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The Moldovan Association for Biosafety and Biosecurity (MDBBA) is a scientific and practical, instructive and educational, non-governmental, apolitical and non-profit professional organization, created in 2017.

The main objective of the association is the development of good practices and culture in the field of biosafety and biosecurity and the promotion of knowledge within professional and research-innovation groups.

Biosafety – includes security principles, technologies and rules to be followed to prevent unintended exposure to pathogens and toxins or their accidental release/leakage.

"Protection of personnel, population from unintended exposure to pathogens/biohazardous material".

Biosecurity - includes a wide spectrum of measures (biosecurity policies, regulatory regime, scientific and technical measures) applied in an organized framework, necessary to minimize risks (prevention of actions, terrorist attacks by the intentional release of pathogens or toxins as well as loss, their theft or misuse).

"Protection and prevention of theft, intentional misuse of pathologies/biohazardous material".

Risk management – is a decision-making process in which the results of risk assessment (the process of estimating workplace hazards) are integrated with economic, technical, social and political principles to generate strategies for risk reduction.

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One Health Concept , the Future of Global Health Perspective

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When we look at the origin of all epidemics that are followed as a public health threat today, it is seen that human-environment interaction plays a major role. When epidemics such as pandemic influenza, avian influenza, Mers CoV, zikavirus, ebola, and COVID-19 in our world are examined, it is seen that they are of zoonotic origin or spread through vectors.

This situation has shown us that health policies can no longer be formed independently of the concepts of environment, climate and nature, and that only human beings cannot be the focus of health planning.

In recent years, the number of multicenter studies and researches that bring together sectors such as environment, veterinary and human health, climate has been increasing.

One Health Concept, supported by all institutions and organizations, especially the World Health Organization, will determine the basis of the measures to be taken against all possible infectious agents or very important problems such as antimicrobial resistance that may threaten humanity in the future. Collaborative networks to be established under the guidance of this approach will be an indispensable part of early warning systems. The most important responsibility of healthcare professionals and decision makers is to adapt and implement these new approach models as soon as possible.

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CLINICAL, IMMUNOLOGICAL AND GENETIC RESEARCH ON THE PARTICIPANTS IN MITIGATING THE CONSEQUENCES OF THE CHERNOBYL NUCLEAR ACCIDENT

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Keywords: *ionizing radiation, nuclear accident, chromosomal aberrations, immunological effects.*

Introduction. *The paper presents the results of research on the influence of IR sources on the health of professionally and accidentally exposed personnel. Particular emphasis is placed on: identifying the relationships between the level of radiation dose and the development of oncological diseases; use of contemporary biological dosimetry methods for retrospective analysis of irradiation doses; the impact of parental irradiation on the health of the offspring; the structure of morbidity, including oncological diseases in patients exposed to IR sources.*

Material and methods. *The study material was used by subjects professionally and accidentally exposed to IR sources: medical staff and PMCCNA born in the Republic of Moldova, Ukraine and the Russian Federation, as well as their descendants, victims of the accident at the Fukushima Daiichi nuclear power plant (Japan) and residents of the region. Chelyabinsk. Clinical, immunological and cytogenetic methods were used.*

Results. *A wide range of mental pathologies was observed at PMCCNA, at the victims of the accident at the Fukushima Daiichi nuclear power plant, etc. The structure of immune deficiency syndromes was dominated by allergic and autoimmune syndromes. Based on the analysis of stable chromosomal aberrations, exposure doses were established.*

Conclusions. *In the absence of dose information received by staff professionally/accidentally exposed to IR sources, assessment of stable chromosomal aberrations makes it possible to reconstruct the radiation dose for PMCCNA exposed to low doses of IR.*

Cuvinte cheie: *radiații ionizante, accident nuclear, aberații cromozomiale, efecte imunologice.*

CERCETĂRILE CLINICE, IMUNOLOGICE ŞI GENETICE ASUPRA PARTICIPANȚILOR LA DIMINUAREA CONSECINȚELOR ACCIDENTULUI NUCLEAR DE LA CERNOBÎL

Introducere. *În prezenta lucrare sunt descrise rezultatele cercetărilor influenței surselor de radiații ionizante asupra stării de sănătate a personalului expus profesional și accidental. În mod prioritar, în studiu se pune accent pe: identificarea relațiilor dintre nivelul dozei de iradiere și dezvoltarea maladiilor oncologice; utilizarea metodelor contemporane de dozimetrie biologică pentru analiza retrospectivă a dozelor de iradiere; impactul iradierii părinților asupra stării de sănătate a descendenților; structura morbidității, inclusiv a maladiilor oncologice la pacienții expuși la surse de RI.*

Material şi metode. *Ca material de studiu au servit subiecții expuși profesional și accidental la sursele de RI: personalul medical și PDCANC, născuți în Republica Moldova, Ucraina și Federația Rusă, cât și descendenții acestora, victimele accidentului de la centrala nucleară Fukushima Daiichi (Japonia) și locuitorii din regiunea Celiabinsk. Au fost utilizate metode clinice, imunologice și citogenetice.*

Rezultate. *O gamă largă de patologii psihice a fost observată la PDCANC, la victimele accidentului de la centrala nucleară Fukushima Daiichi etc. Structura sindroamelor de deficiență imună a fost dominată față de sindroamele alergice și autoimune. În baza analizelor aberațiilor cromozomiale stabile au fost determinate dozele de expunere.*

Concluzii. *În absența informațiilor privind doza primită de către personalul expus profesional/accidental la sursele de RI, evaluarea aberațiilor cromozomiale stabile face posibilă reconstrucția dozei de radiații pentru PDCANC expuși la doze mici de RI.*

ABBREVIATIONS: PMCCNA – participants in mitigating the consequences of the Chernobyl nuclear accident; **IR** – ionizing radiation; **CVD** – cardiovascular diseases; **MCB** – the microcirculatory bed; **CA** – coronary arteries; **AH** – arterial hypertension; **MFC** – the microvascular form of coronary artery disease; **AF** – atherosclerotic form; **CNA** – the Chernobyl nuclear accident; **DEP** – dyscirculatory encephalopathy; **FAC** – fractional anisotropy coefficient; **CG** – the control group; **AOA** – antioxidant activity; **WHO** – World Health Organization; **MN** – malignant neoplasms; **NRER** – National Registry of Epidemiology and Radiology; **CM** – congenital malformation; **OR** – the odds ratio; **NMR** – nuclear magnetic resonance; **DT-NMR** – diffusion tensor NMR; **IG** – the investigated group; **DNA** – deoxyribonucleic acid; **microRNA** – ribonucleic acid (micro – small molecules); **UNSCEAR** – United Nations Scientific Committee on the Effects of Atomic Radiation; **ICRP** – International Commission on Radiological Protection; **RHR** – relatively high risk of radiation exposure; **RSN** – radiation safety norms.

INTRODUCTION

The Chernobyl nuclear accident resulted in a significant contingent of people exposed to "low" doses (<100 mGy) of radiation, followed by the long-term development of various digestive, cardiovascular, neuropsychiatric and psychosomatic disorders. These violations subsequently led to disability and increased mortality among victims. The lack of information on the external exposure dose for many PMCCNA determined the need for retrospective dosimetry. The evaluation of the possibilities of using cytogenetic dosimetry methods to determine stochastic effects and external exposure doses in PMCCNA exposed to low doses of IR is of particular interest from a scientific point of view. A fundamental scientific interest presents the results of the clinical monitoring of the manifestation of stochastic effects, namely CVD and the particularities of their manifestation in PMCCNA who were exposed to "low" doses of ionizing radiation. An important role is to address the issues of the impact of IR on occupationally and accidentally exposed personnel, including medical personnel. It is worth highlighting the relevance of studying the stochastic effects of small doses of radiation, due to the increasing use of IR sources in various fields of scientific and practical human activity, including medicine. At the same time, scientific research is focused on the control and supervision of the health status of the PMCCNA descendants, in order to assess the immunological and genetic effects in the targeted contingent.

The aim of the paper consisted in conducting a synthesis of personal results and the respective literature of studying the influence of exposure to IR on the reaction of the human body at the individual, cellular and molecular level with the manifestation of stochastic effects.

MATERIAL AND METHODS

Subjects professionally and accidentally exposed to RI sources served as study material: medical personnel and PMCCNA born in the Republic of Moldova, Ukraine and the Russian Federation, as well as their descendants, victims of the accident at the Fukushima Daiichi nuclear power plant (Japan) and residents of the region Chelyabinsk, Russian Federation. Clinical, immunological and cytogenetic methods were used. The structure of morbidity and mortality, including oncological diseases, was studied. The immunological effects of those exposed to IR were studied by *flow cytometry* using monoclonal antibodies. Cytogenetic effects were studied by analyzing stable and unstable chromosomal aberrations, which later contributed to the reconstruction of the doses of those exposed to IR (1, 2).

RESULTS

Effects of IR on medical staff and PMCCNA

It has been shown that operating physicians working with IR sources often do not have a sufficient level of protection of the thyroid tissue against radiation. The vast majority of studies in the field of thyroid pathology in medical personnel working with X-rays are aimed at identifying the risks of thyroid cancer and do not take into account the pathology of non-tumor genesis. Based on the analysis of experimental data, it was established that under the influence of low-dose X-radiation, the intercellular contacts of thymocytes are disconnected, which can lead to a decrease in the synthesis of thyroid hormones and, as a result, to the development of hypothyroidism (3).

A wide range of mental disorders was observed in PMCCNA, in the victims of the accident at the Fukushima Daiichi nuclear power plant (Japan), etc. Of particular interest is the study of primary inci-

dence indicators of mental and behavioral disorders (F00–F09, F20–F99) for the period 2005-2018 of residents of the municipal districts of the Celiabinsk region (Kunashaksky, Krasnoarmeysky, Arga yashsky, Kaslinsky districts) exposed to radioactive contamination in years 1949-1951, and in 1957 as a result of the activities of the Mayak Production Association. These data were compared with indicators for the non-radioactively contaminated Etkulsky district, comparable in terms of population and nature of economic activity. Mental disorders were matched to the groups of the fifth class "Mental and behavioral disorders" according to the International Classification of Diseases, 10th revision (ICD-10). The calculation of morbidity cases was made per 10 thousand people or in 0/000. A descriptive analysis of statistical data was presented, which reflects the recorded primary incidence of mental disorders in the inhabitants of the mentioned districts of the Chelyabinsk region. The level of primary incidence of mental disorders in the Chelyabinsk region (49.1±3.6)0/000 significantly exceeded ($p<0.01$) the indicators in the Sverdlovsk regions (30.3±4.2)0/000, Kurgan (30.2±6.9) 0/000 and in general the average in the Russian Federation (32.9±3.8)0/000. In the remote time, the level of primary morbidity with mental disorders in a number of districts among the population living in radioactively contaminated territories was higher than among residents of the Etkulsky district, but it turned out to be significantly lower than the general indicators for the Chelyabinsk region ($p<0.001$) (4).

In order to summarize the results of a clinical examination of PMCCNA with diseases of internal organs, in the years 1996-2002, 117 participants were examined, who since June 1986, in 1987 and 1988 were engaged in various work operations in the emergency area of 30 Kilometres. The absorbed dose of external radiation varied between 50.0 and 270.0 mGy (average value being 163.06±9.54 mGy). The study demonstrated that the pathologic effect of IR consists of widespread SMC damage of variable severity, ranging from vasculopathy to the manifestation of productive vasculitis, established in right ventricular myocardium biopsy specimens. In the post-Chernobyl period, high mortality at working age was established in the mentioned cohort. Until 2002, death was caused by external factors and diseases of the circulatory system, later by diseases of the circulatory system and malignant neoplasms of the lungs (5).

Of particular interest is the analysis of the incidence and risk of cataracts caused by radiation. The results of the study demonstrated that the use of doses of different types of IR exposure on a person led to different values of cataract risk. An excess relative risk of 1 Sv suggests that cataracts caused by IR may be associated with senile cataract. Only the use of the total dose received as a result of occupational, medical, natural exposure and doses during work in the Chernobyl area will make it possible to correctly assess the risk of radiation-related cataract. For a correct analysis of the incidence and calculation of the risk of radiation-induced cataract, it is advisable to apply the diagnosis code "Radiation cataract" in ICD-10 (6).

402 PMCCNA were examined, of which 185 people were investigated for 15 years (1994-2009), undergoing primary and repeated cardiological examinations: CA angiography, endomyocardial biopsy, helical computed tomography of the CA, echographic examination (ultrasound) of the peripheral arteries , evaluation of hormonal and carbohydrate metabolism and the functional state of the cardiovascular system. The results show the predominance of AH – 70.3% and/or MFC – 58.9% among PMCCNA in the first monitoring stage (1995-1999). 10-15 years after the initial examination, the frequency of diagnosis of AH remained unchanged. The number of cases of AF of CVD has increased, including due to the development of CA atherosclerosis in patients with early-onset MFC. The severity of the clinical manifestations of CVD, their form and results depended on a number of factors: age, length of stay in the accident area, the dose of IR, and after returning – on the lifestyle and conditions of professional activity. Based on the study, the authors state that the stabilization of the pathomorphosis of AH and the prevention of the atherosclerotic evolution of MFC CVD was facilitated by the timely diagnosis of the early stages of vascular disorders and the appropriate measures of preventive interventions (7).

The clinical and epidemiological study of the ininfluence of risk factors on the development of diseases of the circulatory system and cerebrovascular diseases in PMCCNA established that the probability of developing diseases of the circula-

tory system in the nominated contingent was much higher than in non-irradiated men in the control group. The priority pathology in the structure of diseases of the circulatory system returned to cerebrovascular ones, the proportion of which in the PMCCNA of 1986 was 1.7 and 3.6 times higher, respectively, than in the PMCCNA of 1987 and 1988. It was demonstrated that the diseases of the circulatory system and cerebrovascular disease in PMCCNA developed at a younger age than in unirradiated men in the control group, 7.5 and 12.8 years earlier, respectively. The age difference between the 1986 PMCCNA for cerebrovascular disease and men in the comparison group with the same diagnosis was 13.6 years. Positive correlations of cerebrovascular disease with radiation dose and hypertension were revealed, with a negative correlation with age. The influence of the radiation factor was confirmed by the results of neuroimaging and neurophysiological studies (8).

A clinical and laboratory study was carried out on a group of PMCCNA who manifested the main forms of bronchopulmonary pathology (pneumonia – 32, chronic bronchitis – 44, bronchial asthma – 34), being irradiated with an average dose of 25 R. The results of the study were compared with similar indicators of the control group – patients identical to the patients in the main group from the point of view of nosology, sex and age. It was detected that the pathology in the investigated patients – former PMCCNA was distinguished by concomitant violations of the disease underlying the functional state of the main homeostasis systems – immunological, hormonal, antioxidant, blood coagulation, which, apparently, determined its slowing down, progressive course, regardless of the nature of nosology (9).

Descriptions of the mental effects of the influence of extreme factors of CNA, the stages of formation and clinical forms of psychosomatic radiation sickness, the characteristics of its diagnosis, treatment and prevention occupy an important role in studying the medico-biological effects of nuclear accidents (1, 10).

Cytogenetic biological assay and biological dosimetry of IR was performed at PMCCNA 27-30 years after participation in liquidation works. Stable and unstable chromosomal aberrations in peripheral blood lymphocytes were analyzed. In the analysis of unstable chromosomal disorders in 45.1% of PMCCNA, radiation markers were identified with a frequency exceeding the control group, a fact indicating the impact of IR. In 18% of those examined, using analysis of chromosomal aberrations, biological exposure doses to IR were determined (2, 11).

In order to identify the peculiarities of the long-term changes of the cerebral tract in PMCCNA with DEP, using magnetic resonance imaging, a detailed examination of 41 patients in the PMCCNA groups and 49 patients in the control group with a history of stage II DEP was performed, whose average age was 68.0±6.9 years in the PMCCNA group, and 68.6±5.8 years in the control group. All patients underwent: routine MRI, diffusion tensor MRI (TD-MRI), clinical examination to identify DEP stage, presence of diabetes and AH. The proportion of diseases leading to cerebrovascular damage, such as diabetes and AH, in the structure of diseases in both groups was comparable. According to routine MRI, all patients in both groups had severe dyscirculatory changes in the brain – multifocal white matter lesions and periventricular leukoaraiosis, mixed substitution of hydrocephalus. The results denote that, compared to the control, the PMCCNA group demonstrated a decrease in FAC in the neocortex structures of the frontal and temporal lobes of the brain. FAC in the PMCCNA group was statistically significantly reduced in four brain tracts: in the superior longitudinal fasciculus ($p<0.02$); anterior parts of the radiant crown ($p<0.02$); in the anterior limb of the internal capsule ($p<0.01$) and in the inferior longitudinal fasciculus ($p<0.01$). The authors state that the structures of the neocortex of the frontal and temporal lobes responsible for cognitive functions are the most sensitive to the impact of the factors used to eliminate the consequences of a nuclear accident. The changes in these brain structures revealed in the main group are similar to those in the elderly with DEP, however, they are more pronounced clinically, supporting the hypothesis of early brain aging in PMCCNA (12).

Previous studies have shown that in PMCCNA, brain dyscirculatory changes were observed more often than in the general population. The assessment of the brain substance status in 45 PMCCNA (IG) in the remote period and CG – 49 patients of the same age and sex, who had no history of radioactive damage demonstrated that the level of severity of DEP in the groups was similar.

High-field magnetic resonance imaging was performed on a Magnetom Verio tomograph with a magnetic field strength of 3 Tesla. Brain morphological changes in IG were more pronounced than in CG. Thus, external substitution hydrocephalus in IG was visualized statistically more often than in CG, in 84% and 65% respectively ($p<0.05$), mixed – in 51% and 16% respectively ($p<0.005$). In 14.2% of CG cases examined, the changes were absent. In both groups, lacunae of the consequences of infarcts were identified as cysts in the area of the basal ganglia, surrounded by areas of gliosis. In IG, these changes occurred with a more pronounced frequency than in CG ($p<0.05$). The results of magnetic resonance imaging of the brain help to research the evolution of DEP in PMCCNA in the long term, but also to correct treatment and rehabilitation measures (13).

The comparative analysis of the main performance indicators of the Chelyabinsk Regional Interdepartmental Council of Experts for establishing a causal relationship between diseases and radiation exposure in the periods 1990-2009 and 2010-2015 is of interest. Annual reports on activities, standard meeting protocols and decisions of the Chelyabinsk Interdepartmental Council of Experts served as sources of information. During the analyzed period, there was a natural aging of all investigated population groups, the proportion of cases considered repeatedly increased, mainly due to the cases found as a result of the disability or death of the exposed persons. In the years 2010-2015, a causal relationship was established more often than in previous periods: 43.3% and 67.1%, respectively. Most frequently, positive decisions were made in relation to people with malignant neoplasms (62.9% of the total number of positive decisions made). The association of disability and death with radiation exposure in the years 2010-2015 was detected more frequently, while the share of classified causes decreased significantly (14).

Comparison of the effect of increased levels of IR on a number of agricultural animal species and bioindicator species of small murine rodents after CNA with the results of studies of human populations after CNA and Fukushima demonstrated that there is a similarity between the main identified targets of IR in humans and agricultural animals (cardiovascular system, kidneys) (15, 16, 17).

Recently, research on the possible mechanisms associated with the threshold of damage accumulation for the induction of DNA repair systems, changes in the ratio of young and old cell populations and mitochondrial dysfunctions is gaining momentum. The concept of horizontal and vertical consequences of increasing the level of IR for biological objects was introduced. The results indicate that in different species of murine rodents (*Microtus arvalis, Clethrionomys glareolus*) and lines of laboratory mice (C57BL/6, CC57W/Mv, BALB/c) under conditions of more than 100-fold increase of IR in bone marrow cells, an increase in the frequency of only those cytogenetic abnormalities is observed, the relatively increased variability of which was typical for the studied objects and in relatively "clean" areas. The main and apparently underestimated vertical consequence of increasing IR levels is a decrease in the reproductive success of irradiated animals. The importance of the transgenerational transmission of PTSD signs revealed in recent years and its mechanisms are discussed, including the transmission of microRNA (mediator of the stress response) through spermatozoa (18), changes in the microbiota of parents and offspring, as well as cultural heritage, to explain the complexity of observed radiobiological effects and their transmission over generations (19, 20).

Domestic and international approaches to standardizing exposure doses for personnel and individuals involved in mitigating the consequences of radiation accidents are of interest. For the purpose of standardization, on the example of the real accidents at the Chernobyl and Fukushima nuclear power plants, the problems of the influence of increased exposure on long-term consequences on the health of emergency personnel were considered. Standardization of existing IR dose and applicability of international criteria are discussed (21).

