et al., 2005).

Among the viral agents, Human polyomavirus, Adenovirus, Cytomegalovirus and Herpes simplex virus types 1 and 2 are encountered. The most common sources of infection in cystitis are viruses. They may cause hemorrhagic cystitis in immunocompromised patients (Jerardi and Jackson, 2021).

While *Candida* species are the first among the fungal causes of UTI, *Cryptococcus neoformans, Aspergillus* species, and endemic mycoses may also be causative. It can show pictures ranging from asymptomatic candidauria to clinical sepsis. While the incidence of pathogenicity alone is low, its ability to cause infection along with the primary disease is high. These diseases; diabetes mellitus, urinary system anomaly, and malignancy (Kauffman et al., 2000).

It is possible to encounter a wider variety of microorganisms in complicated urinary tract infections. It is possible to encounter *E. coli* in 50% of mixed infections caused by two or more microorganisms (Jerardi and Jackson, 2021). The incidence of bacteria such as *Klebsiella spp., Proteus spp., Enterococci, Pseudomonas aeruginosa* is higher than uncomplicated urinary tract infections (Table 2) (Stamm et al.,2001).

Table 2 : Distribution of Urinary System Infection Factors (Stamm et al.,2001).

Microorganisms	Characteristic
E. coli	All UTIs and nosocomial infections
Group B streptococci	Diabetics and newborns
S. epidermidis	In those undergoing urinary catheterization
S. aureus	Secondary to bacteremia
Pseudomonas	Congenital and acquired anatomical anomalies
Adenoviruses	Epidemic hemorrhagic cystitis
Anaerobic bacteria	In vesicocolic or vesicovaginal fistulas

1.3. Pathogenesis

Pathogenesis, respectively; entry to the host, escape from the primary defense mechanisms of the host, adhesion to the host cells, adaptation of the microorganism to the environmental conditions and proliferation, damage to the host by the spreading microorganism by creating toxin or inflammation, and escape from the secondary defense mechanisms of the host occur (Strohl et al., 2001).

It is expected that the urinary system of a healthy person, outside the distal end of the urethra, will be sterile under hygienic conditions. The type of bacteria, virulence factor, amount of pathogen, and the function of the host's defense mechanism are important in the formation

of infection. The infection seen in UTI occurs by hematogenous spread, mostly the ascending distribution of bacteria in the periurethral area (Jantausch et al., 2007). Non-sterile parts of the urinary system should also be present, and have a healthy flora. The flora in the urethra, periurethral region and vaginal entrance are colonized by aerobic and anaerobic uropathogens (disease-causing). At the distal end of the urethra, *Lactobacilli*, coagulase (-) *Staphylococcus, Corynebacterium* and 25% aerobic bacteria take part in the defense against pathogenic microorganisms in the flora. Flora changes occur as a result of antibiotic use, hormonal changes, and metabolic and personal hygiene conditions. The microorganism *E. coli*, which is the main cause of contamination in the deterioration of the flora balance, is 80-90% effective (Sussman, 1998; Bollgren and Winberg, 1976).

1.4. Diagnosis

Rapid evaluation of patients with symptoms of urinary tract infection, correct diagnosis, and initiation of treatment are very important in terms of preventing the development of renal scarring. In the diagnosis of urinary system infection, besides the history, symptoms and clinical findings, hemogram, biochemical analysis of blood, complete urinalysis, and urine culture, if necessary, radiological studies should be performed.

Pyuria; it is the definition that indicates the presence of 10 leukocytes in one mm3 of urine. Pyuria is present in most symptomatic and asymptomatic patients. In symptomatic infection, hundreds of leukocytes can be detected in 1 ml. The diagnosis of UTI in the absence of pyuria should be strongly questioned. One of the rapid tests for UTI is the leukocyte esterase test. Today, this test is very advantageous compared to microscopic methods. If the test is negative, culture and microscopy should be performed in the presence of symptoms of pyuria and UTI. The presence of erythrocytes in the urine may indicate other abnormalities such as stones, tumors, vasculitis, glomerulonephritis,

and renal tuberculosis. If bacteriuria is present, a microscopic examination of the urine is preferred. (Pappas, 1991; Bent et al., 2002).

As in all system infections, a good evaluation of UTI begins with a careful anamnesis. Parameters at the time of illness to be questioned in the history; acute disease history, duration and severity of fever, antipyretic response, urinary symptoms, abdominal pain, suprapubic discomfort, anatomical and physiological changes, and sexual activity. Presence of chronic symptoms suggesting bladder dysfunction such as previous urinary tract infection or undiagnosed febrile illness, recent co-morbidities and antibiotic use, incontinence, voiding dysfunction, frequent and urgent need to urinate, withholding maneuvers, constipation, Presence of chronic disease, previous operation, regularly used drugs, antenatal diagnosed anomaly or presence of VUR should be questioned (Stein et al., 2015).

1.5. Treatment

When the treatment of UTI is started, the microorganism causing the infection and the sensitivity of this agent to antibiotics are not known. In addition, it may not be determined whether there are different symptoms and abnormalities other than infection that may complicate the UTI. An example of this complicated situation is the formation of a stone in the system. Therefore, in the approach to treatment, it is necessary to follow established clinical practice guidelines when the initial choice of antimicrobial therapy is appropriate. After choosing the appropriate treatment according to the guideline, if there is no response to the treatment, culture and antibiotic susceptibility tests of the agent are performed. According to the results of these tests, the previous treatment is changed. In the management of UTI, surgical treatment is performed by endoscopic or open surgical correction methods in case of obstructive lesions, stones, and severe reflux. Prophylactic treatment primarily targets recurrent symptomatic reinfections (Yılmaz, Tanrıverdi and Çelepkolu, 2016).

These guidelines need to be periodically reviewed and rearranged. Because the pathogens in the system develop a constant resistance to antibiotics. In addition, a guide should be developed according to the results of the studies on the development of new antimicrobial agents, and the studies related to them and the results of the studies that define the appropriate treatment times for the infection.

The first things to consider in the treatment are;

- Effective and rapid response to treatment should occur.
- Recurrence should be prevented in treated patients
- The rapidly increasing resistance to antimicrobials in microorganisms should be prevented.
- The type of urinary system infection should be determined in the selection of treatment.
- Treatment selection is initially empirical.
- Treatment should be planned considering the presence of complicating factors and the pattern of antimicrobial resistance in the patient (Yılmaz, Tanrıverdi and Çelepkolu, 2016).

2. RISK FACTORS

The risk factors that stand out effectively in all age groups can be listed as follows; history of previous UTI, presence of a pathogen in the lower genital tract, quality of living space, and urinary system anomalies increase the risk of urinary system infection. Low socioeconomic status, kidney transplant, and prostatic causes also cause UTI. Surgical interventions and catheterizations applied to the urethra cause UTI in all age groups. The incidence of infection is also increasing due to neurological bleeding (Table 3) (Sobel and Brown, 2020).

Women are a special group in the occurrence of UTI and the number of risk factors attracts attention. The risk of bacteriuria is doubled in women with sickle cell anemia compared to women without hemoglobin S (Inci and Davarcı, 2011). Although multiple births and

the incidence of bacteriuria in pregnant and non-pregnant women are the same, the incidence of pyelonephritis is higher due to the anatomical and physiological differences brought about by pregnancy. The use of the diaphragm in women and the deterioration of the vaginal flora due to loss of estrogen are specific reasons for this group (Zor and Savaşçı, 2014; Nicolle et al., 2005).