Evaluation of cytogenetic parameters and markers of oxidative stress (malondialdehyde, plasma AOA) in women who lived in the territories of Russia with various levels of radioactive contamination, as well as resettled in 1991 from the areas recommended for relocation to the "clean zones" demonstrated the increased individual frequency of dicentrics and the significant decrease in the level of plasma AOA ($p<0.005$) in women residing in localities with increased radioactivity, compared to the control group and emigrants (22).

Analysis of structural damage of chromosomes in workers of the Mayak enterprise, exposed to occupational combined irradiation using the modern molecular cytogenetic method mFISH, demonstrated that the frequency of stable chromosomal aberrations in the group of workers exposed to external gamma irradiation with the total absorbed dose in the red bone marrow of over 0.2 Gy was statistically significantly higher, compared to the frequency of stable chromosomal aberrations in a group of workers exposed to radiation at a dose lower than 0.2 Gy, due to the increased frequency of translocations – biological markers of external exposure. In the group of workers with a content of 239Pu in the body greater than 1.48 kBq, the frequency of stable chromosomal aberrations was statistically significantly higher due to a significant number of cells with complex chromosomal rearrangements. Linear dependences of the frequency of translocations on the absorbed dose of external gamma radiation and the frequency of complex chromosomal rearrangements on the content of 239Pu in the body were established. The frequency of chromosomal aberrations (complex chromosomal translocations and rearrangements) detected by mFISH has been shown to be an effective biological indicator of combined exposure (23).

Some researchers studied the identification of the biological dose in a group of employees of the Ministry of Emergency Situations of the Russian Federation who participated in the work in Japan in connection with the accident at the Fukushima-1 nuclear power plant and several journalists who covered this event. The authors conclude that there is no significant overexposure of this contingent during their stay in Japan, however, it is necessary to perform a preliminary analysis of chromosomal aberrations if it is assumed that the assay of biological dose will be performed when people return from the area of possible exposure (24).

An analysis of the incidence of thyroid cancer among PMCCNA (men), residents of Russia, was performed for the observation period 1992-2010. It was shown that the contribution of exposure to 131I in the development of thyroid cancer among PMCCNA, who worked in May 1986, was 53% (25). Thus, a special emphasis is placed on protecting health and preventing diseases associated with IR (26).

In the period 1992-2019, by the collaborators of the National Registry of Radiology and Epidemiology, a study was carried out on the assessment of the risks caused by IR using the indicators: morbidity/mortality from solid malignant neoplasms, and morbidity from leukemia (excluding chronic lymphocytic leukemia) in the PMCCNA cohorts. The number of subjects in the cohort was more than 65 thousand, the average age at the time of entering the Chernobyl zone was 34 years, the average accumulated dose of external gamma irradiation of the whole body during the working period of the liquidator was approximately 0.133Gy. Results denote statistically significant radiation risks of solid cancer morbidity and solid cancer mortality that increased with increasing cohort observation period (27).

Mathematical models of IR risk forecasting and their calculation methods are currently at the stage of scientific research and have not yet been standardized for solving practical risk forecasting problems. At the international level, radiation risk models have been developed by UNSCEAR, ICRP and WHO, using epidemiological data (28).

Statistically significant radiation risks of solid malignancies, including radiation risk of breast cancer in women, were not identified for the population in the most radioisotope-contaminated territories. The radiation risk of leukemia among the population was also not revealed, although for the male population the average value of the excess radiation risk still exceeded zero. Further observations of a cohort of people who lived in territories contaminated with radioisotopes will help to increase the statistical power of epidemiological studies in the research of radiation effects and will refine estimates of human radiation risks during chronic exposure to low doses (29).

To identify the group with relatively high risk of radiation exposure (RHR), special studies were conducted on the incidence of solid malignant neoplasms in the reference cohort of PMCCNA, registered in the NRER. It is recommended that the value of the absolute/relative risk factor criterion does not exceed 10% (30).

In the period 1986-2019, an interesting study was carried out on the PMCCNA contingent with a view to the radio-epidemiological classification of the incidence of MN according to the diagnosis, using statistical relationships between the incidence and the radiation dose. Cancer incidence

data on PMCCNA men who were in the exposure area during 1986-1987, accumulating an average total external gamma radiation dose of 0.128 Gy, were analyzed. The total number of cases was 9542. The average age at diagnosis was 57.4 years. The data were accumulated in the NRER. The authors conclude that in order to clarify the cause-effect relationship between the incidence of malignant neoplasms and radiation dose, further assessment of radiation risks using more precise methods of epidemiologic study of stochastic effects is needed (31).

In order to study the possible impact of the IR on the life expectancy of PMCCNA, the mortality monitoring of people enrolled in the NRER was carried out. The analysis of the obtained results demonstrated that when studying the impact of exposure on changes in life expectancy, it is necessary to consider the contribution of each of the main causes of death, even if it was not related to the exposure. In PMCCNA who received a radiation dose of 150 mGy or more, there was a radiation-related decrease in life expectancy due to mortality from solid malignancies. During 27 years of follow-up, the reduction in life expectancy was 0.3 years per unit dose (32).

A criterion was developed, demonstrating the existence of radiation risk, determined from cohort observational data under a linear multiplicative hazard model (in terms of radiation dose). Based on the calculation, according to the PMCCNA cohort data observed in the NRER from 1986 to 2019, a list of diseases was developed, the mortality from which is statistically associated with the radiation dose. The criterion developed for the excess of average doses for deaths over doses averaged with weighted observation times over the entire cohort was a sufficient condition for the existence of radiation risk in a linear multiplicative model. The use of the developed threshold criterion made it possible to formulate two new hypotheses about patterns of radiation risks of human mortality. (i) For the radiation risk of mortality from solid malignancies, the existence of two time phases for the implementation of this stochastic effect (up to 9 years and beyond 24 years after exposure) was demonstrated for the first time. Further testing of the statistical significance of the observed dependence on risk is required – the time. (ii) For the first time, the existence of an irradiation risk for mortality from diseases of the digestive system and the presence of a latent period for this risk of approximately 9 years was demonstrated (33).

The calculation of the damage caused by radiation was carried out for the population currently living (year 2020) on the territories of the Russian Federation contaminated with ^{137}Cs after the Chernobyl accident in 1986. The damage caused by radiation was calculated in two ways: according to the original ICRP methodology and by the method of using as the product of a nominal risk coefficient RSN-99/2009 per effective dose (nominal radiation damage). For ICRP calculations, equivalent doses were estimated using dose factors from the US Environmental Protection Agency. The number of the studied population from the contaminated territories at the beginning of 2020 was 142,676 people (65,205 men and 77,471 women). Basically, this constituted the population of Bryansk and Tula regions: 85.5%, and 10% of the total number, respectively. The mean cumulative effective dose for the population was 30.6 mSv, and the maximum individual cumulative dose was 707 mSv. In 2020, for 44-year-old men and 55-year-old women, the nominal radiation damage was approximately equal to the amount of radiation damage calculated using the ICRP methodology. At the same time, the nominal damage was significantly (up to 2.3 times) underestimated for the young and overestimated for the older ages. In 2020, the critical population groups with the maximum accumulated doses and maximum radiation damage were men aged 34 and women aged 35. For these population groups, the average cumulative effective doses were 35.3 and 39.2 mSv, and the average radiation damage was 2.6×10-3 and 4.2×10-3 respectively for men and women. For 11.8% of the population (8.3% men, 14.8% women), individual radiation damage calculated using the ICRP methodology exceeded the value of 3.5×10-3, which corresponds to the RSN-99/2009 for normal conditions of exposure. The maximum radiation damage of 3.9×10-2 was found for a 37-year-old woman from Krasnogorsk district of Bryansk region with an effective accumulated dose of 392 mSv. The results of the study in question can be used in the preparation of recommendations for health authorities to improve the medical monitoring of citizens living in territories contaminated with radionuclides, as well as in the development of regulatory documents for the provision of targeted medical care to people from groups at increased risk of radiation exposure that use personalized medicine methods (34).

Current studies are focused on the comparative analysis of modern risk assessment models for exposure to IR, developed by UNSCEAR, ICRP and WHO (35).

In the period 1992-2017, based on the NRER, the assessment of IR risks of mortality from solid cancers was carried out in a PMCCNA cohort in 1986-1987 (36).

Using the method of statistical relationships, work is being done on the radio-epidemiological classification of the causes of death by malignant neoplasms of the population most contaminated with radionuclides due to CNA in Bryansk, Kaluga, Tula and Oryol regions. Data on mortality from malignant neoplasms for the observation period 1993-2017 were used. The number of men who died during the observation period was 30,771 people (5,407 deaths from cancer), women – 29,033 people (3,472 deaths from cancer). The results show that for the population of the four regions, being the most polluted as a result of CNA, no statistically significant relationships were found between the causes of death by malignant neoplasms and the radiation dose both for the entire class of malignant neoplasms and for the ICD-10 rubrics of three digits in this class. Statistically significant relationships with radiation dose were observed for certain diagnoses of the causes of death: among the male population – for gastric cancer of unspecified location (C16.9) and for pancreatic cancer, unspecified (C25.9); in the female population – for stomach cancer (C16.9) and for lung cancer of unspecified locations (C34.9). These diagnoses of the causes of death should first of all be subject to a more sensitive and specific radio-epidemiological analysis, taking into account possible risk factors. The presented methodology defines the priority areas of research for a more precise radio-epidemiological analysis of dose-effect relationships among the population living in areas contaminated with radionuclides as a result of CNA. The methodology and the results of its use can be useful in the work of expert councils to establish a causal relationship between a disease (death) and the impact of man-made factors (37).

Research evaluating median survival time of the subjects after diagnosis of solid cancers in a cohort of PMCCNA exposed to IR is of interest. Individual medical and dosimetric information of male subjects for the observation period from 1991 to 2015 was analyzed. The cohort size in 1991 was 142,871 individuals. These were the participants, who worked in the area in 1986-1987. The number of solid cancers with documented stages of the disease was 7,652 cases, of which deaths constituted 5,085 (4,351 people died from cancer). The internal classification of cancer prevalence stages was used in the research. The median survival time after diagnosis of solid cancers (ICD-10: C00-C80.9) with documented tumor prevalence stages for the period 2000-2015 was 4.73 years; for malignant neoplasms of respiratory organs (ICD-10: C30-C39.9) – 2.57 years and for malignant neoplasms of digestive organs (ICD-10: C15-C26.9) – 3.55 years. The median survival time during the observation period was continuously increasing, despite the aging of the cohort and the increase in overall mortality. The dependence of the median survival time on the stage of tumor development was studied. In the case of solid cancers, the survival time when moving from stage 1 to stage 4 decreased 7 times (from 8.62 to 1.22 years). The dependence of the median survival time in cases of solid cancers on the radiation dose was studied. Two dose groups were considered: ≤100 mGy (average dose 56.6 mGy) and >100 mGy (average dose 187.9 mGy). The median survival time was 4.66 and 4.72 years, respectively, and their difference was not statistically significant. Studies conducted in a cohort with a changing size and age structure were relevant in the context of assessing the consequences of the impact of man-made factors on the health of individual populations and groups of people (38).

Effects of IR on children whose parents were irradiated

The review of data from the literature on the issue of hereditary effects in offspring due to parental contact with mutagenic risk factors highlighted various agents that cause adverse effects on the hereditary apparatus, including chemical, infectious, physical and biological (39).

The CM analysis in the offspring of Mayak Production Association employees who accumulated preconception doses of external gamma radiation is of a fundamental scientific interest. A retrospective study was conducted on 1190 children born in 1949-1969, of which 238 were descendants of workers in an enterprise with a high risk of radiation exposure. Maximum comparability of

groups formed from the infant population of Ozyorsk was achieved by selection by gender, year of birth and age of parents at birth. CM frequency comparison was performed using the χ^2 test, Fisher's exact test. The OR was calculated with a 95% confidence interval. To identify the latent factors, factor analysis was applied, using the method of principal components, followed by the normalized Varimax rotation. The range of external gamma irradiation doses to the gonads of workers at the Mayak Production Association was 0.09-3523.7 mGy; the average cumulative dose was 373.6±34.2 mGy. In the group of offspring of irradiated mothers, every tenth child was born to a mother who had a cumulative preconception dose of external gamma irradiation to the gonads (ovaries, sex glands) of more than 1 Gy. The comparative analysis did not reveal statistically significant differences between the groups regarding the incidence of CM, including the nosological forms. The OR indicator as a whole was 0.86 (0.46-1.59), in boys it was 0.88 (0.35-2.2), and in girls – 0.84 (0.36-1.94). Differences in the CM structure were noted: CM of the nervous system, respiratory organs (23% each) and CM of the musculoskeletal system (15.3% of all defects) predominated among the offspring of irradiated mothers; among children of intact parents, CM of the musculoskeletal system (23.3%) and nervous system (21.7%) were predominant. Gender differences in CM structure were observed in the compared groups. Chromosomal pathology was not recorded in both groups. In the cohort of offspring of exposed mothers, CMs were diagnosed in children whose mothers accumulated preconception doses of external gamma irradiation to the gonads in the range of 1.9–1635.5 mGy, with an average dose of 307.5 mGy. The factor analysis in the group of offspring of workers in the nuclear risk area revealed four factors that characterize the mothers' pre-conception exposure (21.5% of the variance), the antenatal period of the offspring (17.1% of the variance), the obstetric and gynecological antecedents (12.9% of the variance) and mothers' bad habits – alcohol consumption and smoking (11.0% of the variance). Considering the identified characteristics, it is necessary to continue the study with the expansion of the group of descendants and the period of their observation (40).

Scientific studies demonstrate that during intrauterine development, the developing organism is particularly sensitive to adverse effects. Radiation-mediated immunological disturbances play an important role in shaping not only the early but also the long-term effects of exposure. The structure of immune deficiency syndromes was dominated by allergic and autoimmune syndromes. Immune status has been studied most fully in individuals who lived in the Techa River Basin and were subjected to chronic radiation exposure as a result of liquid radioactive waste spills from the Mayak Production Association. During the long-term follow-up period, various disorders of innate and adaptive immunity were recorded in this group of individuals. It is of interest to evaluate the composition of the lymphocyte subpopulation in individuals exposed to intrauterine irradiation in the long-term post-irradiation period, using flow cytometry. In the study, the main subpopulations of lymphocytes were analyzed in 56 subjects: 29 being exposed to intrauterine irradiation (23 women and 6 men) and 27 from the control group (25 women, 2 men). The age of the people examined varied from 52 to 72 years. Results show that there were no deviations in the absolute and relative content of lymphocyte subpopulations. A tendency to decrease the number of lymphocytes expressing the early activation marker CD25 was established as a function of the increase in the total dose of external γ-irradiation of the mother during pregnancy. The data obtained on the composition of the lymphocyte subpopulation were consistent with the results of a survey of people who lived in the Techa River basin and were subjected to intrauterine exposure as a result of liquid radioactive waste spills from the Mayak Production Association, during which no changes were also detected in the composition of the lymphocyte subpopulation in prenatally irradiated individuals. The tendency to decrease the relative number of lymphocytes expressing the early activation marker CD25 in individuals exposed to intrauterine irradiation as a function of the increase in the total dose of external γ radiation absorbed in the bone marrow during maternal pregnancy was consistent with the results of experimental studies. Thus, the authors note that the study of people exposed to intrauterine irradiation did not detect abnormalities in the main lymphocyte subpopulations. A tendency to decrease the relative number of lymphocytes expressing the early activation marker CD25 in individuals exposed to intrauterine irradiation was observed as a function of the increase in the total dose of external γ radiation absorbed in the

bone marrow during the mother's pregnancy (41).

The author's data on the genetic consequences on the population of the reproduction of various mammal species under conditions of high levels of ionizing radiation following the accident at the Chernobyl and Fukushima nuclear power plants were also taken into account. Such conditions contribute to the genomic instability of the parental population, which was directly exposed to increased doses of ionizing radiation, and to the basic reproduction of offspring with a relatively increased stability of the chromosomal apparatus. It is assumed that the use of the relative reproductive "success" of carriers of increased genomic stability under conditions of environmental change as an integral indicator of resistance to the selective action of environmental stressors can be used to identify radioresistant individuals.

The importance of transgenerational transmission of PTSD features and its mechanisms, including microRNA transmission through sperm, changes in parental and offspring microbiota, and cultural inheritance, are discussed to explain the complexity of observed radiobiological effects and their transmission across generations. Man-made accidents (such as Chernobyl and Fukushima) and more frequent economic crises pose the risk of biosocial consequences in the fabric of society, based on the legacy of epigenetic, cultural and micro/macrobiotic changes (42).

The assessment of the effect of ionizing radiation on the offspring of exposed parents is one of the most discussed problems in the epidemiology and radiobiology of radiation effects research. In the last three decades, the main focus of research on the radiological consequences on the descendants of people exposed to radiation as a result of the Chernobyl accident has focused on the studies of the possible relationship between the development of congenital malformations and their dependence on the radiation dose received by their fathers – liquidators of the consequences the Chernobyl nuclear accident. The study of 11,698 liquidating fathers and 15,450 of their children did not detect an effect of fathers' exposure. No statistically significant risks of radiation morbidity were found for most disease classes and headings, including the classes "All neoplasms" and "Congenital anomalies (malformations), deformities and chromosomal disorders". It should be noted that the average external gamma irradiation dose of the fathers was 130 mGy. Three methods of statistical analysis were used in the study: descriptive analysis in dose comparison groups, a method for determining statistical relationships based on data mining, and morbidity modeling (43).

Research conducted in Japan after the Fukushima nuclear accident detected a significant association between effective external dose rate and thyroid cancer detection rate in youth and adolescents living in Fukushima prefecture: the detection rate ratio per μSv/h was 1.065 (44), the same trend being observed in studies performed on PMCCNA children (45).

The epidemiological study of the incidence of thyroid cancer in the Russian Federation after the CNA established that only the population of children in the territories of the country contaminated with radionuclides, which received a thyroid dose of more than 100-150 mGy due to the incorporated exposure to 131I, should be included in the radiation risk group. Taking into account data from Chernobyl and the recommendations of the International Commission on Radiological Protection, predictive estimates of the occurrence of an increased incidence of thyroid cancer in the Japanese population living near the Fukushima-1 nuclear power plant were made. The authors conclude that large-scale studies conducted and included in the National Epidemiological Radiation Registry found that only children who received radiation doses greater than 100-150 mGy due to incorporated irradiation of the thyroid gland with the radionuclide 131I should be included in the group at high risk of radiation exposure for additional induction of thyroid cancer. Taking into account post-Chernobyl epidemiological data and accepted international standards, a forecast was made for a possible additional incidence of thyroid cancer among the Japanese population living near the Fukushima-1 nuclear power plant, depending on the radiation doses received (20).

DISCUSSIONS

The mechanisms of action of high doses on the human body were elucidated by studying the acute effects. To detect the action of small doses, a series of researches were carried out on PMCCNA, victims of the accident at the Fukushima Daiichi

nuclear power plant (Japan), residents of the Chelyabinsk region, etc. and important fundamental results were achieved. Thus, it was shown that the mental morbidity of the population in areas that were exposed to accidental radioactive contamination more than 70 years ago now reflects the general trend of deterioration of the mental health of the population of the Chelyabinsk region. It can be assumed that the incidence of mental disorders in the population of the Chelyabinsk region is determined by a complex set of interacting factors (socio-economic, individual-psychological, radiation, informational, etc.), among which the most important are from a social point of view, determining the mechanisms of formation of mental pathology.

As a result of occupational exposure of the thyroid gland to low-dose IR sources, under the influence of X-rays, the intercellular contacts of thymocytes are disconnected, which can lead to a decrease in the synthesis of thyroid hormones and, as a result, to the development of hypothyroidism – the pathology of non-tumor genesis. An adequate approach to the problem in question is missing in the literature.

During the post-Chernobyl period in the targeted cohort, a large number of deaths at working age were established, determining that until 2002, mortality was caused by external factors and diseases of the circulatory system, later by diseases of circulatory system and malignant neoplasms of the lungs. It can be assumed that damage to MCB vessels of the brain and heart has important targets of the harmful effect of "low" doses of IR. Their changes in the remote period from the accident exacerbate (intensify) the psychosomatic maladjustment of these patients and can explain the high mortality from various external causes and diseases of the cardiovascular system (5).

The problem of hereditary effects in offspring, due to parental contact with mutagenic risk factors, highlighted those of a chemical, infectious, physical and biological nature. At the same time, the influence of smoking and parents' age on the occurrence of *de novo* mutations is essential. The role of the radiological factor in the genesis of hereditary disorders in the offspring of parents exposed to IR has been demonstrated. The stages of the development of radiation genetics, the evolution of ideas about IR damage by presenting the results of experimental, cytogenetic, molecular genetic and epidemiological studies, analyzing the contribution of parental exposure to inherited pathology in offspring were described. Attention is focused on the "non-target" effects of radiation, with evidence being presented about the possibility of transgenerational transmission of genome instability. The special contribution of the studies of the descendants cohort of the victims of the atomic bombings of Hiroshima and Nagasaki should be noted, being considered the main scientific platform for radiation risk assessment. Hereditary effects are known in the descendants of people who suffered as a result of radiotherapeutic exposure, that is, had professional contact with IR, or were exposed to radiation as a result of CNA, nuclear weapons tests at the Semipalatinsk test site, chronic exposure on the territory of the radioactively contaminated Techa River and areas with increased natural radioactivity. Despite numerous confirmations of radiation-induced effects in offspring through experimental and genetic-molecular studies, the results of epidemiological research remain contradictory. We can consider possible reasons for explaining these discrepancies: the evolution of views on legacy effects in the international radiation safety system; the ICRP new approach to hereditary risks; the dynamics of the weighting factor for gonads in the evaluation of the effective dose; methods of evaluating hereditary effects (direct method and double dose method). In order to reduce the uncertainties in the modern assessment of radiation genetic damage, further study of radiation-induced heritable effects is probably necessary. It is worth highlighting the possibility of analyzing the inherited effects on the example of a cohort of descendants of the workers of the production association "Mayak" – the first enterprise in the nuclear industry of the Russian Federation (39).

The study of radiation-induced effects in children of persons exposed to radiation at work is important for the standardization of man-made exposure doses of persons of reproductive age and for predicting the adverse effects of parental exposure in offspring.

The current environmental situation imposes the priority of the protection of the genome and the human body as a whole over the action of IR. Primary disease prevention is a priority component of human health protection. In some localities, the primary prevention of radiogenic cancer in the post-Chernobyl period was insufficiently carried characterized by frag out, and certain stages of its

development are mentation, the lack of a scientific basis, etc. The global distribution of radiation sources, modern ideas about the aetiology and pathogenesis of radiogenic cancer indicate the dominant carcinogenic risk of low doses of radiation. The scientific and practical aspects of the primary prevention of radiogenic tumors are of interest. This reduces the carcinogenic risk in people with increased individual radiosensitivity. Such a strategy should be carried out in parallel with general preventive measures aimed at reducing the incidence of cancer (46).

The lack of information on external exposure dose for many PMCCNA necessitated retrospective dosimetry. Evaluation of the possibilities of using cytogenetic dosimetry methods to determine external exposure doses for PMCCNA exposed to low doses of IR in a remote period of time is of particular interest from a scientific point of view. Biological cytogenetic indication and biological dosimetry of ionizing radiation were performed at PMCCNA 27-30 years after participation in liquidation works. Stable and unstable chromosomal aberrations in peripheral blood lymphocytes were analyzed. In the analysis of unstable chromosomal rearrangements in about 50% of PMCCNA, radiation markers were identified with a frequency exceeding those of the control group, which indicates the impact of IR. In the absence of information on the received dose of external exposure, the assessment of stable chromosomal aberrations makes it possible to reconstruct the radiation dose for PMCCNA exposed to low doses of ionizing radiation, which can be the basis for solving the problem of compensation for the damage caused to their health (2, 11).

Some researchers say that the structures of the neocortex of the frontal and temporal lobes, responsible for cognitive functions, are the most sensitive to the harmful factor of a nuclear accident. Changes in these brain structures revealed in PMCCNA were similar to those in the elderly with DEP, however, they are more pronounced clinically, supporting the hypothesis of early brain aging in PMCCNA (12).

CONCLUSIONS

1. The studies carried out on PMCCNA originating from the Republic of Moldova, Ukraine, Belarus, the Russian Federation and the Baltic countries; victims of the accident at the Fukushima Daiichi nuclear power plant (Japan), residents of the Chelyabinsk region, etc. highlight the harmful impact of ionizing radiation on the health of the exposed, manifested by clinical, genetic and immunological effects.
2. Important fundamental results were obtained in establishing the mechanisms of action of small doses of ionizing radiation, with the identification of biological markers of the radiation factor.
3. The analysis of the results of studies of clinical, immunological and genetic effects in those exposed to occupational/accidental ionizing radiation and their descendants, highlight significant fundamental results and emphasize the importance of investigations aimed at elucidating some markers of the radiological factor and the priority of biological dosimetry in ascertaining exposure doses.
4. The study of ionizing radiation-induced effects in children of persons exposed at work or accidentally is important for the standardization of man-made exposure doses for persons of reproductive age, as well as for predicting the adverse effects of parental exposure in offspring.
5. The lack of information on external exposure dose for many PMCCNA necessitated retrospective dosimetry. Evaluation of the possibilities of using cytogenetic dosimetry methods for determining external exposure doses for PMCCNA exposed to low doses of ionizing radiation in a distant period of time is of particular interest from a scientific point of view.
6. Considering the importance of the problem and the possibility of new radiation accidents, studies in this field urgently need to be continued.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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ETHICAL APPROVAL

Approval from the Research Ethics Committee no. 87 of 27.06.2017, *Nicolae Testemiţanu* State University of Medicine and Pharmacy, of the research topic, the project protocol, the informed consent.

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THE NEED TO IMPLEMENT *ONE HEALTH* APPROACH IN CONTROLLING VECTOR-BORNE DISEASES IN NIGERIA

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Keywords: *One Health, vector-borne diseases, public, Nigeria, human.*

Introduction. *The primary goal of this article is to raise attentiveness about the critical need for vector-borne disease control in Nigeria in relation to One Health, as well as to examine existing understanding on this subject matter, which is quickly befitting as a valuable resource for public health policymakers and specialists across the country. Globally, there has been an increase in the number of vectors, which has resulted in an increase in vector-borne diseases. Thousands of individuals die every year as a consequence of vector-borne diseases, and there is an urgent need to manage these vectors.*

Material and methods. *The present research used PubMed, ResearchGate, WHO and other online databases with the following keywords "Climate change in Nigeria", "Public health in Nigeria", "Vector-borne Diseases", "Nigeria population" and "One Health in Nigeria".*

Results. *We observed that there has not been an implementation of the One Health approach against Vector-borne diseases in Nigeria.*

Conclusions. *The One Health strategy has the potential to address this issue. One Health is the concept that the health of human is intertwined with that of animals and our collective environment. Malaria is a vector-borne disease that is one of Nigeria's biggest health issues. However, land use changes such as deforestation, mining, and other activities have increased in Nigeria, while climate changes have increased internationally.*

Cuvinte cheie: *One Health, boli transmise prin vectori, public, Nigeria, om.*

NECESITATEA DE IMPLEMENTAREA O ABORDĂRII *ONE HEALTH* ÎN CONTROLUL BOLILOR TRANSMISE DE VECTORI ÎN NIGERIA

Introducere. *One Health este conceptul potrivit căruia sănătatea umană este interconectată cu cea a animalelor și a mediului. Scopul principal al acestui articol este de a spori atenția cu privire la nevoia critică de control al bolilor transmise de vectori în Nigeria, în legătură cu abordarea One Health, precum și de a examina înțelegerea acestui subiect, care trebuie să devină, în scurt timp, o resursă valoroasă pentru factorii de decizie din domeniul sănătății publice și a specialiștilor din întreaga țară. La nivel global, creșterea numărului de vectori, a favorizat o creștere a bolilor transmise de aceștia. Mii de persoane mor în fiecare an ca urmare a bolilor transmise de vectori și este imperios de a gestiona acești vectori.*

Material si metode. *Pentru realizarea studiului au fost explorate bazele de date online PubMed, ResearchGate, OMS, aplicând cuvintele cheie: „schimbarea climatică în Nigeria", „sănătate publică în Nigeria", „boli transmise prin vectori", „populația din Nigeria" și „One Health in Nigeria".*

Rezultate. *S-a observat inexistența implementării abordării One Health împotriva bolilor transmise de vectori în Nigeria, strategia One Health având potențialul de a aborda problema în cauză.*

Concluzii. *Malaria este o boală transmisă de vectori, care este una dintre cele mai mari probleme de sănătate din Nigeria. Cu toate acestea, în timp ce schimbările climatice au crescut la nivel internațional, schimbările în utilizarea terenurilor, cum ar fi defrișările, mineritul și alte activități au crescut și în Nigeria.*

INTRODUCTION

Malaria, arboviruses, Lyme diseases, leishmaniasis, and other vector-borne diseases (VBDs) have become a key public health distress around the world. This is because of rapid climate change, as well as further non-climatic elements such as expansion, land-use change, habitat invasion, and human relocation, which partake in the increment of the dispersal of intrusive vector species and the development of new disease-causing organisms in the human populace (1, 2), altering the geographical distribution of vectors, and amplifying the spread of diseases. VBDs are thought to account for more than 17% of the universal disease burden, with more than 700,000 deaths detailed each year around the world (3).

The World Health Organization (WHO), the World Organization for Animal Health (OIE), and the United Nations Food and Agriculture Organization (FAO) launched the "One World, One Health" initiative in 2008, with the expression "One Health" being proposed as a notion to validate the close-knit of animal, man and environmental health (4). It now embraces the link between man, animal, and environmental wellbeing in a multidisciplinary methodology denoted by a multifaceted biotic and societal structure that comprises numerous actors and procedures, as well as their connections over time at the native, nationwide, and international levels (5).

Population health and quality of life are both dependent on an ecologically balanced environment. Great contingents are susceptible to endemic communicable and parasitic infections because of an absence of basic hygiene combined with rain, overflows, municipal garbage, and a high population concentration (6).

In most developed countries, implementing One Health has been simple because all twelve pillars of the global health security agenda are present; however, in developing countries (such as Nigeria) and low-resource settings, achieving the goals of One Health is a major challenge, as there is little or no knowledge about zoonoses due to limiting factors such as a lack of trained medical professionals and surveillance systems (7).

It is essential for Nigeria's human and commercial growth to adopt universal public health priorities. In Nigeria, the Federal Ministry of Health, formed in 1954, is responsible of the entirety of health-related matters (8).

The purpose of this paper is to raise consciousness on the need of One Health implementation.

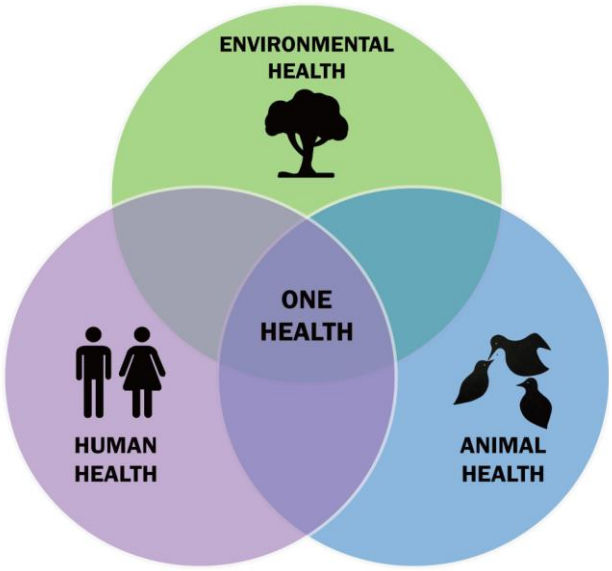

Figure 1. One Health Triad (9).

MATERIAL AND METHODS

For this paper, a literature search to identify articles reporting One Health Initiative in Nigeria was conducted.

Period

The present study reviewed the relevant literature between the year 2008 and 2022 (with only one exception). 2008, was the year that the World Health Organization in conjunction with other organizations launched the “One World, One Health” initiative. This paper was written in 2022.

The search strategy and selection criteria

Relevant publications, 38 in total, were searched in electronic databases including PubMed, ResearchGate, WHO, CDC, Semantics scholar and other online databases (table 1). The following keywords were used “Climate change in Nigeria”, “Public health in Nigeria”, “Vector-borne Diseases”, “Nigeria population” and “One Health in Nigeria”.

RESULTS

Out of the 38 publications reviewed, only 13 pertain to the One Health approach in Nigeria. Out of the 13, only two directly mention the need to implement One Health approach in Nigeria, while

only one stated that action has been taken on the subject matter (tab. 2).

A nationwide One Health strategic plan was launched in Nigeria, integrating the security of human, animal, and environmental health. The Federal Ministry of Health, the Federal Ministry of Agriculture and Rural Development, and the Federal Ministry of Environment collaboratively prepared the strategy, which was issued as a One Health strategic plan. It affirms Nigeria's resolve to intensify cross-sectoral cooperation for health security (10).

According to research made in 2019 by Ogunsola and Yaya, climate change in Nigeria is causing temperatures to rise. The degree of temperature change or volatility affects the spread of vector-borne diseases, such as malaria. The temperatures that support the development of malaria parasites are at their optimum and greatest levels (11, 12, 13). If there are no effective initiatives created to address these disorders, Nigerians will continue to be exposed needlessly to these diseases that otherwise can be prevented.

The World Health Statistics for 2011 show that Nigeria has a malaria fatality rate of 146 per 100,000 people. It also revealed that there were more recorded cases of malaria in 2009 than there were in 2008 (2,834,174 cases vs. 4,295,689 cases) (14). The implementation of One Health will significantly slow the spread of diseases that are transmitted by vectors.

Nigeria's lack of health infrastructure means that fighting the threat of communicable illnesses will be challenging (15). According to the 2000 world ranking, Nigeria is placed as 187 out of 191 countries evaluated, the Nigerian health sector being in a poor condition (16). Therefore, One Health is required in order to address the issue of communicable illnesses in Nigeria as the CDC was not established to do so on its own.

Isere EE, et al. (2015) go into great length about the importance of disease surveillance in Nigeria. Ifeanyi M. Nsofor (2022) reaffirms Nigeria's demand for One Health.

Table 1. Online Databases Used For the Study.

Databases	Number of papers reviewed
PubMed	9
ResearchGate	4
WHO	4
CDC	2
NCDC	1
Semantics Scholar	10
Others	8
Total	**38**

Table 2. The trend of One Health in Nigeria.

Year of publication	Action taken	References
2008 - 2018	None	(8), (17), (19), (21), (22), (23), (26), (27), (28), (33)
2019	Launched	(10)
2021	Reiteration	(7)
2022	Reiteration	(18)

DISCUSSIONS

With a populace of around 191,000,000 people and a gross domestic product of $375.75 billion in 2017, Nigeria is Africa's most populous country (19). With an unemployment rate of 13.4%, Nigeria is classified as a low and middle income nation. Nigeria's population is growing at a rate of 2.43 percent, with a birth rate of 36.9 per 1,000 people (20).

The temperature rising, variable rainfall, increasing sea levels and overflowing, drought and desertification, land dreadful conditions, more common excessive weather occasions, impacted

fresh water resources, and damaged biodiversity are all signs that Nigeria's climate is altering (21, 22, 23), which could lead to an increase in disease vectors.

Ecosystem change encompasses climatic change, ecological change, and their interrelationships, and is thought to be linked to a slew of new diseases (24). The link between healthiness, farming, and ecological units give the "One health" concept a wide opening as illustrated in Figure 1 above.

The ecosystems in and nearby the inter-tropical convergence region, specifically, are thought to be the most vulnerable to climate change. They are thought to be linked to the emergence of a number of new diseases, particularly vector-borne diseases. Though there is an indication that climate change is aggregating the prevalence of vector-borne diseases and may add to the pathogens' virulence, there have been few researches in Nigeria on the association between vector-borne diseases and climate change (25).

Balthazard-Accou K. et all (2021) conducted a study that found evidence of climate change in the Southwestern part of Nigeria by analyzing temperature and rainfall trends in the area.

According to Ogunsola OE (2019), global warming, which is caused by an increase in temperature as a result of climatic changes, may have an impact on many ecological systems in this part of Nigeria. Many communicable diseases, including vector-borne diseases carried by blood-feeding arthropods like Zika, Dengue fever, Malaria, and Chikungunya, are caused by climate change.

Changes in land-use and cover reflect decisions made in natural resource strategy and development. It has real-world ramifications for non-urban livelihoods, environmental health, and the ecosystems and biodiversity it sustains (28).

Universally, zoonotic illnesses are a strain on poor countries' healthcare structures, such as Nigeria's. According to the US Centers for Disease Control and Prevention, zoonoses account for 60% of infectious diseases and 75% of new or emerging infectious diseases (29). The transmission of zoonotic illnesses could be aided by highly infectious human-human encounters (30).

In developing countries like Nigeria, the human-animal-environment interaction of disease spread poses a significant threat to the popular tion's welfare, leading to an increase in the appearance of illnesses that are difficult to treat (31).

This strategy aims to control global public health challenges that are involvedly interrelated and inter-reliant on the expanding human populace, their activities on the environs, and their interactions with faunae. According to Zinsstaga J. (2011), the growing human population has resulted in fast urbanization, increased livestock output, globalization, and intensive environmental exploitation.

Malaria remains one of Nigeria's foremost source of death. It is accountable for virtually 25% of ages below five mortality, 30% of juvenile mortality, and 11% of maternal death. Insufficient plans to address Nigeria's numerous health concerns have ensued in slight change in our health standing (33). Only a good disease monitoring system, in which suspected and confirmed arbovirus infections are frequently reported to the health authority, allows for a quick response.

VBDs exacerbate the poverty cycle by restricting efficiency and the capability to generate foodstuff or earn enough money to buy food or medicinal care. VBDs are well suited to the 'one health' paradigm for addressing communicable infections because of the effect of human undertakings on disease occurrence and the unswerving and incidental impact on human health and livelihoods. Foremost risk reasons for geographical advancement to fresh places include amplified human movement, population development, occupation, and climate change. Sub-Saharan Africa, which bears a large portion of the global disease burden, has a population growth percentage of roughly 2.6% per year, which is greater than the current global average of 1.2 percent (34).

While One Health is supposedly – and possibly economically – appealing, it will require substantial political drive and state capability to overwhelm prevailing institutional and financial obstacles to its execution, especially in developing nations where multiple health and expansion significances contend for consideration and programmatic funding. If One Health is to be seen as something other than an "effort to grab funding on the tail-end of the avian influenza bonanza" (35), current examples must be identified and critically analyzed.

Disease surveillance is the ongoing monitoring of (36). the manifestation of diseases and health-concerned happenings in order to provide timely action for disease management. VBD surveillance consists of observing the infection, the disease-causing organisms, the disease-transmitters, and environment (including climate) in order for the established order to make well-versed judgments about whether or not to interfere, depending on the circumstances (36).

Surveillance systems, in general, deliver actionable information via a feedback mechanism that incorporates observing and involvement operations. Surveillance feedback systems are widely used in the medical field. European countries, for instance, are receiving signals that the measles rate is rising while vaccine coverage is declining (37).

However, the public can be rescued from the threat of these prevalent diseases if adequate preventative and curb methods can be introduced by stakeholders appertained to, as has been done in the US (38) and as described in this paper.

ADVANTAGES of an effectively implemented One Health approach for zoonotic diseases.

1. Quicker and more effective response to zoonotic disease outbreaks and emergencies.
2. All divisions have access to the data they require.
3. Decisions are built on precise and pooled scenario evaluations.
4. Accountability to one another and to decision-makers guarantees that all sectors take action.
5. All sectors may agree on, approve, and implement regulations, policies, and recommendations.
6. Everyone in the partnership is aware of their individual roles and responsibilities.
7. Technical, human, and financial resources are employed efficiently and fairly.
8. Infrastructure, capacity, and data gaps are identified and addressed.
9. Funding, policy, and program advocacy is more effective (4).

CONCLUSIONS

1. Disease surveillance, educated medical professionals, government funding for research, policy-making, and extension initiatives are all critical needs in the country.
2. In order to save lives in Nigeria, entomologists, epidemiologists, public health workers, veterinary and medical doctors, and other health care experts need to work together on VBDs.
3. As this review article reveals, there is still a need for more research.

CONFLICT OF INTERESTS

None.

ETHICAL APPROVAL

Not applicable.

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Not applicable.