UTI in children is another issue that needs to be addressed. Being uncircumcised in boys aged 0-6 months increases the risk of UTI 10-12 times. The short and straight urethra seen in girls increases the incidence of UTI as it is close to the anus. UTIs are rare in boys compared to girls due to the antibacterial properties of prostatic secretions (cationic proteins containing heat-stable zinc) as well as the anatomical structure of the urethra in boys (Hansson et al., 2004). Procedures applied to the urethra and bladder facilitate the ascending spread of bacteria (Sussman, 1998).

The presence of primary disease is one of the reasons that facilitate the formation of infection. In patients with diabetes mellitus, autonomic dysfunction involving the bladder particularly affects the frequency of ascending infections. When a partially obstructed kidney becomes infected, parenchymal damage develops more rapidly. Urinary system obstructions, especially stones, can be a source of infection. They provide adhesion and colonization of bacteria as a result of irritation to the urinary system epithelium. These stones can fill the pelvicalyceal system and may be called staghorn. Vesicoureteral reflux (VUR) is a radiological finding that means the urine from the bladder backs up into the ureters. Physiological disorders that normally prevent the reflux of urine into the ureters cause VUR. It shows that there is less need for virulence factors in the presence of VUR. (Milliner, 2004; Alon and Srivastava, 2007).

Nephrotic syndrome Changes occur in the cellular and humoral immune responses of patients with idiopathic nephrotic syndrome, which predisposes these patients to infections (Ulinski et al., 2010).

The best example of distinguishing immunological factors is defense factors shared between mother and baby. The risk of infection is lower in infants who are breastfed, due to the high number of immunoglobulins, compared to those who do not receive breast milk (Cattell et al., 1998).

Some patients with recurrent UTIs with anatomical and functional abnormalities also have some predisposing factors, such as urinary stasis or impaired urinary flow (Rubin et al., 1996). The inability to empty the bladder due to urinary obstruction, mechanical narrowing, neurological dilatation, increases bacterial growth in the urine and facilitates the spread of infection to different parts of the urinary system. While renal cortical scars do not affect susceptibility to infection, renal papillary scars increase the frequency of infection by causing an intrarenal tubular obstruction. (Uzun and Önal, 2001).

Abnormal voiding without urinary system anomaly is called voiding disorder. In these patients, the frequency of UTI was found to be increased due to secondary vesicoureteral reflux that occurs when the bladder is not completely emptied and the intra-bladder pressure increases. There is a significant correlation between recurrent urinary tract infections and constipation (Honkinen et al., 1999).

Tablo 3: Risk Factors by Age Groups (Sobel and Brown, 2020).

Age	Women	Men
All age groups	▪ History of previous UTI ▪ Urological surgical procedures ▪ Catheterization procedures ▪ Urinary system obstructions (including stones) ▪ Neurogenic bleeding ▪ Kidney transplant ▪ Socioeconomic status ▪ Hygiene conditions ▪ Diabetes mellitus	▪ The state of being uncircumcised ▪ History of previous UTI ▪ Urological surgical procedures ▪ Catheterization procedures ▪ Urinary system obstructions (including stones) ▪ Neurogenic bleeding ▪ Kidney transplant ▪ Socioeconomic status ▪ Hygiene conditions

	▪ Vesicoureteral reflux (VUR)	▪ Diabetes mellitus ▪ HIT
Adult	▪ Pregnancy ▪ Use of anti-sperm contraceptive gel ▪ Diaphragm use ▪ sickle cell anemia ▪ Estrogen loss ▪ Sexual activity (number of partners)	▪ Sexual activity (number of partners) ▪ Homosexual activity
Old age	▪ Estrogen loss ▪ Bladder prolapse ▪ Functional or mental impairment	▪ Prostatic problems ▪ Functional or mental impairment
Children	▪ Proximity of anatomically short and flat urethra to the anus	▪ The state of being uncircumcised

2.1. Factors of Microorganism

In a healthy person, the periurethral area is colonized by aerobic and anaerobic bacteria of the fecal flora. Colonization of the flora by gram-negative bacteria, especially *E. coli,* is a step in the formation of UTI. Studies in *E. coli* have shown that the most important virulence factor is adherens. Adhesion to uroepithelial cells is achieved by adhesins in the capsule or special pili known as P fimbriae. P fimbriae adheres to receptors that are identical to P blood group antigens contained in human uroepithelial cells. Another function of the P fimbriae is to increase the amount by mediating the release of endotoxin in the capsule of *E. coli.* It was found that the P fimbriae number of Pyelonephritisogenic *E. coli* was higher (76-94%) than the strains causing cystitis or asymptomatic bacteriuria (19-23% and 14-18%, respectively) (Kallenius et al., 1980; Vaisanen-Rhen et al., 1984).

Uropathogenic *E. coli* (UPEC) colonizes the bladder using various virulence factors. These virulence factors can be listed as lipopolysaccharide, polysaccharide capsule, flagella, outer membrane

vesicles, pili, curli, non-pili adhesins, outer membrane proteins, toxins, and secretion systems. (Omerovic et al., 2017).

Table 4: Virulence Factors of Uropathogenic *Escherichia coli* (UPEC) (Vaisanen-Rhen et al., 1984)

Virulence Factors	**Mechanism of Action**
Flagella	Up in the urinary system
Type-1 Fimbriae	Adhesion between bacteria and between bladder epithelial cells
P fimbriae	Adhesion to the renal epithelium
Dr fimbriae	Cell invasion
Cytotoxic necrotizing factor-1	Adhesion, invasion, host cell apoptosis
Hemolysin	Invasion, tissue damage
Lipopolysaccharide (LPS)	Immune response activation
Curly fimbriae	Adhesion, biofilm formation, invasion
Cellulose	Biofilm formation
Iron and zinc	Nutrition
Capsule	Resistance to phagocytosis

Virulence factors other than P fimbriae are supportive for tissue invasion. These; α-hemolysin is a cytolytic protein that destabilizes the cell membrane. Sidephores are iron-binding proteins necessary for bacteria to survive. Capsular polysaccharides: Attenuate alternative activation of complement and protect bacteria from phagocytosis. Vitamin K prevents lysis and phagocytosis of bacteria and ensures its continuity in the residential area. Colicin: bacteriocin produced by some strains of *E. coli* and kills other *E. coli*. Most bacteria cannot survive in human living fluids. Some gram-negative bacteria may show

resistance and cause sepsis (Sobel et al., 1997). Cell wall polysaccharides: Lipopolysaccharides cause local and systemic inflammation, fever, classical complement pathway activation, and inhibition of ureteral peristalsis (Marild et al., 1980). Toxins contribute greatly to the formation of inflammation. In this way, it increases the permanence of the bacteria in the area. The coexistence of these factors makes bacterial virulence strong and shows the inflammatory ability of the bacteria. However, interestingly, the fact that bacterial virulence factors are found to be significantly less in patients with VUR indicates that there is less need for virulence factors in the presence of VUR (Table 4) (Kallenius et al., 1980).

2.2. Factors of the Host

Virulence factors must attack the host's defense mechanism for the bacteria to be localized and cause infection after reaching the urinary system. The defense mechanism prevents adequate infection formation. While the virulence factor of the bacteria responsible for the first UTI may be involved, recurrent UTI may be an indication that the host's defense mechanism does not take an active role. The urinary tract contains protective mechanisms. These; physiologically and anatomically, it does not present a condition for bacterial spread, the content of the urine, the presence of bacterial flora, the protective factors of the bladder, ureters and kidney, and immunological, and cellular defense features (Özsüt and Çalangu, 1996).