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THE SPECTRUM OF LIVER PRESENTATION IN WILSON'S DISEASE: A LITERATURE REVIEW

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Keywords: *Wilson disease, liver presentation, acute liver failure, cirrhosis, acute Wilsonian hepatitis, chronic liver disease.*

Introduction. *Wilson's disease represents one of the genetic diseases that has lifelong treatment, which significantly improved the quality of life for patients and reduced the disabling complications associated with the lack of an early diagnosis.*

Material and methods. *A structured search was performed in PubMed and HINARI, using English search terms: "Wilson's disease", "acute liver failure", "cirrhosis", "acute Wilsonian hepatitis", "hepatic manifestation", "chronic liver disease", "asymptomatic Wilson's disease", and "active chronic hepatitis".*

Results. *Wilson's disease can occur at any age and can mimic the presence of other chronic liver diseases. The hepatic expression is highly variable, ranging from asymptomatic presentation to severe liver diseases, such as decompensated cirrhosis and acute liver failure. Any patient with transaminitis and abnormal parameters of cooper metabolism should be comprehensively and carefully evaluated to identify Wilson's disease early and to prevent misdiagnosis or unnecessary therapies. Confirmation of the diagnosis should not exclude the coexistence of other liver diseases.*

Conclusions. *The use of validated and adapted scores for Wilson's disease may facilitate diagnosis, but they cannot be used in acute liver failure. Considering that Wilson's disease presents itself with great phenotypic diversity and can evolve under the mask of other pathologies, it is necessary to carry out a careful differential diagnosis.*

Cuvinte cheie: *boala Wilson, prezentare hepatică, insuficiență hepatică acută, ciroză, hepatită acută Wilsoniană, hepatită cronică.*

VARIETATEA FENOTIPULUI HEPATIC ÎN BOALA WILSON: REVIUL LITERATURII

Introducere. *Boala Wilson reprezintă una din bolile genetice care la moment beneficiază de tratament pe tot parcursul vieții, ce îmbunătățește semnificativ calitatea vieții pacienților și reduce complicațiile invalidizante cauzate de întârzierea diagnosticului.*

Material și metode. *A fost efectuată o căutare structurată în PubMed și HINARI, folosind termenii în limba engleză: „Wilson's disease", „acute liver failure", „cirrhosis", „acute Wilsonian hepatitis", „hepatic manifestation", „chronic liver disease", „asymptomatic Wilson's disease", and „active chronic hepatitis".*

Rezultate. *Boala Wilson poate apărea la orice vârstă și poate imita alte boli hepatice cronice. Expresia hepatică este foarte fluctuantă, variind de la forma asimptomatică până la boli hepatice severe, cum ar fi ciroza decompensată și insuficiența hepatică acută. Orice pacient cu citoliză și parametri anormali ai metabolismului cuprului ar trebui să fie evaluat detaliat și atent pentru a identifica precoce boala Wilson și a preveni stabilirea unui diagnostic greșit sau terapii inutile. Confirmarea diagnosticului nu trebuie să excludă coexistența altor boli hepatice.*

Concluzii. *Utilizarea scorurilor validate și adaptate pentru boala Wilson poate facilita diagnosticul, dar acestea nu pot fi utilizate în insuficiența hepatică acută. Având în vedere că boala Wilson se prezintă cu o diversitate fenotipică mare și poate evolua sub masca altor patologii este necesar de efectuat un diagnostic diferențial atent.*

ABBREVIATIONS: WD – Wilson's disease, **ATP7B** – ATPase copper transporting beta, **CLD** – chronic liver disease, **KFR** – Kayser-Fleischer ring, **HAI** – autoimmune hepatitis, **ACLF** – acute-on-chronic liver failure, **ALF-WD** – classic Wilsonian acute liver failure, **ALI** – acute liver injury, **ALT** – alanine aminotransferase, **AST** – aspartate aminotransferase, **LT** – liver transplantation, **EASL** – European Association for the Study of the Liver, **MELD** – a model for end-stage liver disease, **NAFLD** – nonalcoholic fatty liver disease, **PNPLA3** – patatin-like phospholipase domain-containing 3 genes.

INTRODUCTION

Wilson's disease (WD) was first described in 1912 by neurologist Samuel Kinnear Wilson as "progressive lenticular degeneration," a lethal family-transmitted neurological disease associated with chronic liver disease leading to cirrhosis (1). He noticed that "*the most curious and remarkable feature of this familial nervous disease is the constant presence of a profound degree of cirrhosis of the liver*", but he considered that "*This hepatic cirrhosis does not reveal itself by any symptoms during life"* (2). In his article, it was reported that one patient suffered from ascites, another died as a result of hematemesis, and two patients had episodes of jaundice before neurological manifestations, but at that time, hepatology was a lesser-known branch of medicine, and it was difficult to make a well-defined link between cirrhosis and its complications. Taking into consideration that the disease occurred in young people and most often in members of the same family, Samuel Wilson assumed several causes, such as toxins, and viruses, but was unable to confirm their implication (3).

WD, also known as hepatolenticular degeneration, is an autosomal recessive inherited disorder caused by the mutation in the ATPase copper transporting beta (ATP7B) gene that encodes ATP7B protein synthesis responsible for the incorporation of copper into apo-ceruloplasmin, as well as its biliary excretion. The occurrence of a pathogenic mutation in the Wilson gene causes the synthesis of a non-functional protein leading to a toxic accumulation of copper ions in various tissues, especially in the liver and brain (4).

WD is a disorder with multisystemic involvement characterized by an unpredictable clinical picture, and the time of appearance of clinical signs depends on the severity of organic lesions. Due to dysfunction in many systems, clinical and laboratory features are often subtle and can mimic alternative diagnoses, and in the process of evaluating a patient with a variety of signs, symptoms, and laboratory abnormalities it is necessary to make a differential diagnosis with WD (5). WD should be suspected in any person with altered liver parameters of unknown etiology or unidentified movement disorders or any patient with unexplained liver disease in association with neurological or neuropsychiatric disorders. Despite the clinical variety, the key features of WD are chronic liver disease (CLD), neuropsychiatric disorders, the presence of the Kayser-Fleischer ring (KFR), and episodes of acute haemolysis often associated with acute liver failure (ALF) (6).

WD is a potentially fatal disorder if not diagnosed early. Therefore, early recognition of symptoms and their confirmation by laboratory data, as well as the initiation of timely treatment are essential in preventing the progression of the disease and irreversible sequelae (7). Currently, WD is one of those rare genetic disorders that benefit from effective treatment throughout life, which has dramatically changed the prognosis of the disease and the quality of life in these patients (5).

The purpose of the study is to perform a literature analysis to recognize all variants of the liver phenotype in WD, identify chronic liver pathologies that can evolve with clinical, laboratory, and histological signs similar to WD, and highlight the distinctive features of the evolution of liver lesions in WD.

MATERIAL AND METHODS

An advanced search was performed in the PubMed and HINARI databases – the Research4Life program, using English search terms: *"Wilson's disease", "acute liver failure", "cirrhosis", "acute Wilsonian hepatitis", "hepatic manifestation", "chronic liver disease", "asymptomatic Wilson's disease", "active chronic hepatitis".* All publications offered by these platforms were selected, and articles in English were prioritized, although no language limits were set. Articles published in the Republic of Moldova and Romania are also included. A preliminary analysis of the titles was performed and selected original articles, narrati-

ve syntheses, meta-analyses, systematic reviews, series of cases relevant to the research topic, book chapters, which addressed CLD, a hepatic manifestation of WD, concomitant disease in WD, diagnostic criteria and clinical approach of WD patients.

RESULTS

According to the search criteria and after analyzing the information from the HINARI and PubMed databases, 50 relevant sources were chosen with representative content for this literary review. Articles with content not relevant to the topic, as well as those not available for free viewing through the HINARI database were excluded from the search list.

Epidemiological data

Wilson's disease is found worldwide. The reported prevalence is traditionally 1:30,000 people (6), although research by Coffey and colleagues (8) has shown a genetic prevalence of more than 1: 7026 people, in socio-culturally isolated communities (9) as well as in populations with a high risk of inbreeding (10) was observed a very high prevalence. The mismatch between clinical and genetic prevalence can be explained by the presence of several potential factors that can influence it: incomplete genetic penetration, failed diagnosis, and epigenetic and metabolic factors (11). WD has high phenotypic variability, and research that has studied genotype-phenotype correlations has had contradictory results (12). Although the genetic aspects of the disease have been elucidated, the interaction of epigenetic, metabolic, and habit factors (diet, traditions, exposure to toxic environment) may contribute to clinical diversity (13). Hepatic impairment is present in 40-50% of patients (14), although data ranging from 18-84% at the time of primary diagnosis have been reported (15, 16). In the study conducted by Ferenci P. et al. (12) on 1357 patients (children and adults; index patients and siblings), they observed that in index patients, the hepatic presentation was present in 61% and more frequently in women. The difference in data could be explained by the predominant symptomatic manifestations presented by the patient and the specialist to whom he was referred for primary evaluation (ex. the neurologist reports only neurological impairments), and the present untreated asymptomatic forms manifest themselves as the disease progresses being described late, in the stage of complications.

Age at onset of symptoms

The age of onset depends on the type of mutation and the degree of impairment of ATP7B protein function, mutations in functionally important regions are associated with severe evolution, early onset, and a predominant hepatic presentation, while mutations in less important regions of the gene are associated with late-onset and a predominant neurological or psychiatric presentation (7). WD can occur at any age, but primary hepatic presentations are most commonly diagnosed between the ages of 5-35 although may occur earlier or later (6). It is most frequently found in adolescence, but some cases of hepatic pattern have been reported in very young children (<5 years old) (17-21). The diversity of liver disease in these very young children with WD is remarkable because they can present any clinical pattern from asymptomatic to severe disease. Some children are diagnosed with WD when they have had an intercurrent health problem (22), while others are detected by family screening (12). Analyzing the data from the French Wilson's disease registry, which contained 604 patients, it was observed that 51.6% of patients at the time of diagnosis were up to 16 years old; 47% had liver damage, 32.2% had a neurological phenotype and 20.8% were identified in family screening (22).

Clinical evolution

The disease expression is highly variable, ranging from asymptomatic subjects to patients with severe liver disease (11). The symptomatic variant presents hepatic, neurological, or mixed phenotype, considering that the liver and brain injury predominates, but other organs such as the heart, kidneys, osteoarticular system, endocrine glands, genitals, and skin may also be involved. The silent variant (without any symptoms: apparently well) includes those patients conventionally described as asymptomatic or presymptomatic (it assumes that the disease will become clinically manifest), being identified through family or occasional screening, following a routine evaluation, or intercurrent diseases (13).

Hepatic manifestations often occur with a non-specific picture for a long time and precede the onset of neurological symptoms by as much as 10

years. They are characterized by a wide clinical variety: asymptomatic form (increased transaminases, isolated hepatomegaly and/or splenomegaly), CLD (hepatitis or cirrhosis), and ALF. Some clinical features are found in all types of hepatic presentations, which should raise suspicion for WD. A research on a group of 55 patients in Romania, highlighted that 25.4% of patients had the asymptomatic disease, 52.8% had CLD and 21.8% had ALF (23). The research in our country revealed that the average age at diagnosis was 20.0±1.25 years (the interval being between 9-38 years); the duration of establishing the diagnosis varies from 6 to 36 months; 52.5% of patients had liver damage as an initial clinical symptom, and in 72.5% of patient's liver manifestations such as liver cirrhosis, steatosis, hepatomegaly, hepatic failure was determined (24).

Most patients with neurological symptoms will have some degree of liver damage at presentation. The duration from the onset of symptoms to diagnosis is two to three times longer in patients with a neurological phenotype than in those with liver damage (44.4 months versus 14.4 months) (25), which denotes that neurological symptoms may be subtle and easily missed by patient and specialist, as well as the rapidly progressive evolution of the disease in patients with hepatic manifestations leads to initiate paraclinical evaluation with the identification of WD specific changes.

A working group of the 8th International Meeting on Wilson disease and Menkes disease in Leipzig/Germany (April 2001) agreed to classify symptomatic patients corresponding to the organ predominantly involved in their presented symptoms (tab. 1). They also proposed a scoring system for the diagnosis of WD, which is an important tool in examining a patient suspected of WD and includes clinical, haematological, biochemical, histological and genetic data; a score ≥4 points may establish the diagnosis (26). This classification can help to assign patients to the appropriate group, but a score <4 guides clinicians on which tests are needed to establish and put the final diagnosis.

Table 1. Phenotypic classification of Wilson's disease (26).

Hepatic presentation (H) – requires the exclusion of neurological symptoms by performing a detailed neurological clinical examination at the time of diagnosis.		
H1	Acute hepatic WD	Acute onset of jaundice in previously apparently healthy subjects, either due to hepatitis-like illness or Coombs-negative haemolytic anaemia or a combination of both. May progress to ALF requiring emergency liver transplantation.
H2	Chronic hepatic WD	Any manifestation of CLD, with or without symptoms. It may progress to or even present as decompensated cirrhosis. The diagnosis is based on standard assessments: biochemical and/or radiological or liver biopsy.
Neurologic presentation (N) – patients with neurological and/or psychiatric symptoms present at the time of diagnosis.		
N1	Associated with symptomatic liver disease.	Patients usually have cirrhosis of the liver at the time of diagnosis of neurological WD. CLD may precede the onset of neurological symptoms for several years or may be diagnosed during the diagnostic work-up of the patient with neurological symptoms.
N2	Not associated with symptomatic liver disease.	Documentation of the absence of marked liver disease (steatosis/fibrosis may be present at any time) requires a liver biopsy.
Nx	The presence or absence of liver disease is not investigated.	
Other (O)		

Acute hepatic presentation

Acute-WD predominantly affects children or young adults and may develop as acute non-viral hepatitis, acute-on-chronic liver failure (ACLF), or ALF. The common symptoms in children are frequently nonspecific, ranging from fatigue, anorexia, nausea, vomiting, and weight loss to abdominal pain and jaundice. Jaundice is typically more by decompensation CLD or due to intravascular haemolysis associated with classic Wilsonian acute liver failure (ALF-WD) (25, 26). Wilsonian acute hepatitis is more common in women with a female-to-male ratio 2:1 ratio in some series and even higher in others, possibly due to hormonal factors (27).

Acute liver injury (ALI) is a condition when the patients develop coagulopathy, without any alte-

ration of consciousness, while ALF describes the subjects who develop both coagulopathy and altered mentation (28). Dhawan and colleagues (29) reported that 2 of 15 patients that died had ALI which emphasizes the need to identify a patient at an early stage without encephalopathy and to initiate procedures for liver transplantation (LT). A rapid worsening of ALI can lead to ALF related to Wilson's disease that manifests as ALF-WD, and ALF without a classic profile. ALF-WD is characterized by jaundice, moderate to severe Coombs-negative haemolytic anaemia, low serum alkaline phosphatase, mild to moderately elevated serum aminotransferases, coagulopathy, hepatic encephalopathy, rapidly progressive renal dysfunction (acute tubular injury with renal failure). ALF without a classic profile includes jaundice, coagulopathy, and hepatic encephalopathy; such an evolution does not exclude WD etiology. ACLF can be noted in cases with pre-existing advanced liver damage and can be induced by intercurrent infections, hypotension, discontinued WD therapy against medical advice, or acute worsening of WD. Any type of ALF is a medical emergency and it is essential to differentiate them because the algorithm for care and treatment differs (6, 22, 28). ALF due to WD represents about 6-12% of all cases referred to urgent LT and often is the only therapy option (6).

ALF was classified by O'Grady et al. (30) into 3 groups: hyperacute (1-7 days), acute (8-28 days), and subacute (5-12 weeks), being differentiated by the time when hepatic encephalopathy occurred concerning the onset of symptoms (usually jaundice), although the European Association for the Study of the Liver (EASL) guidelines summarize other classifications as well (31). The hyperacute and acute phases are usually easy to diagnose due to obvious clinical and paraclinical changes, while the subacute phase can be confused with decompensated cirrhosis, losing the opportunity to do LT. Acute hepatic presentation is a special condition accepted by EASL guidelines as having ALF if they develop hepatic encephalopathy even if an underlying CLD is present (31).

The values of copper metabolism parameters are accurate indicators in WD but are less relevant in ALF. Being an acute-phase protein, ceruloplasmin can be normal or elevated, total serum copper and urinary copper in 24 h are increased due to massive liver necrosis with the release of copper in the blood (32). However, urinary copper excretion is statistically significantly higher in WD patients compared to ALF from other causes, while the values of ceruloplasmin and total copper did not show a statistical difference (33, 34).

Several indicators have been proposed to differentiate ALF due to WD from other causes. Alkaline phosphatase and alanine aminotransferase (ALT) have been observed frequently in these patients, respectively, a ratio of alkaline phosphatase to total bilirubin <2.0 and aspartate aminotransferase (AST) to ALT>4.0 was identified to recognize the ALF due to WD, but comparative studies did not show significant differences between groups (ALF due to WD and other causes) (33, 34). However, WD patients present clinically significant low levels of haemoglobin, transaminases, and cholinesterase, the latter being explained by the pre-existence of CLD with significant functional lesions and advanced hepatopriv syndrome (34).

ALF can cause significant diagnostic difficulties due to useless information from the diagnostic criteria of the WD, in a critical time window, and the lack of sensitive and specific criteria for the rapid diagnosis. The New Wilson's index, a model for end-stage liver disease (MELD), and the Child-Pugh score are useful tools to make decisions about LT; however, none of them is an independent decisive tool (33). The New Wilson Index predicts mortality without LT, using serum bilirubin, international normalized ratio, AST, albumin, and white cell count; a score ≥11 points is fatal without LT (29), while a Child-Pugh score ≥7 points can show the need to include the patient in the waiting list for LT (33).

The mortality rate in ALF due to WD is around 100% if LT is not performed urgently. This phase is associated with poliorgan failure which aggravates the patient's condition. It is essential to differentiate ALF due to WD from other causes because awaiting LT one of the goals of therapy is to reduce acute injury caused by the massive release of toxic copper (33, 34). The procedures for rapidly decreasing the amount of copper are known as bridging techniques for LT and include: therapeutic plasma exchange, albumin dialysis, plasmapheresis, hemofiltration, fractioned plasma separation and absorption, liver dialysis with single-pass albumin dialysis, early institution of renal replacement therapy, and molecular adsorbents recirculating system. They stabilize the patient and improve biochemical parameters to pass LT successfully (35).

Chronic hepatitis

A clinically important presentation is chronic active hepatitis that can evolve under the mask of nonalcoholic fatty liver disease (NAFLD) or autoimmune hepatitis (HAI) (30). Being characterized by an active inflammatory process, it can progress over time to advanced fibrosis with a negative impact on the patient's prognosis. Clinical evolution is difficult to distinguish from that observed in chronic hepatitis of other causes, and nonspecific biochemical results associated with borderline changes in copper metabolism may lead to a delayed diagnosis (6, 13).

Histological examination in WD is polymorphic and nonspecific, and only the correlation of clinical and paraclinical data guarantees a correct diagnosis (36). As a result of the mutation, copper is not eliminated by the bile and accumulates in toxic amounts in the liver. That affects the function of mitochondria and causes beta-oxidation of fatty acids, leading to steatosis. The hepatocyte contains fat droplets of different sizes from macro- to microvesicles and the histological picture changes with the progression of the disease. In the initial phase, macrovesicular steatosis is observed. With the progression of the disease morphological features of either steatohepatitis or chronic active hepatitis can be determined. Later the histological appearance acquires similarities with chronic active hepatitis of autoimmune or viral type, and finally, cirrhosis can be detected (25, 37).

Nonalcoholic fatty liver disease and WD

Whereas obesity is a global epidemic, clinicians must take into account that all patients suggestive of steatohepatitis, regardless of age and weight, need to be evaluated for WD before starting treatment. Comparative studies (NAFLD due to WD versus metabolic NAFLD) have shown that the severity of hepatic steatosis increases with the amount of copper in the liver tissue, respectively hepatic steatosis in WD is not induced by the associated metabolic conditions, but directly by the accumulation of copper. However, the pathogenesis of NAFLD due to WD is a multifactorial one, and the involvement of metabolic conditions as cofactors in this process is not excluded (37). A recent study on 98 Caucasian WD patients, highlighted the involvement of the G allele of the genetic polymorphism in rs738409 in the patatin-like phospholipase domain-containing 3 genes (PNPLA3) in the development of NAFLD associated with WD, as independent predictors of moderate to high-grade steatosis (38).

Autoimmune hepatitis and WD

Some WD patients may summarize diagnostic criteria for HAI type 1, such as cytolyzes, positive anti-nuclear antibody and/or smooth muscle antibody, and histologically compatible findings on liver biopsy, but compared to patients with HAI, the response to corticosteroid treatment is poor. The appearance of autoantibodies is not known, possibly as a consequence of hepatocyte necrosis or as a concomitant disease, but such an association usually delays the diagnosis of WD. The coexistence of WD and HAI is rare, but, if necessary, the combined therapy of prednisolone and d-penicillamine is recommended (39, 40, 41).

Cholestatic liver diseases and WD

Differential diagnosis between WD and cholestatic liver diseases represents a real challenge in clinical practice due to clinical and laboratory similarities. It is known that cholestatic syndrome, especially primary sclerosing cholangitis, can occur with secondary systemic copper accumulation associated with changes in copper metabolism parameters. It is important to note that in WD, the level of ceruloplasmin is significantly reduced compared to cholestatic cases (6 vs. 16 mg/dL), the 24-hour urinary copper is increased much more (322.3 vs. 74.5 μg/day) and after the initiation of therapy with chelators, a decrease in liver copper content is observed and the level of ceruloplasmin may decrease more (42).