Infection susceptibility in periurethral and uroepithelial cells is related to the receptor density and affinity of the cell. The accumulation of antiadhesive receptors and individual differences in receptor expression play an important role in susceptibility to infection (Hannson et al., 2004). Antiadhesive receptors act to prevent the pathogen from binding to the cell. The binding of *E. coli* with type 1 fimbriae to the mucosal surface is inhibited by the binding of Tamm-Horsfall glycoproteins and secretory IgA to terminal mannose residues

(Wold et al., 1990). The incidence of UTI was seen in the following order from high to low; uncircumcised boy, girl, circumcised boy. The reason for this is the removal of the mucosal surface (preputium) necessary for the adhesion of the bacteria with the circumcision and the failure of the bacteria to adhere. Bacteria that could not colonize the perineum could not form UTI (Inci and Davarcı, 2011).

Urine, which is sterile in a healthy person, is a suitable environment for bacterial growth at 37ºC. An easy breeding environment for microorganisms reaching the bladder is reached with human body temperature. Thanks to the glucose in the urine and proline betaine and glycine, which have osmoprotectant properties, favorable conditions are provided for microorganisms (Principi et al., 1977). In addition, urine has properties that prevent bacterial growth in vivo and in vitro. Urine has a pH of 8, 600 mOsm/kg osmolality, secretory IgA content, Tamm-Horsfall proteins, hippuric acid provides bacteriostatic effect, oligosaccharides and urea are substances that give antibacterial properties. At the same time, anaerobic bacteria rarely cause UTI through stool because the oxygen pressure of the urine is 3.7 kPa in the bladder: kilo pascal and 7.2 kPa in the kidneys. While mandelic acid takes on the task of lowering the pH, low molecular weight polyamines in the urine prevent hemagglutination and adhesion of *E. coli* type 1. Anaerobic bacteria found in the flora of the urethra can exert an adhesive effect on scarred kidney tissue where the oxygen pressure is very low, bladder tumors, and necrotic kidney papillomas (Jones et al., 1998). Uromucoids or uromodulin, defined as Tamm Horsfall protein (THP), is the most abundant protein in the urine. This substance covers the uroepithelial tissue as a thin layer. THP in the form of this layer in the loop of Henle and the distal tubule is released into the urine. The increased amount of dissolved THP in the urine prevents *E. coli* carrying type 1 fimbria from adhering to the uroepithelium. The characteristic of THP as a defense mechanism is that it binds to neutrophils and initiates phagocytosis. Complement

activation and increasing arachidonic acid metabolism are also among the tasks of THP. The decrease in THF affinity has been associated with increased virulence of some *E. coli* strains (Principi et al., 1977; David et al., 1991).

The bladder is not surrounded by infection by every organism that reaches the ascending route. Microorganisms are cleaned within 2-3 days by effectively periodically emptying the bladder. This cleaning process is also provided by the bacteriostatic substances in the urine and the intrinsic protective mechanisms in the bladder. In case of loss of effectiveness of these protective mechanisms, infection formation in the bladder may occur (Uzun and Önal, 2001). In recurrent UTI, lack of protective molecule release may be the cause of infection. The mucopolysaccharide layer of the bladder mucosa prevents adhesion and colonization. Microorganisms cannot adhere to the epithelium and are excreted during the periodic discharge of urine (Strohl et al., 2001).

Urine is carried to the bladder by peristaltic movements in the ureter. Decreased ureteral peristalsis facilitates the passage of microorganisms to the kidney. Bacteria that develop heat-resistant calcium ionophores may cause pyelonephritis by reducing this mobility (Saatçi, 1994).

The regions in the kidney show different sensitivity to bacteria. It was observed that the cortex region showed more resistance compared to the medular region. To infect the microorganism injections into the kidney, a process is applied to the cortex at a concentration of 10,000 times the medullary region. Renal medulla is more susceptible to infection because it is the first stop in the kidney by bacteria. Low blood flow and pH, high osmolarity, and ammonium concentration are factors that increase the probability of infection in the renal medulla, which is the first stop of the bacteria in the kidney. These factors reduce the resistance to infection by inhibiting complement activation and leukocyte chemotaxis. It also disrupts the cellular and humoral system. (Erdoğan and Öner, 1995; Rubin et al., 1996).

Due to low IgA and IgM defense mechanism substances seen in newborn blood, their immune systems have not developed compared to other age groups. IgG passes from mother to baby through the placenta, but decreases over the 6-month period. When the secretions and urine of breastfed infants are examined, a higher amount of immunoglobulin is seen compared to infants receiving adapted milk, and this is passed from mother to infant through breast milk. Lymphocytes and immunoglobulins that pass through breast milk competitively inhibit the adhesion of microorganisms to the uroepithelium. Breastfed infants are more resistant to bacterial infections than infants using alternative foods (Cattell et al., 1998).

Opsonization is achieved by the stimulation of complement by bacteria. While the host is clearing this bacterial infection, the tissues that belong to it may be damaged. Bacterial lipopolysaccharide structures stimulate monocytes and macrophages to secrete proinflammatory cytokines (Springall et al., 2001).

CONCLUSION

Urinary system infections are an important disease that affects the quality of life of a significant amount of people all over the world, regardless of age and gender. This disease, which is mostly seen in women, is a disease that can progress with mild symptoms and can cause symptoms up to kidney failure.

In researches, personal care, various fungi, viruses, and bacteria can also cause urinary tract infections. Urinary tract infections are the leading cause of bacterial infections. 40% of women experience a UTI at least once in their lifetime. 27% of this group show signs of recurrent UTI within 6-12 months following the infection. Recurrent UTI result; Irreversible conditions such as proteinuria, hypertension, renal scarring and renal failure may occur. Although the incidence of bacteriuria is the same in pregnant and non-pregnant women, the incidence of pyelonephritis is higher due to the anatomical and physiological

differences brought about by pregnancy. When the relationship between asymptomatic bacteriuria and the gestational week is examined, it is statistically higher in the first and second trimesters.

Being uncircumcised in boys aged 0-6 months increases the risk of UTI 10-12 times. The short and straight urethra seen in girls increases the incidence of UTI as it is close to the anus.

The prevalence of UTI, which is 1% in boys and 1-3% in girls, changes with age. While the male/female ratio is 2.8-5.4/1 in 0-24 month-old babies, the male/female ratio is 1/10 after 1 year of age, and the probability of UTI in girls increases.

Gram-negative bacteria and *Escherichia coli* included in this group are the most isolated microorganisms from uncomplicated urinary tract infections. According to the information obtained from the patients, the most common bacteria in UTI infections is *E. coli*. The reason for this is thought to be the special adaptation of *E. coli*. While the incidence of *E. coli* increased with increasing age, the opposite course was observed in other pathogens.

Procedures applied to the urethra and bladder facilitate the ascending spread of bacteria. In this study, as in other studies, it was observed that the presence of menopause and vaginal atrophy significantly increased the prevalence of UTI.

Considering the results obtained; In empirical antibiotic selection, since resistance to antibiotics varies between regions, each region should determine its resistance rates and direct its treatments accordingly. First and second- generation cephalosporins, semi-synthetic penicillins with or without β-lactamase inhibitors, fosfomycin, and nitrofurantoin are the most commonly used antibacterial agents in the treatment of urinary tract infection in pregnancy. Treatment with NSAIDs delays antimicrobial therapy and effective treatment cannot be provided. It has shown that the use of vaginal estrogen is effective in preventing re-infection. Nitrofurantoin

and fosfomycin trometamol are recommended as first-line therapy for uncomplicated cystitis.

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CHAPTER 6

THE SIDE EFFECTS OF BREAST CANCER RADIOTHERAPY

MSc Aleksandra SANČANIN[1]

MSc Sofija SANČANIN[2]

Assist.Prof. Branislav SANČANIN[3]

[1] Oncology Institute of Vojvodina, Sremska Kamenica, Serbia
ORCİD: ID/0000-0002-9770-9264, aleksandra95sancanin@gmail.com, 381 66 199502
[2] Hamad General Hospital - Doha, Qatar
ORCİD: ID/0000-0002-6397-6567,ssancanin@hamad.qa, 974 5026 3980
[3] Union - Nikola Tesla University - Belgrade, Faculty of Management, Sremski Karlovci, Serbia, branislav.sancanin@famns.edu.rs, 381 65 8981898
ORCİD: ID/0000-0002-4255-2634,
branislav.sancanin@famns.edu.rs, 381 65 8981898

INTRODUCTION

Breast cancer is the leading cause of death from cancer in women, with this diagnosis being established, on average, in one of eight women. Compared to most female malignant diseases where there is a decline, the incidence of breast cancer has been steadily increasing over the last half century.

About 1.5 million new cases of breast cancer are registered on a global level, with over 570,000 deaths (Stewart and Wild 2014). In a large number of countries, breast cancer accounts for 25% of all malignant diseases of the female population, while it accounts for up to 15% in the mortality structure. The lifetime risk, *i.e.*, the cumulative probability of the disease, is about 12.5%, which practically means that one in eight women can be expected to develop breast cancer during her lifetime (Pekmezonic and Dojke 2013).

The highest age distribution is among postmenopausal women, with an average age of around 60, with a tendency of younger women becoming ill. The highest mortality from malignant diseases is recorded in women under the age of 40, specifically from breast cancer (Kurs and Dojke 2018).

The most pronounced difficulties during treatment arise as a result of the issue of how to predict its behavior in the future based on the clinical classification and morphological characteristics of the tumor, which represents a great scientific and practical challenge. Therefore, after surgical treatment, there is a continuous need for the prompt detection of all indicators that would enable us to identify patients with an increased risk of relapse.

Patients usually tolerate the radiation well, but sometimes, in certain localizations, side effects may occur in the form of itching, burns, difficulty in breathing or swallowing, and pain. As minor changes can occur on the blood vessels, spider nevus can be seen as a side effect on the skin.

In case of damage to the lymph nodes, a swelling of the hands can occur - lymphedema, as the inability of the lymphatic system to support lymphatic circulation and drainage (Stolldorf et all 2016).

Statistically speaking, lymphedema, with the accompanying feeling of discomfort, disruption of numerous bodily functions and reduction in quality of life, occurs in about 20% of irradiated patients, that is, on average, in one in five. Manual massage, as a harmless method of reducing lymphedema, can be useful [**5.]**, with the application of compression bandages being even more

useful over an extended period of time. Namely, the swelling decreases significantly more with the application of compression bandages than with manual massage alone. Some studies have shown that combining manual massage and compression bandages helps patients with a milder form of swelling.

On a global scale during 2018, there were approximately 2.1 million newly diagnosed female breast cancers, which is approximately one in four cases of cancer in women. Breast cancer incidence rates are the highest in Australia and New Zealand, Northern and Western Europe (Great Britain, Sweden, Finland, Denmark, Holland and France) (Bray and Ferlay et al 2018).

According to the World Health Organization's indicators, breast cancer in women is the most frequently diagnosed cancer (11.6%) and the leading cause of death among the female population. The incidence of breast cancer in women in the region is highest in Serbia - 5,422, followed by Croatia - 2,641, and Bosnia and Herzegovina - 1,152. Common to all three countries is that breast cancer, among all malignant female diseases, occupies the leading position [**7.].**

COMPLICATIONS FROM RADIOTHERAPY

Radiotherapy (RT) is one of the three basic approaches to the treatment of breast cancer, and it was received in almost all cases after breast-conserving surgery, as well as for those patients with an increased risk of disease relapse after a radical mastectomy (Senkus and Kyriakides et al 2015).

In order to improve the results of treatment, combining radiotherapy with chemotherapy is performed, where a negative interaction is possible. By applying chemotherapy as a radiosensitizer, the radiosensitivity of healthy cells can be increased by applying much lower doses than usual. In addition, the harmful effect of systemic therapy on the target organ can be enhanced by the use of radiotherapy. Subclinical damage that is acceptable with the application of one oncological modality can become manifest by combining these two therapeutic modalities.

Adverse effects of radiotherapy may occur during radiation therapy. The most common symptoms occur on the skin in the form of redness. These

are actually early symptoms that occur during radiation therapy (Sciubba and Goldenberg 2006).

Acute or early complications can develop as early as two to three days after the start of radiotherapy and end two to three days after the end of radiotherapy. Chronic or late complications manifest themselves a few days after the end of radiation therapy and up to several months or years after the end of radiation therapy.

A scale recommended by the association of radiation oncologists is used to monitor acute complications during radiation therapy (*Acute Radiation Morbidity Scoring Criteria-RTOG*).

Most patients have side effects that occur during oncological treatment, especially during and immediately after the end of radiation therapy. During the first year after the end of treatment, these side effects are diluted to the extent that most of them almost completely disappear. Some consequences remain permanently. In a smaller number of patients, ignoring possible side effects during therapy is one of the treatment approaches. Being informed about all possible side effects can help the patient mitigate them when they occur.

Late complications visible on the skin after radiological treatment of breast cancer, after a mastectomy and conserving surgery, can occur even after several years. They are manifested by the presence of visible capillaries on the skin of the amputated breast (Telangiectasia), a thickening of the epidermis of the skin (Scleroderma), a darker discoloration of the irradiated region (Hyperpigmentation), the creation of permanent scar changes at the site of the surgical incision (Fibrosis), the thinning of the superficial layers of the cutis (Atrophy), an inflammatory reaction on the skin and remaining breast tissue (Mastitis), and the presence of enlarged lymph nodes on the neck and armpits (Lymphadenitis).

Skin damage (radiodermatitis)

Skin damage caused by radiation can be acute and chronic, with permanent aesthetic changes. Skin changes can appear in the region of the input and output radiation fields and depend not only on radiation therapy parameters (dose, fractionation, field size, type of radiation), but also on the

characteristics of the patient (age, innate sensitivity to ionizing radiation, previous skin damage, hygiene habits, *etc.*).

Radiation therapy damages skin cells as well as hair roots, sebaceous and sweat glands, blood vessels of the dermis, so there is a possibility of temporary or permanent alopecia and dryness of the skin over the irradiated region (Mileusnim and Durbaba 2012).

Skin redness, dryness and itching may appear during radiation therapy as a direct reaction of the skin to radiation. When redness or itching occurs on the skin, cold compresses made of water or Rivanol solution can be used (2-3 times a day for 20 minutes). Creams, lotions and powders should not be applied to skin.

In order to care for the skin of the breast during radiation therapy, a short shower with lukewarm water is recommended. The skin of the irradiated breast area should not be rubbed with a sponge, terry cloth or exfoliating net, but should be done with the palm of the hand and the fingers. The skin should be allowed to dry naturally or the region to be irradiated should simply be pat-dried with a soft terry cloth (Obrenovac and Lecenja 2020).