Cirrhosis

The progression of liver fibrosis leads to the installation of liver cirrhosis which is initially compensated, but with the aggravation of portal hypertension, there is decompensation of cirrhosis associated with classic complications such as ascites, encephalopathy, and haemorrhage from esophageal varices. The evolution of cirrhosis in WD is no different than other etiologies being present fatigue, confusion due to hepatic encephalopathy, spider angiomas, gynecomastia, palmar erythema, bruising, bleeding, and muscle wasting (43). Therefore, patients with advanced CLD need to be screened periodically for signs of portal hypertension (esophageal varices and splenomegaly) (43), but also liver neoplastic processes (44).

Decompensated cirrhosis increases the risk of bacterial infections or sepsis, as well as spontaneous bacterial peritonitis. Hepatic encephalopathy, hepatorenal syndrome, acute kidney injury, hepato-pulmonary syndrome, porto-pulmonary hypertension, cirrhotic cardiomyopathy, and relative adrenal insufficiency are complications associated with a high degree of mortality (30, 43).

In a large Austrian cohort that included 229 patients, delay in diagnosis was observed to reduce survival time, and the presence of cirrhosis at the time of diagnosis was the lead predictor of mortality. It should also be highlighted that cirrhosis was present in 47% of cases (66/140 patients) with the hepatic presentation, in 34% of cases (21/61 patients) with the neurological presentation, and in 9% of cases (2/23 patients) in asymptomatic patients (45).

Hepatobiliary malignancies and WD

Although the occurrence of liver neoplasia is a frequent event in CLD, the association of tumors with WD is rare, and the risk of development is not clearly defined, even in cirrhotic patients (44). Toxic accumulation of copper might be expected to be associated with the induction of carcinogenesis, but high copper content in the liver has been hypothesized to have a protective effect, although studies are conflicting (46). A European multicenter cohort study that included 1186 patients with WD identified 8 cases with hepatocellular carcinomas and 6 cases with intrahepatic cholangiocellular carcinomas and found that the prevalence of liver neoplasia in WD was 1.2% and the incidence was 0.28 per 1000 person-years (44). A Dutch retrospective cohort study found that the annual incidence of hepatocellular carcinoma in WD is 0.09% in all patients and 0.14% in those with cirrhosis, therefore dynamic cancer surveillance in WD patients is not recommended, unless there are additional risk factors (e.g., hepatitis B or C, alcohol) (47).

Asymptomatic presentation

Most asymptomatic patients are identified through family screening, which is performed in stages, initially evaluating siblings, then first-degree relatives (and not just first-degree ones). This allows the initiation of treatment as early as possible, preventing the progression of the disease and the appearance of irreversible sequelae (48). Currently, many patients are detected in the asymptomatic phase due to the awareness of this disease in clinical practice and the routine performance of liver function tests (23). No clinical signs or symptoms of manifest liver disease may be observed in these patients, sometimes isolated mild hepatomegaly and/or splenomegaly may be their only findings on physical examination, but they may have some abnormalities of liver tests as mild persistent elevations of ALT or AST (48). Up to 11% of asymptomatic patients had advanced CLD not previously diagnosed (49).

Asymptomatic patients are a real challenge in diagnosing WD, as they may present asymptomatic liver test changes, but with normal values of some of the copper metabolism tests, which reduces the accuracy of the Leipzig score. The diagnosis of WD in asymptomatic patients with minimal changes in copper metabolism or overweight/obese patients with hepatic steatosis should be re-evaluated by histological examination and genetic testing, to exclude a mild phenotype or a modifier gene intervention that regulates copper metabolism in the presence of malfunctioning ATP7B protein (36).

DISCUSSIONS

Any patient presenting increased transaminases needs to be considered for clinical work-up similarly to any other acute/chronic hepatitis and cirrhosis, as the usual histological examination is not pathognomonic (6). The differential diagnosis should be done between WD and viral hepatitis (B, C, D), HAI, cholestatic syndromes, drug-induced liver injury, nonalcoholic or alcoholic steatohepatitis, hemochromatosis, alpha-1-antitrypsin deficiency, ischemic liver damage, and indeterminate causes. In the case of the patient suspected of WD, attention will be paid to the physical examination to identify the stigmas characteristic of liver disease and subtle extrahepatic manifestations, history of unexplained episodes of jaundice or hemolysis, unexplained liver disease with or without neurological or neuropsychiatric disorders in family members (siblings, parents, grandparents, aunt, uncle), current alcohol consumption (a presence of alcohol withdrawal), recent administration of hepatotoxic drugs, unexplained amenorrhea or delayed puberty (30, 50). No single test is specific for diagnosis, but a range of tests must be applied. Given that the parame-

ters of copper metabolism can be influenced by many conditions (e.g., acute inflammation, hyperestrogenemia, increased zinc uptake, healthy heterozygotes, chronic cholestasis, etc.), the diagnosis of copper findings can only be interpreted plausibly in the context of other findings (clinical, laboratory and genetic) to avoid false-positive or false-negative results (32). Ferenci P. and colleagues (12) reported that 3-10% of patients present a normal histological appearance or mild changes, therefore when performing a liver biopsy, it is necessary to do liver copper quantification. To make a differential diagnosis, it is recommended to perform an imaging examination: abdominal ultrasonography, magnetic resonance, or computed tomography, and in the advanced stages, it is important to evaluate the degree of liver fibrosis by transient elastography and biochemical scores of fibrosis (25, 27). The KFR is seen in 45-50% of patients with liver damage and 95% of neurological damage (6).

The presence of WD does not exclude other liver pathologies and vice versa. Reduced vigilance in special clinical cases can lead to several situations. First, the simultaneous presence of another disease may delay the diagnosis of WD, with progressive liver damage. Second, targeted untreated concomitant liver disease can lead to the worsening of lesions despite adequate treatment with chelators for WD. Third, the patient with WD may have overdosed with chelating agents developing secondary copper deficiency and various side effects, while the concomitant disease remains unrecognized (1, 13, 50).

CONCLUSIONS

1. The evolution of liver lesions in WD can be very variable, ranging from an asymptomatic state to decompensated cirrhosis, accompanied by its classic complications, therefore establishing the diagnosis in the early stages can prevent the occurrence of irreversible sequelae.
2. The examination of the patient with changes in liver function tests should be extensive and objective, using validated scores and carefully analyzing the results of investigations.
3. Acute liver failure due to WD is rather difficult to differentiate from other etiologies due to the lack of sensitive and specific criteria for diagnosis in this type of presentation, which complicates potential decisions regarding the type of treatment required.
4. Confirmation of the diagnosis should not exclude the co-existence of other liver diseases, and the complex and systematic approach of the patient prevents delayed diagnosis, irreversible injury with organ failure, and administration of ineffective therapies.

CONFLICT OF INTERESTS

None to declare.

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EVALUATION OF YOUTUBE CONTENT REGARDING *VARENICLINE* AND SMOKING CESSATION

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Keywords: *cessation, content, education, misinformation, decision-making.*

Introduction. *The use of varenicline, an effective way to quit smoking, has become a subject of discussion in the mainstream media, social media, and the internet due to the growing number of people trying to quit smoking. YouTube videos serve a significant purpose in the drive towards smoking cessation with the aid of the drug mentioned above. This study aimed to evaluate the content of videos related to varenicline on YouTube.* ***Material and methods.*** *Six different research terms were created for a search on YouTube. Two reviewers evaluated the videos for quality, reliability, and usefulness. After detecting useful and misleading videos, the factors affecting them as video parameters in these groups were compared.* ***Results.*** *Of the videos evaluated, 78% were classified as useful, while the remaining 22% were misleading. Video length, number of likes and the likes ratio showed no significant difference between these two groups (p=0.264; p=0.075; p=0.798). The DISCERN (DS) and Global Quality Scale (GQS) scores of misleading videos were significantly lower (p=0.001). The DS and GQS scores of the useful videos were 2.58±1.11 and 2.67±1.05, respectively.* ***Conclusions.*** *Misleading videos had a higher number of views and comments compared to useful videos. It was observed that independent YouTube users and media sources are producing more misleading videos. It is very concerning that cigarette quitters do not develop awareness in decision making about the misleading information from media and independent users. In contrast, health pages, physicians, and patient experiences can guide those trying to stop smoking.*

Cuvinte cheie: *renunțare, conținut, educație, dezinformare, luarea deciziilor.*

EVALUAREA CONȚINUTULUI DE PE YOUTUBE CU PRIVIRE LA *VARENICLINĂ* ȘI RENUNȚAREA LA FUMAT

Introducere*. Utilizarea vareniclinei, ca modalitate eficientă de a renunța la fumat, a devenit un subiect de discuție, care suscită un viu interes, în mass-media mainstream, pe rețelele de socializare și internet, datorită numărului tot mai mare de persoane care încearcă să renunțe la fumat. O serie de videoclipurile de pe YouTube își propun ca scop popularizarea medicamentului respectiv drept un remediu eficient în tentativa de renunțare la fumat . Acest studiu și-a propus să evalueze conținutul videoclipurilor de pe YouTube care descriu acțiunea vareniclinei.* ***Material si metode.*** *Au fost creați șase termeni diferiți de cercetare pentru căutarea pe YouTube. Doi recenzenți au evaluat videoclipurile sub aspectul calității, fiabilității și utilității. După delimitarea videoclipurilor utile de cele cu un conținut înșelător, au fost comparați factorii care le afectează, precum parametrii video.* ***Rezultate.*** *Dintre videoclipurile evaluate, 78% au fost clasificate ca utile, în timp ce restul (22%) au fost înșelătoare. Durata videoclipului, numărul de aprecieri și raportul de aprecieri nu au arătat nicio diferență semnificativă între aceste două grupuri (p=0,264; p=0,075; p=0,798). Scorurile DISCERN și GLOBAL QUALITY ale videoclipurilor cu un conținut înșelător au fost semnificativ mai mici (p=0,001). Scorurile celor utile au fost 2,58±1,11 și, respectiv, 2,67±1,05.* ***Concluzii.*** *Am constatat că videoclipurile cu un conținut înșelător au avut un număr mai mare de vizionări și comentarii în comparație cu videoclipurile utile. S-a determinat că utilizatorii independenți ai YouTube și ai surselor media produc comparativ mai multe videoclipuri care induc în eroare. Este alarmant faptul că cei care decid să renunțe la țigări nu dau dovadă de spirit critic și vigilență în selectarea informațiilor care apar în mass-media sau sunt difuzate de utilizatorii independenți, ignorând, în mare parte,, paginile de sănătate, medicii și experiențele pacienților care îi pot ghida, în mod eficient,să se debaraseze de viciul fumatului.*

INTRODUCTION

Smoking is the cause of 7.10 million deaths worldwide and 182 million Disability Adjusted Life Years (DALY) in 2017, which makes it the most significant behavioral element of the global healthcare burden and the second most predominant causal factor among all the contributors to this burden (1). Quitting methods and the war against nicotine addiction are continuously improving. Varenicline, bupropion, and nicotine band or psychotherapy consist part of effective addiction treatment. Studies have biologically proven these agents (2). Varenicline is receiving much more attention because of its effectiveness in treating nicotine addiction. It does not produce significant neuropsychiatric side-effects compared to a placebo, bupropion, or nicotine bands (3).

With the advent of the information age and the widespread use of smartphones, the internet has become an electronic communication network that provides easy access and usage regardless of location and time. Due to broad coverage and ease of use, it provides health information with effortless accessibility. It can be considered a form of media and is a common source of information. YouTube is an omnipresent website for the sharing and viewing of videos. According to Amante DJ et al., one in every two people in the USA resort to YouTube as a source of health information (4). These qualities of YouTube should be assessed, the internet can also be a misleading and wrong source of information. Video quality, video content, and the investigation of false information are the areas that have mainly been studied. Determining the characteristics and what defines these will explain how beneficial YouTube can be and which video characteristics can be trusted. In addition to high-quality content, YouTube may also contain misleading content (5).

There has been no evaluation of videos related to varenicline videos yet. Therefore, in the light of the latest developments, an examination of the quality, likes, duration, and comments of the YouTube videos on varenicline has become necessary. Therefore, *this study aimed* to evaluate the varenicline videos on YouTube.

MATERIAL AND METHODS

We searched on YouTube using the following key terms: varenicline, smoking cessation varenicline, quit smoking varenicline, smoking cessation medicine, quit smoking medicine, and medication to quit smoking. Only English videos were included in the study. Videos in languages other than English, videos with poor sound quality, off-topic, and duplicated videos were excluded from the study. The search was performed on March 6, 2021. Some previous studies have used a method similar to this content analysis study (6-8). Video listings are made based on view counts. The studies on YouTube show that a great majority of users (84%) watch videos from the first three pages (9). Therefore, 360 videos were selected from the six search terms. Following the elimination of videos according to the defined criteria, 144 videos were selected for evaluation (fig. 1). Two public health specialists evaluated the videos regarding the informative quality and information content using the Global Quality Scale (GQS) and DISCERN (DS) scale.

Usefulness

Two researchers evaluated the videos under subheadings to determine if they contained misleading information. The videos that did not refer to any of the following topics were considered out of context: usage, side-effects, efficacy, the effect, the mechanism, information on the harms of smoking addiction, the psychology of the addiction, the safety of the active substance, and reference to professional help. We determined the characteristics of the misleading videos by comparing them with the beneficial ones.

Useful videos are any video, the content of which included scientifically proven information and did not contain any unscientific claims on the topics mentioned above.

Misleading videos were considered any video containing information that has not been proven scientifically or that has been scientifically proven to be wrong under any of the topics mentioned above.

Assessment of quality

The GQS is a scale used to evaluate internet streams is also suitable for evaluating the quality of YouTube videos. The GQS has a 5- point system with 1 point given to videos with the lowest quality and 5 points to the highest quality (tab. 1) (10).

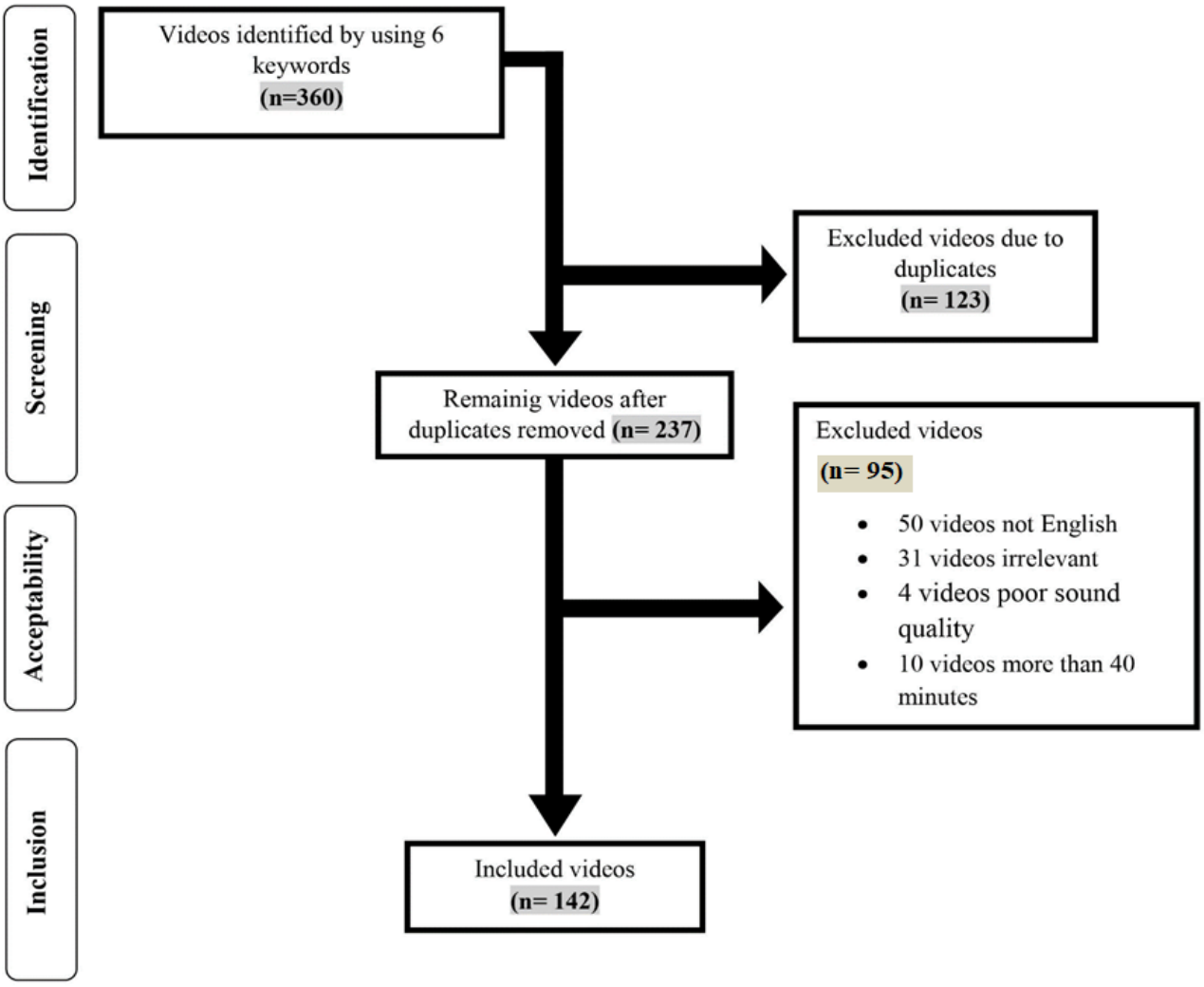

Figure 1.The study process.

Table 1. Global Quality Scale.

1.	Poor quality, poor flow, most information missing, not helpful for patients
2.	Generally poor, some information given but of limited use to patients
3.	Moderate quality, some important information is adequately discussed
4.	Good quality good flow, most relevant information is covered, useful for patients
5.	Excellent quality and excellent flow, very useful for patients

Assessment of reliability

The YouTube videos were evaluated regarding reliability by using the DS tool score. There is a different evaluation for each of the five items, with one point allocated for a "yes" response. The DS tool helps evaluate health information, and along with increasing quality, the score moves from 1 to 5. This reliability tool was previously used by Charnock (tab. 2) (11).

Video parameters

During the research, we recorded the video parameters within a file on March 6, 2021. Then, we calculated the characteristics of the videos [time since upload (days), video length (mins), the number of views, comments, likes, dislikes, and the variables derived from these, viz. views/day, likes/day, dislikes/day, comments/day, and like ratio (like/like+ dislike)].

Table 2. Modified DISCERN* reliability tool.

1.	Are the aims clear and achieved?
2.	Are reliable sources of information used?
3.	Is the information presented balanced and unbiased?
4.	Are additional sources of information listed for patient reference?
5.	Are areas of uncertainty mentioned?

**From charnock (11)*

Video sources

Video sources were categorized under seven main groups: (a) independent users, (b) physicians, (c) health institutions, (d) academic/ journals, (e) consumer/patients, (f) pharmacy companies, and (g) Agencies/ TV channel.

Ethics

Since the video-sharing site YouTube is free and open to everyone, ethical approval was not required for the study.

Statistical analysis

Data processing and statistical analyses were performed using SPSS vn.15 software. Descriptive statistics were stated as mean± standard deviation, median, minimum, and maximum values. When comparing the determinants of video quality, the *Student's t-test* or the *Mann-Whitney U-test*, based on normality tests, and the *Chi-squared test* were used to understand the differences between groups. Conformity of the variables to normal distribution was based on the *Shapiro-Wilk test* and *Kolmogorov-Smirnov test*. A value of $p<0.05$ was considered statistically significant.

RESULTS

Out of 360 videos initially identified, 310) (%86.1 were English, while 50 (%13.9) videos were non-English, thus, being excluded. The duplicates (123 videos), non-English (50 videos), irrelevant videos (31 videos), videos longer than 40 mins (8 videos), and videos with poor sound quality (4 videos) were removed, whereas 144 videos remained. The remaining 144 videos were then analysed and separated into two categories: useful or misleading. The Kappa scores used to examine inter-rater agreement were 0.81 and 0.84 for the GQS and DS tools, respectively. Misleading information was determined in 34 videos (23.6%).

These videos were examined under specific subheadings. It was determined that 76.6% of the videos mentioned the side-effects of the drug, 62.1% contained information about its effectiveness, 40% stated the mechanism of action, 37.9% listed the social, economic and health problems caused by the addiction, 37.3% referred individuals to professional care, 37.3% provided information on the safety of the active substance, 31.7% mentioned other treatment options, 28.5% mentioned the medicine usage, 10.3% stated situations in which the drug is contraindicated, and 17.9% the physiology of the addiction (tab. 3). Videos that did not include any misleading information under any subheadings were accepted as useful.