In case of desquamation without infection, local application of corticosteroid creams is recommended, while a break in radiation therapy is also often recommended. However, in case desquamation is accompanied by infection, the application of local antibiotics or antifungals is mandatory, along with regular management of the infected area with a compress containing silver ions. After the infection subsides, ointments or emulsions that stimulate epithelization are applied locally, and it is useful to expose the deepithelialized skin zone to air flow.

Chronic changes to the skin leave permanent consequences. Oral administration of pentoxifylline in a dose of 800 mg (2x400 mg) has been shown to be useful in preventing permanent vascular changes to irradiated skin. Depigmentation or hyperpigmentation of the skin, loss of hair on hairy parts of the body and skin atrophy are, unfortunately, permanent and aesthetically irreparable forms of skin damage (Mileusnim and Durbaba 2012).

During the radiation period and one month after the end of therapy, the armpit on the side that was irradiated should not be shaved and deodorants should not be applied. Wider cotton clothes should be worn and the use of

tight t-shirts made of synthetics or wool, which can irritate the skin, is not recommended, while under home conditions the skin should be left exposed, as much as possible, and a bra should not be worn. During radiation and one year after, the skin should not be exposed to direct sunlight while later it is necessary to use creams with a high protection factor (over 40 SPF) (Obrenovac and Lecenja 2020).

In case of emesis after radiotherapy, it is necessary to avoid food for several hours before treatment. In case of nausea before radiation, patients are advised to eat smaller portions of dry food (*e.g.*, toast). Emesis caused by radiotherapy is treated with antiemetics, as well as glucocorticoids in combination with other antiemetics. Patients are given recommendations to eat smaller meals, at room temperature, and to avoid fatty and heavy food (Redzovic and Zahirovic et al 2015).

Tiredness (fatigue)

Given that during radiation therapy, patients complain of fatigue and weakness, it is necessary to rest more and avoid strenuous work. In addition, it is recommended to drink more fluids and eat a balanced diet, without strict diets and starvation (Obrenovac and Lecenja 2020). During radiation, it is necessary to control blood count, especially as it shows a decrease in the number of blood cells, most often leukocytes, which usually normalizes after the end of radiation. In case of severe side effects, a decision can be made to stop radiation.

The feeling of fatigue is the main symptom that dominates among breast cancer patients and is highly correlated with distress that accompanies the presence of a malignant disease. The etiology of fatigue is complex and is related to the patient's ability to respond to heavy burdens and worries about the future, but also to depression and other social factors that greatly change life. The complexity of the etiology of fatigue can be seen in the fact that even after the correction of anemia, as one of the possible causes of fatigue, the condition sometimes remains unchanged.

The feeling of fatigue caused by radiotherapy lasts during radiation and for a period after, which can be from three to six months, but it is less pronounced than when radiotherapy is combined with chemotherapy, *i.e.*, less than when chemotherapy is applied as the only form of treatment. In addition

to fatigue, anxiety and depression are often present during radiotherapy and, in some patients, deterioration is possible during the administration of radiation, along with the development of a feeling of chronic fatigue. The treatment of fatigue is complex and directly related to the dominant cause. Physical activity is recommended for patients, considering that in addition to improving mood, it also improves survival, and thus the overall quality of life (Mileusnim and Durbaba 2012).

Taste disorder (dysgeusia)

The state of having an impaired ability to taste often occurs in cancer patients undergoing radiotherapy and/or chemotherapy. Mosel et al indicate that this condition affects a reduced appetite, primarily in the form of a decrease in food intake which results in a decrease in body weight and deterioration of nutritional status. A taste disorder can change qualitatively (distorted perception) or quantitatively (increased or decreased perception of taste change). The change in taste leads to a decrease in intake, which further significantly affects the emotional stability and overall health and life of the patient (Mosel, Bauer and Lynch, Hwang 2011).

Although all the causes that lead to taste disorders have not been identified, it can be said with certainty that certain medications, insufficient oral hygiene, and infections contribute to a change in taste.

Given that breast cancer patients have hyposalivation, drinking more water is recommended. In addition, the slow chewing of food is suggested in order to stimulate the production of saliva. The intake of zinc and vitamin D, with different mechanisms of action, contributes to the improvement of taste.

Dry mouth (xerostomia)

Dry mouth or xerostomia is not directly related to breast cancer but to gland dysfunction and a decrease in the amount of saliva in the oral cavity. Taking into account individual variations, the normal value for the amount of saliva secretion is 0.4 to 0.5 ml per minute, and when saliva secretion decreases by 50%, *i.e.*, when it is below 0.2 ml/l, dry mouth occurs, *i.e.*, xerostomia.

The characteristic of xerostomia is really thick, sticky and viscous saliva, making taking food and swallowing, as well as speaking, difficult. Given the change in the amount and composition of saliva, there is also a

change in the pH of the saliva, *i.e.*, a decrease in the pH value and the creation of conditions for a change in bacterial microflora. As a result, cavities and infections of the mucous membrane of the oral cavity occur (Mosel, Bauer and Lynch, Hwang 2011).

If enough active glandular tissue remains, it can be stimulated locally and systemically. For local stimulation, the use of chewing gum and candies without sugar, paraffin, glycerin lozenges, and toast is recommended, while for systemic stimulation, pilocarpine, hydrochloride, anetoltrition, and pyridostigmine can be used. Insufficient secretion of saliva affects the microbial flora of the oral cavity, so Candida albicans fungi often develop under such conditions, which dictates the use of antimycotic therapy. Ascending infections of the major salivary glands have also been recorded, which necessitated the introduction of antibiotics (Topic and Vucicevic-Boras 1998).

Osteonecrosis

In advanced breast cancer, bones are one of the most vulnerable places for the development of metastases. Particular attention is paid to estrogen in order to preserve bones. However, during the treatment of, primarily elderly, breast cancer patients, aromatase inhibitors are used as adjuvant therapy to inhibit the production of estrogen. In this way, deterioration and loss of bone mass is accelerated, which is clearly seen when diagnosing osteoporosis. Tamoxifen, which is an estrogen antagonist, has the opposite effect as it contributes to an increase in mineral density in postmenopausal women. One should be careful with the final conclusions and recommendations, especially when considering the fact that in premenopausal women, tamoxifen contributes to the occurrence of the risk of a decrease in bone mass density (Taichman et al 2014).

Secondary infections

Microorganisms that are normally found in the body, with a change in conditions, *i.e.*, a decline in immunity, become pathogenic (Zheng et al 2020).

Infections, especially those that are not diagnosed in time, can lead to systemic infections and sepsis, *i.e.*, endanger the life of patients. The immune response to infection includes innate and acquired actions involving various cell types, mediators, and chemical agents, and the exact nature of the response depends on the origin and nature of the antigen (Calder 2007).

When the environment is locally or systemically altered, especially in a situation where the tissue is damaged or when there is a noticeable imbalance of the flora that is common in cancer patients, actual colonies and infections of Candida albicans (Candida albicans) and related fungi occur.

In addition, frequent complications during cancer treatment are viral infections, most often herpes simplex virus (HSV), as well as cytomegalovirus (CMV), varicella-zoster virus (VZV) and Epstein-Barr virus (EBV). These infections, as indicated by Mosel et al. (2011) can result in malnutrition and dehydration (Mosel, Bauer and Lynch, Hwang 2011).

In addition to viruses and infections, there are also bacteria in a healthy body which become pathogenic in the event of a decline in the body's immunity. Sepsis is also possible in case of untimely recognition and treatment, which always carries with it the threat of death.