Table 3. Distribution of items, n (%).

Items*	n	%
Usage	41	28.5
Side effects	111	76.6
Contraindications	15	10.3
Effectiveness	90	62.1
The mechanism of effectiveness	5	40.0
Hazards of addiction	55	37.9
Other treatment options	46	31.7
The physiology of addiction	26	17.9
Referral to professional care	54	37.3
Safety	54	37.3

*There is more than one topic, n: number, %: percentage

The reliability of the information of the video content varied according to the video source. Useful information was provided most often by physicians (90.9%), health institutions (94.7%), academicians/journals (100%), pharmaceutical company (100%), and consumers (patients) (85.2%). News Agencies/TV channels and independent users predominantly uploaded misleading videos (tab. 4).

Table 4. Distribution of useful videos by sources, n (%).

Video Source	Total (n=142) (100%)	Useful (n=111) (78%)	Misleading (n=31) (22%)	p
Independent users	7 (100%)	1 (14.3%)	6 (85.7%)	<0.001
Doctors	11 (100%)	10 (90.9%)	1 (9.1%)	
Health Facility	19 (100%)	18 (94.7%)	1 (4.3%)	
Academic institutions/journals	14 (100%)	14 (100%)	0 (0%)	
Pharmacy company	16 (100%)	16 (100%)	0 (100%)	
Consumer	61 (100%)	52 (85.2%)	9 (14.8%)	
Agencies/ TV channel	14 (100%)	0 (0%)	14 (100%)	

The video parameters were grouped into two categories: useful or misleading. More recent videos were seen to contain less misleading information (p<0.001). The duration of the videos had no significant impact on their usefulness. Videos containing misleading information were significantly longer than useful videos (p=0.014). The number of useful and misleading videos did not differ (p=0.075), however, the misleading videos had more dislikes (p=0.012). The number of comments was higher in misleading videos (p=0.004). Views/days ratio (p=0.327) and dislikes/days ratio (0.069) were not significantly different between the groups. Misleading videos had a higher comments / day ratio (p=0.011) and lower GQS and DS tool scores (p<0.001) (tab. 5).

Table 5. Baseline characteristics of videos.

Parameters	Useful (n=111)		Misleading (n=31)		
	Median (min-max)	Mean± SD	Median (min-max)	Mean± SD	P value
Numbers of days on YT	1940 (76-44258)	2557.9±4212.6	2771 (509-4922)	2678.1±1527.1	<.001
Length of videos (sec.)	218 (34-2220)	333.2±259.1	290.5 (57-962)	363.8±266.7	.264
Numbers of views	3854 (39-477988)	15469.5± 48011.8	8763.5 (287-2705764)	189951.7± 542923.2	.014
Numbers of likes	23 (0-2600)	101.4±277.8	42.5 (1-34000)	2775.8±8297.1	.075
Numbers of dislikes	3 (0-130)	6.5±15.0	6 (0-896)	61.1±176	.012
Numbers of comments	10 (0-473)	32.3±65.6	15 (0-1829)	144.3±351.8	.004
Views/day	2,13 (0.0-198.8)	10.3±25.5	3.5 (0.0-2365.2)	162.6±474.8	.069
Likes/day	0.0 (0-1.7)	0.1±0.2	0.0 (0.0-43.9)	2.7±8.9	.327
Dislikes/day	0.0 (0-0.1)	0.0±0.0	0.0 (0-0.8)	0.6±0.2	.069
Comments/day	0.0 (0-0.5)	0.0±0.1	0.0 (0.0–2.4)	0.2±0.4	.011
Like ratio	0.9 (0-1.0)	0.8±0.2	1.0 (0.5-1)	0.9±0.2	.798
Modified DISCERN	3 (0-5)	2.6±1.1	1 (0-3)	1.3±0.7	<.001
GQS score	3 (1-5)	2.7±1.1	1 (1-3)	1.5±0.6	<.001

DISCUSSIONS

In the previous studies regarding the internet and health, participants mostly used the internet as a primary source of health information without consulting doctors or other sources (12). In addition to being increasingly attractive in every field of life, YouTube has an increasing fascination for those seeking health information.

These videos presented by YouTube with open access do not have any supervision mechanism for the quality, content, and information reliability. Anybody with access can be affected by the low-quality videos and content with distorted reality (13).

Evaluations initiated by Keelan J. et al. for the first time are followed and updated by researchers (14-16). YouTube is an independent video archive of thousands of patient experiences on varenicline. The abandonment of a paternalistic attitude in medicine with the effect of improvements in medical approaches has made patients more proactive. YouTube videos determine what stance and tone should be adopted by the patients (17). YouTube can cause the spread of wrong and misleading information (18). These study findings showed that YouTube was used to broadcast videos about varenicline. The parameters examined in this study included the quality of the videos, the sources uploading the high-quality videos, video parameters, and the quantitative characteristics of accurate and misleading information.

Considering the tendencies of viewers on YouTube, it is evident that misinformation is essential in video choice, and these videos had higher viewing rates. In the first step, we assessed whether the videos were beneficial and reviewed them under separate subheadings. Some parameters were added to be able to evaluate the videos more objectively, and this enabled a more beneficial assessment of varenicline. Even if some videos do not meet all the criteria, they might be more beneficial than others regarding the areas covered.

The three leading topics in the videos showed side effects (76.6%), efficacy (62.1%), and the mechanism of action (40%). Side-effects and the mechanism of action of varenicline were very popular on this platform. However, the first-hand source that gave the most information was patient videos.

The researchers examined YouTube videos on different topics. In the analysis of previous studies, the study made by Mcmullan M. et al. found 77% of the useful videos, another study by Erdem and Sisik found 78.3%. In contrast, Singh AG et al. reported a misleading rate of 30.4%. In the current study, the rate of useful videos was 78%, showing a high usefulness rate similar to those recorded in the other studies (14, 19-20). The reasons for such high rates of usefulness were that videos included patient experiences, health pages, physicians and academic sources. The popularity bases behind the misleading videos were that they were uploaded by TV channels/agencies and independent users. Varenicline has been the subject of controversy since it first appeared, so there has been a great deal of misleading information about it. The media, in particular, has encouraged this situation. There was a high usefulness rate in another study of videos that used health web pages, patients, and trainers (13). According to a study conducted by Şahin A. et al., universities and academic institutions or magazines uploaded few videos, whereas healthcare workers produced more useful videos (14). Despite the low participation, it has been shown in studies that information provided by experts proved to be more beneficial, and sources are critical regarding access to accurate information. The producers of misleading and useful videos must be categorized (18). It is concerning that people seek health information on the internet as a primary source, and 75% do not consider the source of information (21). Among the sources categorized in the current study to detect healthy and correct information sources, there was seen to be more distorted information provided by agencies/tv channels and independent users.

The number of likes and comments determines the popularity of videos on Youtube. In the comparisons between misleading and valuable videos in terms of the number of views, there was much higher internet traffic for misleading videos. A high view count does not necessarily imply that the information provided is credible (22). In a study conducted on diabetes, similar results were recorded. Considering the higher popularity of the misleading videos, it presents a high risk that people get information from them without making a qualitative evaluation. There is a need for greater support from the sources of helpful health information or magazines or a user of a particular drug to continue real stories and to increase the viewing of videos produced by these sources. People and institutions should support these people (3). Similar results were obtained in a study in which methotrexate injection videos were examined.

Thus it can be understood that the number of videos containing accurate and useful YouTube information is sufficient. However, at the same time, there is the indisputable fact that the viewers do not watch the videos in a highly competent manner (18).

The popularity and viewings are open to manipulation. They can be increased by viewers referred by a group of people to a video intentionally. Therefore, the number of dislikes of the videos and formulas derived from those should be used as an indication. Erdem H. and Sisik A. reported that helpful and beneficial videos had higher rates of views and likes (19). The manipulation of the viewing numbers is possible; it can be assumed that as the misleading videos were viral. However, by looking at dislikes/days, it is evident that the viewers could not distinguish video quality and did not give likes to valuable videos. In the current study, misleading and useful videos were compared into two categories considering the fundamental qualities. The comments/day rates demonstrated that people talked much more about the misleading and distorted videos as they had more comments and interaction. The distorted information sparks controversies among the viewers. It has also been noticed that information coming from television shows attracts more comments and views (23).

The current study determined significant differences between the groups regarding the DS tool and GQS points. These results showed that videos are of higher credibility when they are of higher quality. The average GQS point and DS tool credibility points were 2.67±1.05 and 1.35±0.73, respectively, for useful videos and significantly lower at 1.47±0.56 and 1.35±0.73 for misleading videos ($p<0.001$). The DS tool and GQS scores of the useful and misleading videos were consistent and similar within the groups. The useful videos of the current study were found to be lower in quality than those in the study by Erdem H. et al., however the mean score points obtained were similar to those in the study made by Singh AG et al. (19, 20).

There were some limitations to this study. Although the study was designed based on the evaluations of two observers and several criteria were applied in the evaluation process, the results were the subjective interpretations of the persons that evaluated the videos. This study constitutes a snapshot of YouTube on a specific date, but the content might have changed as YouTube is dynamic platform. The fact that the study only included English speaking videos can also be considered a limitation as the content of the videos on YouTube may vary with the language.

CONCLUSIONS

1. Smoking is one of the most important preventable causes of death. YouTube still serves as a platform that affects the approach of individuals to smoking cessation and is a primary source of information for those planning to stop smoking. As an information source, it is an active component that may be either a facilitative or an obstructive tool in the fight against smoking.
2. Health researchers and those wishing to stop smoking may encounter new videos of varying content. They may make much use of these videos by evaluating patient videos and informative health clues. However, at the same time, they risk being exposed to and influenced by YouTube videos that try to attract more attention through the spread of misleading and false information. YouTube users typically cannot distinguish between valuable and misleading videos on varenicline use. As a healthy source of information, health pages, healthcare professionals, doctors, and academicians upload only few videos and do not contribute sufficiently to spreading healthy and helpful information.
3. There is a need for greater support and contributions. Comments of varenicline users that include personal experiences result in helpful videos based on the correct narrative of past experiences. Generally, viewers can watch these videos and obtain useful information even if these videos are not very inclusive and highly qualified. There are valuable videos on varenicline, but they might not be of high quality or comprehensive. Individuals who wish to stop smoking are unaware of the distorted information uploaded by the media and independent users. In contrast, health pages, doctors, and patient experiences can be helpful guides for stopping smoking.

CONFLICT OF INTERESTS

The authors report there are no competing interests to declare.

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THE DYNAMICS OF LETHALITY BY THE PROGRESSION OF THE PULMONARY TUBERCULOSIS PROCESS OF THE NEW CASES PATIENTS

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Introduction. *Mortality among tuberculosis patients, both nationally and globally, has decreased significantly in the last 10 years, but the current epidemiological situation in tuberculosis allows to predict a new rise in the endemic state of the disease due to the increase of TB/HIV coinfection and the resistant tuberculosis.* ***Material and methods.*** *The patient's reports were analyzed – new cases, deaths due to the progression of tuberculosis registered in the Municipal Clinical Hospital of Phthisiopneumology, Chisinau, in 2 periods: the 1st period – 2001-2003 and the 2nd period – 2018-2020. During both periods, 64 new cases died in the hospital due to the progression of tuberculosis. Statistical data processing was performed with the application of the Student (t) test, the data were considered statistically true when p<0.05.* ***Results.*** *In the first period, the age between 25-54 years (81.3%) predominated, in the second period between 45-65+ (73.5%) years of the deceased patients. In both periods, a high share of unemployed patients was found. In the 2nd period compared to the 1st period a severe form of disseminated tuberculosis was established – generalized tuberculosis that was not registered in the first period, the number of deaths with TB/HIV increased 6 times, 4.5 times – with stomach resection in anamnesis, 2 times – with diabetes, 1.5 times with chronic alcoholism. Primary multidrug-resistant tuberculosis was confirmed at 21.9%, extensively resistant tuberculosis – at 4.7% of deceased patients.* ***Conclusions.*** *In the second period, the majotiry of the patients new cases due to the progression of tuberculosis died of severe forms of tuberculosis – generalized, increased number of comorbidities (mainly HIV infection), the presence of a large number of cases with primary resistant tuberculosis.*

Cuvinte cheie: *letalitate, mortalitate, tuberculoză, cazuri noi, comorbidităţi, factori de risc.*

DINAMICA LETALITĂŢII CAZURILOR NOI PRIN PROGRESAREA PROCESULUI DE TUBERCULOZĂ PULMONARĂ

Introducere. *Mortalitatea în rândul bolnavilor de tuberculoză, atât în ţară, cît şi la nivel global a scăzut semnificativ în ultimii 10 ani, însă situaţia epidemiologică actuală în tuberculoză permite prognozarea unei noi ascensiuni a endemiei bolii, din cauza extinderii cazurilor de coinfecţie TB/HIV şi a creşterii ponderii tuberculozei, determinate de rezistenţa germenilor la tratament.* ***Material şi metode.*** *Au fost analizate fişele de observaţie a pacienţilor – cazuri noi, decedaţi din cauza progresării tuberculozei, înregistrate în Spitalul Clinic Municipal de Ftiziopneumologie din Chişinău, în 2 perioade: prima perioadă relevă anii 2001-2003 şi a II-a -2018-2020. În ambele perioade, în staţionar, au decedat cîte 64 de pacienţi, dintre cazurile noi cauzate de progresarea tuberculozei. Prelucrarea statistică a datelor a fost efectuată cu aplicarea testului Student (t), datele fiind considerate statistic veridice pentru un p<0.05.* ***Rezultate.*** *În eşantionul de pacienţi decedaţi, în prima perioadă a predominat vârsta cuprinsă între 25-54 ani (81,3%), iar în a II-a perioadă – 45-65+ (73,5%) ani. În ambele decade s-a constatat o pondere înaltă a pacienţior neangajaţi în câmpul muncii. În perioada a II-a, comparativ cu perioada I, s-a stabilit o formă gravă a tuberculozei diseminate – tuberculoza generalizată, care nu a fost înregistrată în prima perioadă, a crescut de 6 ori numărul deceselor cu TB/HIV, de 4,5 ori – cu rezecţia gastrică în anamneză, de 2 ori – cu diabet zaharat şi de 1,5 ori – cu alcoolism cronic. Tuberculoza primară multidrogrezistentă a fost confirmată la 21,9 % dintre cazuri, tuberculoza rezistentă extinsă – la 4,7% dintre bolnavii decedaţi.* ***Concluzii.*** *În perioada a II-a au decedat mai mulţi bolnavi, dintre cazurile noi cauzate de progresarea tuberculozei, ca formă gravă– generalizată, majorarea numărului de comorbidităţi (preponderent HIV infecţia), prezenţa unui număr mare cazuri cu tuberculoză rezistentă primară.*

INTRODUCTION

Tuberculosis (TB) represents one of the priority public health problems, and its prevention and control are strategic objectives of national interest. Mortality due to tuberculosis is one of the main indicators used to assess the epidemiological situation and determine the "burden of tuberculosis". Tuberculosis is the 2nd leading infectious disease killer after COVID-19. According to the UN Sustainable Development Goals and the "The End TB" strategy, by 2035 the incidence is forecast to be reduced by 90% and mortality by 95%, but progress in achieving these goals is becoming slow (1, 2). The COVID-19 pandemic has significantly affected the successes achieved in the fight against this disease throughout the world, for the first time in the last 10 years the tuberculosis mortality rate has increased (3, 4, 5). According to WHO data, TB and HIV-infection are the main causes of population mortality in the world. Globally in 2019, 208,000 TB/HIV died out of 1.4 billion TB deaths, and in 2020 – 214,000 died and contributed to the increase in mortality to 1.5 billion (1).

The occurrence of new cases of tuberculosis are more frequently influenced by the following risk factors: malnutrition, HIV-infection, disorders dependent on alcohol consumption, smoking, diabetes, etc. Social factors such as worsening socio-economic conditions (no place to live, lack of employment, criminal history, drug addiction and alcoholism, migration processes) contribute to late access to medical services and this fact results in the development of extensive, serious processes of fatal tuberculosis. The majority of patients are hospitalized in somatic inpatient units, where they are diagnosed and transferred to specialized hospitals (6, 7).

At the global level in the years 2000-2017, a positive trend in the decrease in the incidence and mortality from tuberculosis is attested. Tuberculosis mortality fell by about 42%. From all WHO regions in the years 2013-2017 in the European Region the decrease in mortality was 11%. However, against the backdrop of the decrease in tuberculosis mortality, there is a radical change in the aspect of the share of tuberculosis patients associated with HIV infection (8). In 2001, the DOTS strategy was introduced in the Republic of Moldova, when tuberculosis mortality was 20.1 deaths per 100,000 inhabitants. The high mortality rate remained until 2013, after which it slowly decreased to 5.1 deaths per 100,000 inhabitants in 2020 (9, 10).

According to international sources, the current epidemiological situation in tuberculosis allows forecasting a new rise in the tuberculosis endemic due to the expansion of HIV co-infection cases and the increase in the share of resistant tuberculosis, which contributes to a high mortality of patients with this disease (11).

Unlike other characteristics, lethality is a precise indicator for assessing the effectiveness of medical-curative actions. Lethality is used to assess the dynamics of the process, its changes over time, sex, age groups.

Aim of study: Studying the dynamics of the lethality of new cases through the progression of pulmonary tuberculosis in 2 periods of organization of the tuberculosis service in the municipality of Chisinau.

Study objectives: We set out to address the particularities of age, sex, social status, comorbidities, forms of tuberculosis, the development of mycobacterial resistance to antituberculosis drugs at the current stage of patients who died in the hospital. These data will allow us to highlight the risk factors that will be used to form groups of people at risk to develop advanced forms of tuberculosis, which can evolve into death.

MATERIAL AND METHODS

Patient observation sheets were analyzed – new cases, deceased due to the evolution of tuberculosis registered in the Municipal Clinical Hospital of Pneumology, Chisinau, in 2 periods: 1st period 2001-2003 (the period of introduction of the DOTS strategy) and 2nd period 2018-2020 (the period of the "The End TB" strategy). In both periods, 64 new cases died in the hospital due to the evolution of tuberculosis. The anamnestic, clinical data, paraclinical results of patients who died of tuberculosis were studied. The statistical processing of the results was carried out in a computerized way.

The data analysis was carried out using the Microsoft Office 2003 Excel component and the Epi Info 7.1 program with the help of the functions and modules of these programs. To estimate

significant differences, the Student test was used (differences are significant in the case of p-values <0.05; P – characteristic rate, SE – standard error) in 2 periods: 1st period 2001-2003 (the period of introduction of the DOTS strategy) and 2nd period 2018-2020 (the period of "The End TB" strategy).

RESULTS

The distribution of deceased patients by gender in both periods was similar: men – 51 (79.7%), and women 13 (20.3%); M/W ratio = 3.9/1.

Distribution of deceased patients according to age (tab. 1): up to 25 years in both periods the cases of death were similar (no statistical difference for p=0.3). In the 1st period, statistically significantly, young people between 25-44 (52.9%) years predominated (p<0.05). In the 2nd period, the level of lethality was higher in people aged between 55-64 years (p<0.05).

Table 1. Distribution of deceased patients according to age.

Age	1st period (64)		2nd period (64)		p<0.05
	N	(P±SE%)	N	(P±SE%)	
<25	1	1.6±1.6	3	4.7±2.6	0.3
25-34	15	23.4±5.2	7	10.9±3.9	0.05
35-44	17	26.6±5.5	7	10.9±3.9	0.02
45-54	20	31.3±5.8	21	32.8±5.9	0.85
55-64	7	10.9±3.9	17	26.6±5.5	0.02
65+	4	6.3±3.0	9	14.1±4.3	0.14

Table 2. Social status of deceased patients.

Social status	1st period (64)		2nd period (64)		p<0.05
	N	(P±SE%)	N	(P±SE%)	
Employed	3	4.7±2.6	11	17.2±4.7	0.02
Unemployed	53	82.8±4.7	36	56.2±6.2	0.0008
Disabled	3	4.7±2.6	3	6.3±3.0	0.69
Retirees	5	7.8±3.4	14	21.9±5.2	0.02

Most of the patients who died (tab. 2) in both periods were unemployed. In the 2nd period period compared to the 1st period, the number of employees and people of retirement age increased (p<0.05), and the number of people with special needs remained unchanged.

In the 1st period, 11 (17.2%) patients died without living conditions, and in the 2nd period – 15 (23.4%); in the 1st period – 12 (18.7%) and in the 2nd period – 11 (17.2%) were in prison.