RESEARCH OBJECTIVES, HYPOTHESES and WORKING METHODS

The problem in breast cancer radiotherapy is predicting the occurrence of complications and side effects based on the applied radiotherapy technique. In order to implement appropriate radiotherapy for breast cancer, there is a continuous need to find new indicators to detect patients who are at an increased risk of developing side effects during and after radiotherapy.

The subject of research is the analysis of theoretical and research results on the impact of radiotherapy on the occurrence of side effects and emotional reactions.

Hypotheses from which the research starts

Based on the proposed variables and assumptions built on the basis of reference literature, the following hypotheses were proposed:

H1 There is a positive correlation between radiation therapy and side effects: fatigue and exhaustion, loss of appetite, weight loss, nausea and vomiting.

H2 There is a positive correlation between radiation therapy and emotional reactions: feelings of helplessness, fear, listlessness, hypoactivity and anger.

Material and methods used during research

Scientific methods typical for the field of medical science were used in the paper for data analysis and presentation of results: induction, deduction and description. Using a structured questionnaire, an analysis of research data in the area that is the subject of the paper was carried out. Quantitative analysis was used in this research with the aim of finding out the elements of the content and context determined by the subject of the paper.

Appropriate statistical methods were used for statistical data processing, and statistical analyzes were carried out using the SPSS 22 package (*Statistical Package for Social Sciences for Windows 22*). In the first step of statistical analysis, the validity of the used measuring instruments, used when assessing the influence of the mentioned variables on the occurrence of complications and side effects of radiotherapy, was assessed. In order to test the general hypotheses H1 and H2, Kendall's τ correlation coefficient was used.

The unit of observation in the research are patients with verified breast cancer, who underwent radiotherapy during the period July 1 - September 30, 2020.

The research was conducted in October and November 2020 at the Institute of Oncology of Vojvodina in Sremska Kamenica, Republic of Serbia. The basic starting point in the research is to detect the optimal technique of radiotherapy in patients with breast cancer, on the one hand, and the side effects of radiotherapy, on the other hand. The study included patients with pathohistologically verified breast cancer. The research was approved by the director of the Institute of Oncology of Vojvodina and the Ethics Committee of the Institute of Oncology of Vojvodina in Sremska Kamenica.

The total patient sample was irradiated on the linear accelerator Elekta Versa HD, Varian TrueBeam, Varian VitalBeam, Varian 600 DBX. The radiation dose applied to the tumor depends on the radiation technique, the stage of the disease, the TNM classification, and the application of other oncological treatment options according to the recommendations of the *National Comprehensive Cancer Network* (NCCN).

RESEARCH RESULTS

Fifty female patients participated in the research. By age, the most represented were those between 56 and 60 years of age (44%), and only two were between 26 and 40 years of age (4%). There were 17 (34%) in the cohort aged 40-55 years, while among those over 65 there were 9 patients (18%).

In terms of education, more than half of the female patients had a completed secondary education - 33 (66%). Six patients each (12%), had completed elementary school or university, and 5 (10%) a completed college education.

More than half of the patients who participated in this research were from Bačka -31 (62%), 10 (20%) were from the Banat region, 8 (16%) from Srem, and one patient (2%) was from Serbia proper.

The side effects of radiotherapy in patients with breast cancer were observed through side effects during radiation therapy and emotional reactions. Each of these groups of side effects has been represented by five questions on a three-point scale, where a higher number indicates greater agreement with the statement. Scores for groups of side effects of radiotherapy were calculated as an average score.

Table 1: Side effects of radiation therapy

	AS	SD	M_e	Minimum	Maximum	W	p
Side effects during radiation therapy	1.3000	0.37742	1.20	1.00	2.20	0.745	0.000
Emotional reactions	1.3918	0.45085	1.40	1.00	2.60	0.754	0.000

On average, female patients have the most pronounced emotional reactions (M=1.39; SD=0.45) to radiotherapy. Side effects were the

least pronounced during radiation therapy (M=1.30; SD=0.38). The *Shapiro Wilk* test was used to test the normality of the distribution of these three variables. The distribution of the results of both variables cannot be considered normal, $p<0.05$.

To test the correlation between radiation therapy (fractionation) and the side effects of radiation therapy in breast cancer patients (side effects during radiation therapy and emotional reactions), Kendall's τ correlation coefficient was used. Radiation therapy (fractionation) is a dichotomous variable with the following categories: "Hypofractionation" and "Hypofractionation + boost".

The correlation between radiation therapy (fractionation) and fatigue and exhaustion, loss of appetite and body weight is positive, which indicates that with the application of "Hypofractionation + boost" patients have more pronounced fatigue and exhaustion, loss of appetite and body weight compared to the application of "Hypofractionation" alone.

Table 2: Correlation between radiation therapy (fractionation) and the side effects of radiation therapy

	Radiation therapy: fractionation		
	τ	p	N
Fatigue and exhaustion	0.074	0.588	49
Loss of appetite	0.389**	0.035	49
Weight loss	0.310*	0.092	49
Nausea	-0.117	0.158	49

The correlation between radiation therapy (fractionation) and nausea is negative, which indicates that with the application of "Hypofractionation + boost" patients have less pronounced nausea compared to the application of "Hypofractionation" alone. The

correlation between radiation therapy (fractionation) and loss of appetite is significant, $p<0.05$. The correlation between radiation therapy (fractionation) and fatigue and exhaustion, weight loss and nausea is not significant, $p>0.05$.

H1 Based on the obtained results, hypothesis H1 was partially accepted, *i.e.*, there is a significant correlation between radiation therapy (fractionation) and loss of appetite.

To test the correlation between radiation therapy (fractionation) and emotional reactions during radiation therapy in patients with breast cancer, Kendall's τ correlation coefficient was used.

The correlation between radiation therapy (fractionation) and a feeling of helplessness, fear, listlessness and anger is positive, which indicates that with the application of "Hypofractionation + boost" patients have a more pronounced feeling of helplessness, fear, listlessness and anger compared to the application of "Hypofractionation" alone.

Table 3: Correlation between radiation therapy (fractionation) and emotional reactions to therapy

	Radiation therapy: fractionation		
	τ	p	N
Feeling of helplessness	.209	.199	49
Fear	.117	.461	49
Listlessness	.256	.1350	49
Hypoactivity	-.029	.841	48
Anger	.290*	.102	49

The correlation between radiation therapy (fractionation) with hyperactivity is negative, which indicates that with the application of "Hypofractionation + boost" patients have less pronounced

hyperactivity compared to the application of "Hypofractionation" alone. The correlation between radiation therapy (fractionation) and anger is moderate and significant, $p<0.05$. The correlation between radiation therapy (fractionation) and a feeling of helplessness, fear, listlessness and hyperactivity is not significant, $p>0.05$.

H2 Based on the obtained results, hypothesis H2 was partially accepted, *i.e.*, there is a positive correlation between radiation therapy (fractionation) and anger.

DISCUSSION

Both groups of observed reactions to radiotherapy for breast cancer, on average, belong to partially or moderately expressed. Not a single patient had all, very pronounced indicators of radiation therapy side effects. On average, female patients have more pronounced emotional reactions than side effects.

To interpret the research results, it is necessary to differentiate radiation therapy (fractionation) into categories: "Hypofractionation" and "Hypofractionation + boost", given that fatigue, exhaustion, loss of appetite and body weight can be associated with the application of "Hypofractionation + boost". Also, the application of this category increases the feeling of helplessness, fear, listlessness and anger, which indicates the need for a multidisciplinary approach to the radiotherapy process and a significantly greater sensitivity to all observed reactions.

CONCLUSION

This paper should point out the importance of fundamental knowledge about radiotherapy and radiotherapy treatment of breast cancer, as the most prevalent malignant disease in women. Improving the techniques of planning and implementing radiation therapy leads not only to a significant reduction in recurrence, survival without signs of disease and overall survival without significant risks to normal tissue, but also to a reduction of realistically possible side effects.

Unwanted effects of radiotherapy may occur during radiation therapy, and the most common ones appear in the form of skin redness.

Most patients have side effects that occur during oncological treatment, especially during and immediately after the end of radiation therapy. During the first year after the end of treatment, these side effects are diluted to the extent that most of them almost completely disappear.

During the radiotherapy process, the central place belongs to planning given that the errors that occur during the planning of therapy represent systematic errors and include all patients who pass through a system so defined. Compared to conventional treatment and planning based only on computed tomography, qualitatively better results should be expected by introducing a more precise method, the fused conformal 3-D technique of radiation therapy with magnetic resonance imaging, which could result in a reduction of unwanted side effects.

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CHAPTER 7

HOLMIUM LASER ENUCLEATION OF THE PROSTATE (HoLEP)

Assist. Prof. Mesut YEŞIL[1]

[1] Mesut Yesil, e-mail: dryesil@gmail.com
ORCID ID: 0000-0001-8575-9830

INTRODUCTION

Holmium laser enucleation of the prostate (HoLEP) technique has been introduced in 1994 for the first time (Gilling, Cass, Malcolm and Fraundorfer, 1995) for the treatment of benign prostatic hyperplasia (BPH). In order to increase efficiency, HoLEP developed gradually over time from resectional and ablative techniques using the high-powered holmium laser (>60 W). In patients with BPH, a natural tissue plane is created by the expanding adenoma. This tissue can be exploited both during endoscopic HoLEP operation and open prostatectomy. The use of this plane in a retrograde direction has been increased as experience with HoLEP technique has increased. The development of a transurethral soft tissue morcellator tool has enabled entire lobes to be freed safely (Gilling, Kennett, Das, Thompson and Fraundorfer, 1998). In the current HoLEP method, the prostate is divided into its three anatomical lobes. Each lobe is then enucleated in a retrograde approach. Next, the same defect as in open prostatectomy is created. Following hemostasis, a transurethral morcellator tool is used to remove the tissue plane. This chapter begins with a brief description of BPH. It continues with the surgical treatment of BPH with the focus on HoLEP technique. In addition, outcomes of HoLEP and comparison of this technique with the other two commonly used methods are addressed.

1. Benign Prostatic Hyperplasia (BPH)

Benign prostatic hyperplasia (BPH) is one of the most common conditions in elderly men, because this age group constitutes the major portion of the general population (Park et al., 2004). BPH is defined by the abnormal proliferation of smooth muscle and epithelial cells in prostate tissue (Wei, Calhoun and Jacobsen, 2005). This condition leads to a major impact and significant burden on healthcare systems. BPH affected more than 210 million men worldwide as of 2010. It has been reported that 70% of men aged 61-70 years and 90% of men aged

81-90 years have pathologically identified BPH (Lim, 2017; Bray, Ferlay, Soerjomataram, Siegel, Torre and Jemal, 2018; Lokeshwar, 2019). Clinical manifestations of BPH include urgency, frequency, decreased stream, and nocturia, resulting in decreased patients' quality of life (Kapoor, 2012).

The pathophysiology of BPH has not been fully understood. Many factors have been associated with BPH including sex hormones, diet, inflammation, microorganisms, and neurotransmitters (Lokeshwar, 2019). The structure and volume of the prostate vary by aging. Cross-sectional studies have reported that volume of the prostate increased to 35-45 cc in men aged 70 years and to 25 cc in men aged 30 years (Hiort et al, 2014).

1.1. Treatment of BPH

In patients with BPH, the goal of treatment is to increase quality of life. Medical treatment such as 5 alpha-reductase inhibitors or alpha-adrenergic receptors antagonist has been shown to improve symptoms as demonstrated by indications for surgical treatment and a decrease in the acute urinary retention (de la Rosette et al., 2001).

However, surgical intervention is required under certain conditions. Current indications for surgical management are as follows (Budaya and Daryanto, 2019; Ingimarsson, Isaksson, Sigbjarnarson, Gudmundsson and Geirsson, 2014):

1. Failure of medical treatment based on both The International Prostate Symptom Score (IPSS), decreased maximum flow rate and increased residual urine.
2. Complications of the urinary tract (hematuria, bladder stones, recurrent urinary tract infections, recurrent urinary retention, renal failure) and outside urinary tract (hemorrhoid and hernia).
3. Patients' preference of surgical options instead of using medications for a long term.

1.2. Surgical Management of BPH

1.2.1. Open Prostatectomy

Open prostatectomy has remained the standard treatment of BPH more than 50 years (Tubaro and de Nunzio, 2006). It is the oldest treatment for increasing maximum flow and relieving symptoms of BPH. Open prostatectomy involves surgical removal of the inner portion of the prostate through a retropubic and suprapubic approach. In the retropubic approach, a direct incision is made in the anterior prostatic pseudocapsule, allowing direct visualization of the prostatic fossa in order to provide more complete removal of the adenoma. The bladder is left intact with this procedure (Han and Martin, 2007). Suprapubic approach is suitable for patients who have concurrent bladder calculi or a large median lobe. However, open prostatectomy is the most invasive treatment technique and is related to significant morbidity (Gratzke et al, 2007). In addition, perioperative bleeding is the most common complication and blood transfusion poses the risk of transfusion reactions and infectious diseases (Hendrickson and Hillyer, 2009). This method is reserved for patients with anatomical problems such as a large median lobe that protrudes into the bladder or a very large prostate.

Transurethral Resection of the Prostate (TURP)

Monopolar TURP: Based on long-term follow-up studies, the use of monopolar resection has been considered as safe, however it may lead to acute complications such as urethral stricture, electrolyte imbalance, and bleeding (Reich, Gratzke and Stief, 2006). Improvements in the technique has resulted in a decrease in the incidence of adverse events, although concerns remain about complications including urethral strictures, bleeding and TUR syndrome (Hawary, Mukhtar, Sinclair and Pearce, 2009).

Bipolar TURP: In this procedure, a closed electrical circuit is completed during ablation and energy is limited to a pathway between a

resectoscope tip and resection loop. Bipolar TURP is preferred in patients with diabetes mellitus or those with cardiac pace-makers (Tang et al, 2014). In a meta-analysis of 24 studies, no significant difference was found between monopolar and bipolar TURP procedures in terms of IPSS and health related QoL scores (Omar et al, 2014).

The most common complications of TURP include inability to void, secondary infection and clot retention. Long term complications of TURP include partial and total incontinence, impotence and retrograde ejaculation. Nearly 10% of patients require re-treatment within next five years.

2. Holmium Laser Enucleation of the Prostate (HoLEP)

2.1. History of HoLEP

In BPH, holmium laser application was introduced for the first time for sequential tissue ablation in order to enlarge a channel through the prostate. This first technique has been applied with neodymium (Nd:YAG) laser and named "combination endoscopic laser ablation of the prostate" (CELAP) (Gilling, Kennett, Das, Thompson and Fraundorfer, 1998). As experience with holmium lasers increased over time, it has been found that the laser alone had adequate hemostatic effect on vaporization of soft tissue without using Nd:YAG component (Kuo, Paterson, Kim, Siqueira, Elhilali and Lingeman, 2003). This has resulted in the development of HoLAP, in which many short-term voiding difficulties related to the use of Nd:YAG were eliminated. Easy learning of the procedure was its main advantage, while it has an important disadvantage of the absence of tissue for pathological examination. Furthermore, vaporization of tissue is a slow process. Due to these disadvantages, HoLAP has been reserved for patients with smaller prostate glands.