36 people (56.2%) had contact with tuberculosis patients both inside and outside their homes in the 1st period: family – 8 (12.5%), relatives – 3 (4.7%), with friends – 12 (18.7%), at work – 1 (1.6%), in prison – 12 (18.7%). During the 2nd, 35 people (54.7%) had contact: family – 10 (15.6%), relatives – 2 (3.1%), with friends – 10 (15.6%), at work – 2 (3.1%), in prison – 11 (17.1%).

The majority (89.8%) of the deceased patients were transferred from municipal somatic hospitals, and 5.1% were hospitalized through emergency medical assistance and on the recommendation of the phthisiopneumologist.

The number of patients who died from infiltrative tuberculosis decreased significantly in the 2nd period compared to the 1st period (p<0.05) (tab. 3). Generalized tuberculosis was registered in approximately half of the patients who died in the 2nd period, and in the 1st period there was no case. Fibro-cavitary tuberculosis had a non-significant decreasing trend in the 2nd period (p>0.05).

Analyzing the interval at which death occurred compared to the time of hospitalization, it was found that 4.7% of patients died in the first 24 hours; 2-10 days – 42.2%; 11-20 days – 21.9%; 21-30 days – 10.9% of cases. Thus, it can be seen that 79.7% died in the first month.

Table 3. Distribution of deceased patients according to forms of tuberculosis.

TB form	1st period (64)		2nd period (64)		p<0.05
	N	(P±SE%)	N	(P±SE%)	
Infiltrative	12	18.7±4.9	3	4.7±2.6	0.01
Caseous pneumonia	33	51.6±6.2	22	34.3±5.9	0.04
Disseminated	11	17.1±4.7	5	7.8±3.3	0.1
Generalized	-	-	30	46.9±6.2	-
Fibro-cavitary	8	12.5±4.1	4	6.3±3.0	0.22

Table 4. Comorbidities of deceased patients.

Comorbidities	1st period (64)		2nd period (64)		p<0.05
	N	(P±SE%)	N	(P±SE%)	
Chronic alcoholism	19	29.7±5.7	27	42.2±6.2	0.13
HIV infection	2	3.1±2.1	12	18.8±4.9	0.004
Hepatitis and liver cirrhosis	9	14.1±4.3	8	12.5±4.1	0.79
Stomach resection	2	3.1±2.1	9	14.0±4.3	0.02
Diabetes Mellitus	3	4.6±2.6	5	7.8±3.6	0.46
Other pathologies	9	14.1±4.3	3	4.7±2.6	0.067
No pathologies	20	31±2.3	-	-	0.006

The patients who died as a result of the progression of tuberculosis also had other pathologies (tab. 4). The number of deceased with chronic alcoholism in the 2nd period compared to the 1st period increased by 1.5 times, diabetes by 2 times, statistically insignificant (p>0.05), HIV infection increased the probability of death by 6 times, gastric resection in the antecedents of 4.5 times (the data were statistically significant for p<0.05); hepatitis and cirrhosis remained at the same level in both periods. All patients of the 2nd period had concurrent pathologies.

In the 1st period, sputum microscopy for acid-alcohol-resistant bacilli (AARB) was positive in 46 (71.1%), and in the 2nd period – in 29 (45.4%) deceased patients. In the 2nd period, the results of molecular-genetic analyzes and by sputum culture were negative – in 8 (12.5%), positive sensitive – in 27 (42.2%), positive multidrug-resistant (MDR) – in 17 (21.9 %) and extensively drug-resistant (XDR) – in 3 (4.7%), and 12 (18.7%) patients were not evaluated due to their serious condition at hospitalization.

DISCUSSIONS

The comparative study in different time periods of new cases and people who died due to the progression of the tuberculous process in hospital conditions highlighted some clinical and paraclinical peculiarities that occurred during the 20-year interval. The results of the research demonstrated a decrease in the mortality of new cases due to the progression of the tuberculous process in the 2nd period compared to the 1st period, but the number of in-hospital deaths in the 2nd period increased significantly. In the current conditions, concomitant diseases play an important role in the increase in tuberculosis mortality. Low-income countries with high disease prevalence, TB/HIV co-infection and drug-resistant TB will face great difficulties in achieving the mortality indicators proposed by "The End TB" strategy. TB/HIV co-infection is one of the main causes of lethality through the development of severe forms of tuberculosis. Alcohol abuse and chronic alcoholism is another cause, which contributes to the late detection of tuberculosis patients with severe and extensive forms (caseous pneumonia, fibrocavitary tuberculosis), to the development of multidrug resistance of mycobacteria to antituberculosis medication with the progression of lesions and unfavorable outcome. Diabetes mellitus is another concomitant disease, which, through its complications, causes difficulties in antituberculosis therapy and in the successful completion of treatment (1, 3, 8).

All patients in the 2nd period had associated di-

seases (the number of deaths with TB/HIV increased 6 times, 4.5 times – with previous gastric resection, 2 times – with diabetes, 1.5 times with chronic alcoholism. The unfavorable social status in both groups (unemployed, without suitable living conditions) contributes to late treatment and progression of the tuberculosis process. The Republic of Moldova is among the 30 countries in the world with a high burden of multidrug-resistant tuberculosis. Primary multidrug-resistant tuberculosis (MDR) was confirmed in 21.9%, extensively resistant tuberculosis (XDR) – in 4.7% of deceased patients. Tuberculosis patients, who died in the hospital, presented unfavorable living conditions and were also confirmed with concomitant diseases, such as be HIV infection, chronic alcoholism and diabetes, which corresponds to the data of other authors.

In both periods, about ½ of the patients had contact with tuberculosis patients, the number of people previously in detention remaining constant, and in the 2nd period the number of people without adequate living conditions increased insignificantly. Patients who died in the 2nd period compared to the 1st period had an older age.

CONCLUSIONS

1. In the 2nd period, compared to the 1st period, there were more cases of death due to the progression of tuberculosis in advanced, generalized forms, with the presence of comorbidities (HIV infection, diabetes, chronic alcoholism, gastrointestinal tract pathology).
2. Sputum microscopy for AARB was established in over half of deceased patients from both periods. In the 2nd period, primary multidrug resistance to antituberculosis treatment was found in a ¼ of the death cases.
3. In both periods, the deceased patients had an unfavorable social status (unemployed, without appropriate living conditions or with criminal record).

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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The article does not have ethical approval.

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A STUDY OF MENSTRUAL HYGIENE PRACTICES AND ASSOCIATED ENVIRONMENTAL & SOCIAL FACTORS AMONG ADOLESCENT GIRLS IN RURAL PUDUCHERRY

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Keywords: *adolescent girls, menstrual health and hygiene, sanitary pads, genito-urinary illness.*

Introduction. *Adolescent females were prevented from receiving the appropriate knowledge due to social restrictions and traditional beliefs, which in turn led to poor hygiene habits.* ***Material and methods.*** *A community-based descriptive cross-sectional study employing a semi-structured questionnaire was carried out in the rural field practice region of MGMCRI, Puducherry, between the 15th of March 2019 and the 31st of April 2021. The study was done in Puducherry. The comprehensive enumeration yielded a total of 528 countable teenage females.* ***Results.*** *The vast majority of the teenage females (89.2%) reported using sanitary pads, whereas just 6.6% and 4.2%, respectively, reported using fresh or reused towels. 65.3% of the girls changed their wet absorbent between two and five times during the day. The vast majority of the girls, or 60.8% of them, disposed of their spent absorbent by either burying it or burning it. 67.9% of the girls were cleansing their genitalia when they were urinating. 54.4% of people cleaned their hands using soap and water, whereas 1.4% utilized ash soil, muddy dirt, or other types of soil. There was a statistically significant correlation between the style of housing and the availability of sanitary latrines (p<0.005) in relation to menstrual hygiene behaviors.* ***Conclusions.*** *This research also highlighted the absence of sanitary toilet facilities in the majority of residences, which negatively impacted the girls' ability to maintain their privacy and led to bad practices around menstruation hygiene.*

Cuvinte cheie: *adolescente, sănătate, igienă menstruală, absorbante igienice, boli genito-urinare.*

UN STUDIU AL PRACTICILOR DE IGIENĂ MENSTRUALĂ ŞI AL FACTORILOR DE MEDIU ŞI SOCIALI ASOCIAŢI LA ADOLESCENTELE DIN ZONA RURALĂ PUDUCHERRY

Introducere. *Adolescentele au fost împiedicate să primească cunoştinţele adecvate din cauza restricţiilor sociale şi a credinţelor tradiţionale, care, la rândul lor, au condus la obiceiuri de igienă deficitare.* ***Material şi metode.*** *A fost realizat un studiu transversal descriptiv comunitar în regiunea rurală. A fost aplicat un chestionar semistructurat, de practică în teren a Colegiului Medical şi Institutul de Cercetare Mahatma Gandhi, Puducherry, între 15 martie 2019 şi 31 aprilie 2021. În studiu au participat 528 de adolescente.* ***Rezultate.*** *Marea majoritate a adolescentelor (89,2%) au raportat că folosesc absorbante igienice, în timp ce doar 6,6% şi, respectiv, 4,2%, au raportat că au folosit prosoape noi sau refolosite,65,3% dintre fete şi-au schimbat absorbantul umed între două şi cinci ori în timpul zilei. Marea majoritate a fetelor – 60,8% dintre ele au eliminat absorbantul uzat îngropându-l sau arzându-l; 67,9% dintre fete şi-au igienizat organele genitale când urinau; 54,4% şi-au igienizat mâinile folosind apă şi săpun, în timp ce 1,4% au folosit cenuşă, noroi sau alte tipuri de pământ. A existat o corelaţie semnificativă statistic între stilul locuinţei şi disponibilitatea latrinelor sanitare (p<0,005) în raport cu comportamentele de igienă menstruală.* ***Concluzii.*** *Această cercetare a evidenţiat absenţa toaletelor sanitare în majoritatea locuinţelor, ceea ce a afectat negativ capacitatea fetelor de a-şi păstra intimitatea şi a dus la practici nesatisfăcătoare în ceea ce priveşte igiena menstruaţiei.*

ABBREVIATIONS: ***ANM*** – Auxiliary Nurse Midwife; ***AICPI*** – All India Consumer Price Index; ***ASHA*** – Accredited Social Health Activist; ***AWW*** – Anganwadi Worker; ***MGMCRI*** – Mahatma Gandhi Medical College and Research Institute; ***RHTC*** – Rural health training center; ***SPSS*** – Statistical Package for the Social Sciences; ***SES*** – Socio-economic status; ***WHO*** – World Health Organization; ***WASH*** – water, sanitation and hygiene.

VERNACULAR MEANING: Kutcha – temporary houses; **Pucca** – permanent houses; **Semi-pucca** – partially finished house.

INTRODUCTION

According to the World Health Organization (WHO) (1), an adolescent is a person who is between the ages of 10 and 19 years old. In India, this age group accounts for 20.9% of the total population, which means that adolescents make up one fifth of the world's total population (2). In Indian culture, menstruation is seen as a dirty and disgusting activity. The inability to obtain access to the correct sort of knowledge on menstrual hygiene is hampered by social prohibitions, a strong bondage with taboos and traditional beliefs during menstruation, and the reluctance of parents to openly address the relevant problems with their teenage daughters (3). Because of their lack of education, they end up repeatedly using dirty menstrual absorbents, which leads to the harboring of microorganisms and an increased risk of urinary, perineal, vaginal, and pelvic infections. This is a vicious cycle that may be avoided (4). Infertility, ectopic pregnancy, fetal wasting and prenatal infection, low birth weight newborns, and toxic shock syndrome are some of the potential outcomes that might occur if these infections are not treated in a timely manner.

On May 28th, a day that is being commemorated as "Menstrual Hygiene Day" by WASH United is being done so in order to provide greater emphasis on the menstrual health (5).

Since August 2011, the government of India has been running a program to provide subsidized sanitary napkins to teenage girls living in rural areas of the country. This was done in recognition of the significance of the promotion of menstruation hygiene (6).

These members of the younger generation will become India's parents and the country's future. The choices that people make about their reproductive health today will have an effect on the health and welfare of future generations as well as the community as a whole. In spite of the fact that all of these initiatives were carried out by the government of India, a significant proportion of the young women in India do not have a prior awareness about the menstrual cycle and the hygienic practices that are associated with it, which results in poor menstrual hygiene (7). There have been a lot of research done on menstrual health and hygiene, but the factors that matter most, like the availability of sanitary latrines and the kinds of homes that people live in, haven't gotten as much attention as they should have. As a result, this research was carried out on adolescent girls in regards to their menstrual hygiene and related personal hygiene practices, as well as to study the environmental factors related to menstrual hygiene and help them to prevent the gynecological infections and its serious consequences in their future. As a result, it is very important to study the menstrual hygiene practices among adolescent girls in rural Puducherry, as well as to study the associated environmental and social factors relatable. *This study's objective is* to investigate the association between environmental and social factors in rural Puducherry and the menstrual hygiene practices of adolescent females in that area.

MATERIAL AND METHODS

The current research was carried out only after receiving authorization to do so from the Institutional Human Ethics Committee. A community-based descriptive cross-sectional study was carried out with the purpose of conducting research the menstrual and related personal hygiene practices of adolescent girls over the course of one year, beginning in March 2019 and ending in February 2021. The study lasted for a total of twelve months. The study population for this investigation consisted of young women who lived in rural areas, were between the ages of 10 and 19 years old, were willing to take part in the research and provided their informed permis

sion, and belonged to the rural field practicing region. RHTC includes 3 villages in Pondicherry, India, namely Seliamedu, Kudiyirupupalayam and Aranganur. structured questioners who were already prepared to do an analysis of the menstrual hygiene habits and the environmental and social aspects linked with them. It is estimated that there are roughly 528 teenage females living in these three settlements. This survey counted each and every one of the 528 teenage females living in each community as part of its exhaustive census. In the course of the data collecting process, there were 26 teenage girls who did not provide their permission or who did not reach menarche. As a result, these girls were not included in the study, and the final population of the research was 502. The participants' right to privacy was respected at all times. All of the information that was gathered over the course of the study was treated as confidential, and it was only utilised for the purposes of the research.

Socio-economic status SES, Based on Rvised B.G Prasad's social classification for the year 2020, Modified BG Prasad Socio-economic Classification, Updated – 2020 by [BG Prasad SES] Debnath DJ et al. (8).

The range of family monthly income was 1000 to 35,000 Indian rupees. The mean value for monthly family income was 8464.64 Indian rupees, with the standard deviation of 5440 rupees. Percapita income ranges between 250 and 8750 Indian rupees. Mean value was 1830.89 with standard deviation of 1229.18 Indian rupees. According to B.G. Prasad's Classification for socio-economic status, (Jun 2014-AICPI) Majority of the girls were belonging to class IV and class III respectively (tab. 1).

Table 1. Distribution of study population based on socio-economic status (n=502).

	SES	Revised for 2020 (in Rs. /month)	Number	Percentage
Socio-economic status	Class I	7533 and above	10	2.0
	Class II	3766-7532	59	11.8
	Class III	2260-3765	136	27.1
	Class IV	2260-3765	222	44.2
	Class V	1129 and below	75	14.9

Among the total adolescent girls under the study, three fourth of the girls (74%) were belonging to nuclear family and 25.1% were from joint family and 79.3% girls belonged to families having less than 5 members and 7.4% girls were having more than 7 members in their family.

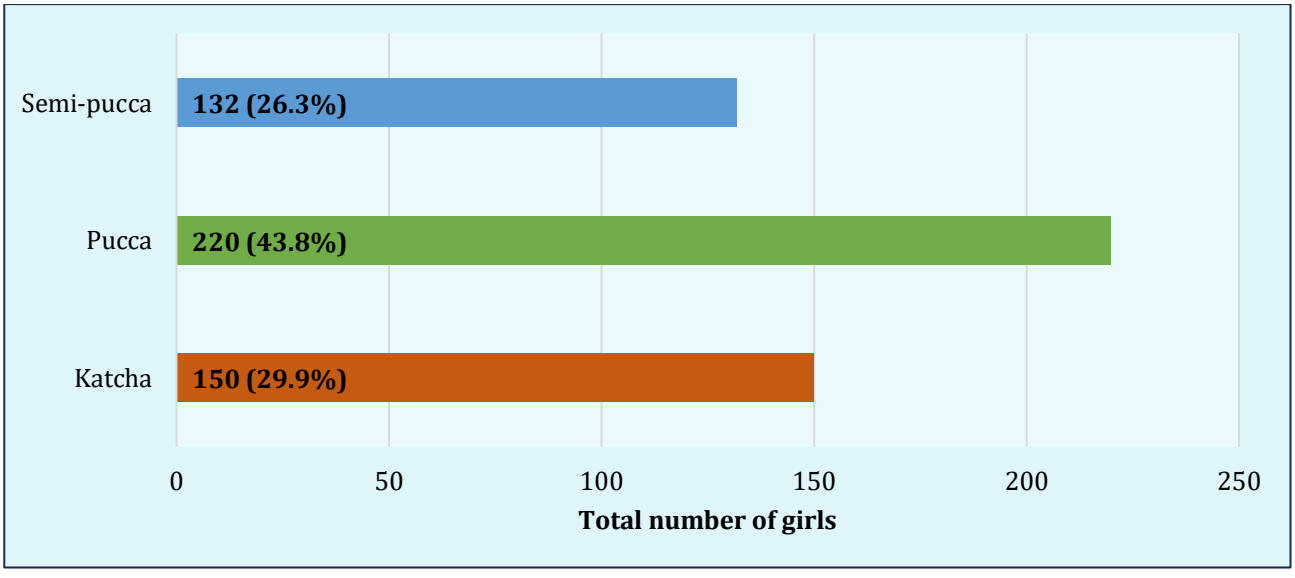

Figure 1. Distribution of adolescent girls according to type of house.

The composition of the teenage girls' families is depicted in Figure 1. 43.8 percent of the females in the sample lived in the pucca house (strong and durable made up of bricks and cement) followed by 29.9 percent of the girls who came from the kutcha house (weak and made up of clay and sand), and finally 26.3% of the girls called the semi-pucca house (stable made up of bricks and clay without cement) their home.

Figure 2 illustrates the prevalence of sanitary latrines among teenage females in the research region. The majority of the girls, 283 (56.4%), did not have access to a hygienic toilet at home, while 43.6% did.

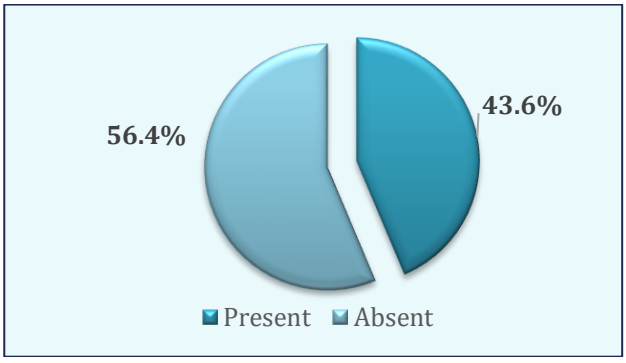

Figure 2. Distribution of the adolescent girls according to presence of sanitary latrine (N=502).

Participants' mean age of menarche was 12.71 years, with a standard deviation of 1.05 years. More over half of females (53.8%) reached menarche between the ages of 12 and 14. The majority of girls (67.5%) had regular menstrual cycles (28-35 days cycle). Nearly three quarters of the girls (77.9%) had typical menstrual flow length of 2-7 days. 57.2 percent of the total number of 502 females had regular menstruation. 51.6% of girls reported clot passing during menstruation.

The majority of the girls, or 70.5%, in a total sample of 502 complained of stomach discomfort during menstruation. Low backache was the next most common complaint, occurring in 51.6% of the females. Muscle cramps were experienced by 29.1% of the girls, while nausea or vomiting was reported by 16.5% of the girls.

Regarding the receipt of any formal or non-formal information regarding menstruation among adolescent girls in the rural study area, among the 502 study participants, 261 (52%) girls had any information regarding menarche and menstruation before attaining menarche, whereas 241 girls (48%) did not know that information. This was the case regardless of whether the information was formal or nonformal. Following the onset of menarche, 32.1% of girls indicated that their mothers were the key source of knowledge, followed by 24.7% of girls who named their sisters, and 23.9% of girls cited their friends and relatives. It's interesting to note that 15.3% of young women said they obtained information from ANM or AWW. Less than half of one percent of the females surveyed said that their professors are their primary source of information.

16.9% of all girls reported following at least one of the menstrual limits, followed by 25.5% of all girls who reported following any two restrictions, and 14.3% of all girls reported following all four restrictions during their periods. Surprisingly, 168 girls (33.5% of the total) claimed that they were not subjected to any restrictions when they were menstruating.