Holmium laser resection of the prostate (HoLRP) was reported for the first time by Gilling et al. in 1996 (Gilling, Cass, Cresswell and Fraundorfer, 1996). In this procedure, cutting properties of holmium

laser was used for the first time to dissect large portions of adenoma tissue. In HoLRP, first the median lobe is resected followed by resection of the lateral lobes. The lobes are broken down into pieces endoscopically, while released from the surgical capsule. However, operative time remains a problematic issue with longer operation time compared to TURP (Kuo, Paterson, Kim, Siqueira, Elhilali and Lingeman, 2003).

In 1998, a prototype of "transurethral tissue morcellator" was developed by Gilling and Fraundorfer (Gilling, Kennett, Das, Thompson and Fraundorfer, 1998). By this new technique, the need for the process of breaking down the lobes into manageable parts, which is a time consuming process, was eliminated. The new resection technique permits enucleation of both lateral lobes and median lobe. Today, this latest modification is known as "holmium laser enucleation of the prostate" (HoLEP), and is combined with the process of morcellation. HoLEP can be used in all BPH sizes or configuration. In addition, bladder calculi can also be vaporized in an easy way during HoLEP. In the last decade HoLEP has been regarded as the gold standard in the surgical management of BPH.

2.2. HoLEP Procedure

2.2.1. Preoperative Considerations

The severity of obstruction is documented through medical history and physical exam, which include flow rate and post-void residue. Laboratory investigations include PSA level, complete blood count (CBC), and urinalysis. Before proceeding, prostate biopsy should be performed in case of an elevated PSA level. A transrectal ultrasound (TRUS) is recommended, because estimation of the size by digital rectal exam can be difficult. In addition, TRUS provides information regarding operation time. A flexible cystoscopy is performed in order to rule out other forms of obstruction. Informed written consent should

be obtained from the patient, emphasizing the likelihood of retrograde ejaculation following the HoLEP procedure.

2.3. Technique

The HoLEP procedure is performed using a high-power holmium laser. There are two separate foot pedals, one for the enucleation process and the other for hemostasis. A 26 Fr endoscope is used with a laser bridge and an off-set nephroscope is used for the morcellation process. The laser fiber is advanced through a laser catheter which is locked at the end to provide a fixed laser fiber length during the procedure. Because of the large need of fluid during the procedure, an inflow port is connected to 3 L of normal saline.

The laser enucleation process involves release of two lateral lobes and one median lobe into the bladder. For this purpose, an incision is made over the urethral mucosa from bladder neck to the verumontanum and the surgical capsule is localized. The median lobe is isolated and dissected off the capsule with a retrograde approach in a distal to proximal direction towards the bladder neck. The median lobe is freed so as to float in the bladder. The lateral lobes are enucleated with the same manner. The incisions are connected from the 12 o'clock to 5 o'clock position to enucleate the left lateral lobe and from 12 o'clock to 7 o'clock to enucleate the right lateral lobe. The enucleated lateral lobes are then placed into the bladder.

Hemostasis must be completed prior to morcellation in order to increase visibility. Hemostasis occurs during the enucleation process of the procedure due to holmium laser fiber that coagulates as it cuts tissue. The tree lobes that are now floating freely in the bladder are retrieved using an endoscopic soft tissue morcellator. The morcellator removes the fragmented pieces through hollow blades into a retrieval device. In order to provide optimal visibility and to avoid damage to the bladder mucosa it is important to have the bladder full during morcellation.

2.3.1. Post-op Care

A 22 Fr 3-way catheter is placed in all patients for continuous bladder irrigation overnight. This catheter is removed on the first post-op day and when patients are able to void twice they are discharged. Patients are advised to avoid heavy lifting and vigorous activity for one week and sexual intercourse for two weeks. The use of anticoagulants is resumed post-op 5th or 7th day once gross hematuria has subsided (Rivera, Frank, Viers, Rangel and Krambeck, 2014).

2.4. Outcomes of HoLEP

In a study by Alkan et al. including patients undergoing HoLEP procedure for treatment of BPH with long-term follow-up (5 years), significant improvements were achieved in PSA levels and PVR volumes, Qmax values, QoL and IPSS (Alkan, Ozveri, Akin, Ipekci and Alican, 2016). In this study, the mean Qmax value was found as 22.9 mL/s at one month following the HoLEP procedure. Naspro et al. stated that postoperative Qmax value was 21.4 mL/s following HoLEP (Naspro et al, 2009). Krambeck et al. found a significant increase in the mean Qmax value with HoLEP (Krambeck, Handa and Lingeman, 2010). Yin et al. stated that there were significant improvements in IPSS scores following HoLEP (Yin, Teng, Huang, Zhang and Xu, 2013). Sun et al. showed improved QoL after HolEP (Sun et al, 2014). Klett et al. reported no adverse effect on sexual functioning with HoLEP (Klett, Tyson, Mmeje, Nunez-Nateraz, Chang and Humphreys, 2014). Jeong et al. reported that there was a slight reduction in sexual functioning, but this improved 12 months later (Jeong, Ha, Lee, Cho, Kim and Paick, 2012).

2.5. Comparison with Other Techniques

2.5.1. HoLEP versus Open Prostatectomy

Various randomized controlled studies have reported significantly longer operation times, a shorter duration of catheterization and shorter length of stay in hospital with HoLEP compared to open prostatectomy.

In addition, perioperative need for transfusion is higher with open prostatectomy than in HoLEP. No significant difference was reported in the rate of re-operations, strictures and incontinence between the two modality. Post-op subjective evaluation by IPSS scores and peak urinary flow rate were similar between the two techniques (Kuntz, Lehrich and Ahyai, 2008; Naspro et al, 2006).

2.5.2. HoLEP versus TURP

HoLEP requires longer operative times including enucleation and morcellation compared to TURP. Perioperative complications are less and the rate of blood transfusion is lower with HoLEP compared to TURP. Serum sodium and hemoglobin levels are better maintained with HoLEP. Furthermore, duration of hospitalization and catheterization is shorter with HoLEP. Shishido et al. reported length of stay in hospital as 6.6 ± 2.3 days with HoLEP and 9.4 ± 2.2 days with TURP (Shishido et al., 2008). There is no significant difference between HoLEP and TURP in terms of postoperative acute urinary retention, urethral stricture, recatheterization, clot retention and storage symptoms (Cornu et al, 2015).

CONCLUSION

Increasing experience with HoLEP procedure worldwide has shown that thişs technique is a safe and effective option for treatment of BPH. This procedure has become the gold standard in many healthcare centers all over the world. Enucleation of prostate adenoma can be carried out using various sources of energy, although only holmium laser has the ideal combination of cutting and coagulation. The need for transfusion is eliminated with HoLEP. Hospitalization is minimal and most patients are discharged catheter free with this technique. It seems that this technique has a potential to become a routinely performed procedure in daily practice of urology community.

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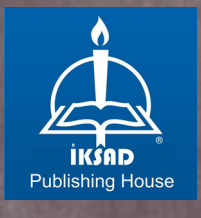

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