The following table details the menstrual hygiene routines of teenage females when they are experiencing their periods. The vast majority of the 502 girls surveyed (89.2%) reported using sanitary pads as a menstrual absorbent throughout their periods. Sixty-five-point three percent of the female students successfully replaced their drenched menstruation absorbent (2-5 times per day). The discarded menstrual absorbent was either burned or buried by the vast majority of the girls (60.8%). A far higher percentage of the female students, 67.9%, stated that they washed their genitalia while they were urinating.

Association of type of house and menstrual hygiene practices were depicted in Table 3. Those who used pads 46% were living in pucca house, whereas maximum number of girls among fresh and reusable cloth users, (42.4% and 57.1% girls respectively), were residing in kutcha house. These observed differences were statistically significant (p=0.012).

There was significant statistical association observed between changing times and type of house (p=0.031). Satisfactory changing times (2-5 times and >5 times) observed highest in 45.7% and 51%, pucca house dwellers. On other hand, unsatisfactory changing times (<2 times) were observed highest (36.5%) among girls living in semi-pucca houses.

The association between disposal of soaked absorbent and type of house having statistical sig-

nificance (p=0.005), that is among the girls who were reusing the cloth material majority (57.1%) of them belonged to kutcha houses, and satisfactory disposal (dust bin, burnt/ buried) were observed highest, 39.7% and 47.5% respectively, among pucca house dwellers.

Table 2. Magnitude of menstrual hygiene practices among adolescent girls during menstruation (n=502).

Menstrual hygiene practices		Number	Percentage
Type of absorbent used during menstruation	Pads	448	89.2
	Fresh cloth	36	6.6
	Reusable cloth	21	4.2
Number of times absorbent changed (per day)	<2 times	96	19.1
	2-5 times	328	65.3
	>5 times	49	9.8
	As per need	29	5.8
Disposal of used menstrual absorbent	Dust bin	146	29.0
	Wash & reuse	21	4.2
	Burn / burry	305	60.8
	Flush in toilet	30	6.0
Cleaning of genital area (per day)	<3 times	44	8.8
	>3 times	56	11.1
	Only during bath	61	12.2
	During micturition	341	67.9
Agent used for cleaning purpose	Only water	202	40.2
	Soap & water	273	54.4
	Dettol	20	4.0
	others	7	1.4

Table 3. Bivariate analysis of association between type of house and menstrual hygiene practices (n=502).

Menstrual hygiene practices		Environmental variable Type of house (%)			P value
		Kutcha (temporary houses)	**Pucca** (permanent houses)	**Semi-pucca** (partially finished house)	
Type of absorbent used during menstruation	Pads (448)	27.7	46	26.3	**0.012**
	Fresh cloth (36)	42.4	27.3	30.3	
	Reusable cloth (21)	57.1	23.9	19	
Number of times absorbent changed	<2 times (96)	31.2	32.3	36.5	**0.031**
	2-5 times (328)	31.4	45.7	22.9	
	>5 times (49)	26.5	51.0	22.5	
	As per need (29)	13.8	48.3	37.9	
Disposal of used menstrual absorbent	Dust bin (146)	24.7	39.7	35.6	**0.005**
	Wash & reuse (21)	57.1	23.9	19.0	
	Burn/ dump (305)	30.8	47.5	21.7	
	Flush in toilet (30)	26.7	40.0	33.3	
Cleaning of genital area	<3 times (44)	35.6	33.3	31.1	0.697
	>3 times (56)	25.0	48.2	26.8	
	Only during bath (61)	33.4	38.3	28.3	
	During micturition (341)	29.3	45.5	25.2	

Table 4 emphasized the association between sanitary latrine facility and menstrual hygiene practices. 85.7% and 63.6% reusable cloth users and fresh cloth users didn't have latrine facilities in their house. Among pad users, highest number of girls (45.5%) were having sanitary latrine in their house and such difference were found to be statistically significant (p=0.013). Similarly, girls who were reusing cloth material 85.7% didn't have latrine and 61% of girls who burnt or buried cloth material didn't have latrine facilities, these differences were having statistical significance (p<0.001). There was statistically significant association found out between family type and cleaning times (p=0.01).

DISCUSSIONS

There are a total of 502 females, and 43.8%, 26.3%, and 29.9% of those girls reside in pucca, semi-pucca, and kutcha houses, respectively. Only 43.6% of the girls lived in homes that had a properly functioning latrine. Despite the fact that approximately three quarters of the girls belonged to classes III and IV, they did not place a great deal of weight on the separate toilet facility; this demonstrates their ignorance and lack of understanding about hygienic toilets in the neighborhood. Only a handful of the studies that were done on menstrual hygiene habits looked at environmental factors like bathing area and site, but they did not look at other factors like the style of dwelling or sanitary toilet facilities. (8,9).

In terms of limitations, out of 502 girls, 61.8% are required to sleep in separate beds, followed by 39.2% who were required to avoid specific kinds of food and 28.5% who were prohibited from playing, respectively. A tiny percentage of girls and women, respectively 1.2% and 0.8%, did not attend school and participated in activities such as weddings; 23.1% of these individuals were prohibited from doing any kind of labor or cooking. A number of investigators, including Emanshoky et al., Abhay et al. in Wardha, Dasgupta et al., Adrija et al., Keerti et al., Narayan et al., Singh et al., and Balasubramani et al., also found different sorts of limits in their research that were equivalent to the results of our current study (7-9).

There was not a statistically significant correlation identified between the educational level of the girls and their menstrual hygiene habits in this research; however, such an association was shown to be statistically significant in a study from southern India 10-14).

Table 4. Association between sanitary latrine facility and menstrual hygiene practices (n=502).

MENSTRUAL HYGIENE PRACTICES		Environmental variable Sanitary latrine		P value
		Yes (%)	No (%)	
Type of absorbent used during menstruation	Pads (448)	45.5	54.5	0.013
	Fresh cloth (36)	36.4	63.6	
	Reusable cloth (21)	14.3	85.7	
Number of times absorbent changed	<2 times (96)	44.8	55.2	0.349
	2-5 times (328)	42.7	57.3	
	>5 times (49)	38.8.	61.2	
	As per need (29)	58.6	41.4	
Disposal of used menstrual absorbent	Dust bin (146)	54.1	45.9	<0.001
	Wash & reuse (21)	14.3	85.7	
	Burn/ dump (305)	39.0	61.0	
	Flush in toilet (30)	60.0	40.0	
Cleaning of genital area	<3 times (44)	44.4	55.6	0.108
	>3 times (56)	57.1	42.9	
	Only during bath (61)	35.0	65.0	
	During micturition (341)	42.8	57.2	
Agent used for cleaning purpose	Only water (202)	43.1	56.9	0.199
	Soap & water (273)	43.6	56.4	
	Dettol (20)	60.0	40.0	
	others (7)	14.3	85.7	

In a previous cross-sectional study that was carried out in south India, it was mentioned that there was an inverse relation between socioeconomic status and the necessity for more information about practices; however, the present study showed that there was no significant association between socioeconomic status and awareness (p=0.129). Although Shabnam et al. (15, 16) found that girls from poor socioeconomic backgrounds were more likely to use inadequate sanitary menstruation absorbents, this link was not shown to be statistically significant in the current investigation (p=0.587). This might be because of the high levels of knowledge, availability, and affordability of sanitary pads in this research location; (yet, a statistically significant correlation was established with SES and the changing times of absorbents being used (p=0.002).

CONCLUSIONS

1. The present study explored menstrual hygiene practices of adolescent girls in rural field practicing area of MGMCRI. It is good to notice that majority of the girls were using sanitary pads, so awareness of menstruation and menstrual hygiene practices, availability, accessibility, and affordability of sanitary absorbent are good in this area.
2. It was found out that there was significant statistical association between poor menstrual hygiene practices and environmental & social factors. Even though sanitary pad users were high, most of the girls reported symptoms that were suggestive of poor menstrual hygiene. So, provision of pads or increased accessibility will not give a complete solution for poor menstrual hygiene related problems. In addition to that more emphasize has to be given on adequate changing times of soaked absorbent, adequate number of times of cleaning of external genitalia, sanitary material used for cleaning purpose, method of disposal of used menstrual absorbent among adolescent girls in this area.
3. The study highlights, majority of the houses didn't have separate sanitary latrine facility even though few families could have afforded, it is mainly due to ignorance, and they felt that it was not necessary. Open air defecation, a public health issue silently prevailing in this community. In addition to that girl couldn't find privacy for their menstrual hygiene management that led to poor practices during menstruation and end up with poor menstrual hygiene related problems. These issues were left unnoticed in this study area since long.

CONFLICT OF INTERESTS

No conflicts of interest.

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ETHICAL APPROVAL

This study was approved by the Institutional Ethics Committee of Mahatma Gandhi Medical College and Research Institute, Puducherry, India, proposal no: MD/MS/2013/10.

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SEROPREVALENCE OF VIRAL HEPATITIS B, C AND E IN TB PATIENTS FROM THE REPUBLIC OF MOLDOVA

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Keywords: *seroprevalence, laboratory investigations, viral hepatitis B, C and E, TB patients.*

Introduction. *For the first time, in the Republic of Moldova, there was established the seroprevalence of viral hepatitis B, C and E markers in patients with tuberculosis depending on gender, age and geographical areas.*

Material and methods*. 200 blood samples were collected from patients with tuberculosis and tested by the immuno-fermentative method for presence of viral hepatitis B, C and E markers.*

Results. *Following the investigations, it was established that the seroprevalence of the anti-HEV Ig marker in patients with tuberculosis is 12.0±2.3%. The seroprevalence of viral hepatitis B marker HBsAg in patients with tuberculosis was 13.5±2.4%, and that of viral hepatitis C anti-HCV – 9.0±2.0%. The study of investigations results for the presence of nominated markers depending on the geographical areas showed a significant statistically difference in the seroprevalence of the anti-HEV IgG marker in TB patients in the Central area of the country compared to the Southern area(p<0.05).*

Conclusions. *Patients with tuberculosis showed a high level of seroprevalence of viral hepatitis marker viz. HBsAg - 13.5±2.4%; anti-HVC - 9.0±2.0% and anti-HVE IgG - 12.0±2.3%. People aged 40-49 and > 60 years were the most affected, including males.*

Cuvinte cheie: *seroprevalenţa, investigaţii de laborator, hepatitele virale B, C şi E, bolnavii cu tuberculoză.*

SEROPREVALENŢA HEPATITELOR VIRALE B, C ŞI E LA BOLNAVII CU TUBERCULOZĂ DIN REPUBLICA MOLDOVA

Introducere. *Pentru prima dată în Republica Moldova a fost stabilit nivelul seroprevalenţei markerilor hepatitelor virale B, C şi E la bolnavii cu tuberculoză în dependenţă de gen, vârstă şi zonă geografică.*

Material şi metode. *Au fost colectate 200 probe de sânge de la bolnavii cu tuberculoză şi testate prin metoda imuno-fermentativă la prezenţa markerilor hepatitelor virale B, C şi E.*

Rezultate. *Urmare a investigaţiilor a fost stabilit că seroprevalenţa markerului anti-HEV Ig la bolnavii cu tuberculoză constituie 12,0±2,3%. Seroprevalenţa markerului hepatitei virale B - AgHBs la bolnavii cu tuberculoză a constituit 13,5±2,4%, iar a hepatitei virale C anti-HCV – 9,0±2,0%. Studierea rezultatelor investigaţiilor la prezenţa markerilor nominalizaţi în dependenţă de zonele geografice a demonstrat diferenţă statistică semnificativă a seroprevalenţei markerului anti-HEV IgG la bolnavii cu tuberculoză din zona de Centru a ţării comparativ cu cei din zona de Sud (p<0,05).*

Concluzii. *S-a stabilit un nivel înalt al seroprevalenţa markerilor hepatitelor virale la bolnavii cu tuberculoză: AgHBs – 13,5±2,4%; anti-HVC – 9,0±2,0% şi anti-HVE IgG – 12,0±2,3%. Preponderent fiind afectate persoanele cu vârsta cuprinsă între 40-49 ani şi >60 ani, inclusiv cele de gen masculin.*

INTRODUCTION

Both viral hepatitis and tuberculosis are major public health issues. Although, literature data highlight on tuberculosis and viral hepatitis co-infection, their convergence is less studied yet. On the other hand, there are almost no studies on the seroprevalence of the hepatitis E virus in patients with tuberculosis, and even fewer studies on the impact of viral hepatitis E on the TB course and treatment.

The most recent and relevant studies in this area have pointed out that the prevalence of chronic hepatitis B (HBV) among TB patients ranges from 0.5% to 44% (1), whereas the prevalence of viral hepatitis C among TB patients is estimated at 3.4-44.6% (2). Co-infection of tuberculosis and viral hepatitis B and C increases the risk of treatment failure (3), activates latent tuberculosis (4), and increases the risk of death (5) and drug-induced injury (6). Hepatitis B infection increases the TB severity by 59.5%, and hepatitis C increases the TB severity by 34.5% (7). It's due to the fact that hepatitis virus reactivates tuberculosis, leading to severe clinical manifestations (8).

Since chronic hepatitis B and C cause liver damage, the treatment of TB patients co-infected with hepatitis viruses is still a health problem in the Republic of Moldova, due to the common anti-TB drug-induced hepatotoxicity (9). Currently, isoniazid, rifampicin, pyrazinamide, and ethambutol are considered first-line drugs, however, they are associated with hepatotoxicity. The incidence of hepatotoxicity and other side effects ranges from 3% to 28% (10).

Regarding viral hepatitis E, recent studies conducted in Europe show a heterogeneity of its spread from 0.6% to 52.5% among the population, where anti-HEV IgG traces have been found. The anti-HEV IgG seroprevalence varies, depending on the geographical region, the type of test used and population under study (11).

On the other hand, as it has been already mentioned above, there are almost no literature data on the prevalence of viral hepatitis E in patients with tuberculosis. Although hepatitis E is usually a self-limiting disease in immunocompetent individuals, it can cause serious complications in risk groups such as pregnant women (12) and organ transplant recipients (13). In cases where viral hepatitis E is associated with another pre-existing viral hepatitis (B or C), it quickly changes from chronic into a fulminant condition, followed by severe liver damage and high mortality rates (14).

The treatment options for viral hepatitis E remain limited so far, and only one vaccine has been developed, which, unfortunately, is still not used in our country.

This research paper is aimed at studying the seroprevalence of viral hepatitis B, C and E in TB patients from the Republic of Moldova.

MATERIAL AND METHODS

A cross-sectional descriptive-epidemiological study was conducted on TB patients admitted to the IMPH Clinical Hospital of Phthisiopulmonology in Chisinau during the period from January to February 2021. 200 blood samples were collected, including 7 samples – from the northern, 178 – from the central and 15 – from the southern districts of the country. The men-women ratio was 131 (65.5%) and 69 (34.5%), correspondingly.

All the samples were studied for the presence viral hepatitis B (HBsAg), viral hepatitis C (anti-HVC) and viral hepatitis E (anti-HEV IgG) markers via the enzyme immunoassay (ELISA). Tests were performed using Dia. Pro Diagnostic Bioprobes kit that has a sensitivity of 99.9% and a specificity of 99.9%. A total of 672 laboratory studies were performed (including repeated studies with doubtful results). Statistical data processing was carried out using Microsoft Excel and EpiInfo programs.

RESULTS

The laboratory findings of TB patients showed that the seroprevalence of the surface antigen HBsAg was 13.5±2.4%, the anti-HCV marker of hepatitis C was 9.0±2.0% and the anti-HEV IgG was 12.0±2.3%. In females, HBsAg seroprevalence was 5.8±2.8%, and in males – 17.6±3.3%, which indicated a significant statistical difference between the sexes ($p<0.05$). The anti-HCV marker was detected almost to the same extent both in females – 8.6±3.4% and in males – 9.2±2.5%. The predominant anti-HEV IgG marker was found in 17.4±4.6% of females and in 9.2±2.5% of males. Thus, the obtained results reveal that TB patients are at higher risk of developing viral hepatitis B, C

and E (tab. 1).

The analysis and assessment of the laboratory findings of the examined TB patients, depending on their geographical areas of origin, revealed that the seroprevalence of HBsAg in the Northern region was 14.3±13.2% and anti-HEV IgG – 14.3±13.2%. There were no cases of anti-HCV marker positivity among 7 patients from this area. At the same time, the seroprevalence of HBsAg was 13.5±2.6%, anti-HCV – 8.9±2.1%, and anti-HEV IgG – 10.1±2.3% within the central part of the country. The seroprevalence of TB patients in the Southern regions was 33.3±12.1%, and 13.3±8.8% for both viral hepatitis E and for HBsAg and anti-HCV markers (tab. 2).

Table 1. The test results on viral hepatitis B, C and E markers of TB patients according to their gender distribution.

Gender	Total	Markers					
		Ag HBs		Anti-HCV		Anti-HEV IgG	
		positive	P±ES	positive	P±ES	positive	P±ES
Males	131	23	17.6±3.3	12	9.2±2.5	12	9.2±2.5
Females	69	4	5.8±2.8	6	8.6±3.4	12	17.4±4.6
Total	200	27	13.5±2.4	18	9.0±2.0	24	12.0±2.3

Table 2. The test results on viral hepatitis B, C and E markers of TB patients according to their geographical origin distribution.

Geographical areas	Total	Markers					
		Ag HBs		Anti-HCV		Anti-HEV IgG	
		positive	P±ES	positive	P±ES	positive	P±ES
North	7	1	14.3±13.2	0	0	1	14.3±13.2
Centre	178	24	13.5±2.6	16	8.9±2.1	18	10.1±2.3
South	15	2	13.3±8.8	2	13.3±8.8	5	33.3±12.1
Total	200	27	13.5±2.4	18	9.0±2.0	24	12.0±2.3

Subsequently, all cases were assessed by age. Thus, the highest level of HBsAg seroprevalence was found in the age group of 40-49 years old viz. 24.5±6.1%, whereas the other age groups revealed as following: 20-29 years old – 7.7±7.4%, 30-39 years old – 7.5±4.2%, 50-59 years old – 10.0±4.7%, 60 and over – 11.3±4.3%.

As regarding the anti-HCV marker, it was mainly determined in the 60 age group and older, showing a seroprevalence of 13.2±4.6%, followed by the age group of 50-59 years, where the same marker was determined in 10.0±4.7% of cases, the age group of 40-49 years exhibited the smallest distribution of 5.6±3.1%, the age group of 30-39 years was found in 7.5±4.2% cases and, finally, only one positive case was determined in TB patients aged 20-29, the marker being of 7.7±7.4% (tab. 3).

The studies related to the age-dependent presence of viral hepatitis E marker in TB revealed that the seroprevalence of anti-HEV IgG in the group aged 20-29 years old was 7.7±7.4%, 7.5±4.2% in patients aged 30-39 years, 14.8±4.8% in those aged 40-49 years, 10.0±4.7% in 50-59 year-old group, and 15.1±4.9% in TB patient older than 60 years old.

Therefore, the obtained results show that the highest seroprevalence of surface antigen (HBsAg) was recorded in TB patients aged 40-49, whereas the anti-HCV and anti-HEV IgG markers showed a higher prevalence in patients aged over 60.

The last task performed in our study was to analyze and evaluate the seroprevalence of viral hepatitis associated with TB patients. Thus, the seroprevalence of the anti-HEV IgG marker associated with HBsAg was 25.9±5.1% of cases (7 people out of 27). The same association of markers was found in male patients in 22.2±8.0% of cases (6 people out of 27), and in females in 3.7±3.6% of cases (1 person out of 27). There is a statistical difference in the prevalence of these associated markers in men compared to women ($p<0.05$). Depending on the age, the highest level of association of anti-HEV IgG and HBsAg markers was found in TB patients aged 40-49 years viz. 8±8.8%

(4 people out of 27). The seroprevalence of patients under study revealed the anti-HEV IgG associated with HBsAg, which was 20.8±8.3% (5 patients out of 24) in the Central region and 100% in the Southern region (2 patients out of 2). No cases of these associations have been registered in the Northern regions.

Table 3. The test results on viral hepatitis B, C and E markers of TB patients according to their age distribution.

Age (years)	Total	Markers					
		Ag HBs		Anti-HCV		Anti-HEV IgG	
		positive	P±ES	positive	P±ES	positive	P±ES
20-29	13	1	7.7±7.4	1	7.7±7.4	1	7.7±7.4
30-39	40	3	7.5±4.2	3	7.5±4.2	3	7.5±4.2
40-49	54	13	24.0±5.8	3	5.6±3.1	8	14.8±4.8
50-59	40	4	10.0±4.7	4	10.0±4.7	4	10.0±4.7
≥60	53	6	11.3±4.3	7	13.2±4.6	8	15.1±4.9
Total	200	27	13.5±2.4	18	9.0±2.0	24	12.0±2.3

The anti-HEV IgG marker associated with anti-HCV was detected in 22.2±9.8% of cases (4 out of 18 people). Depending on gender, this association showed a seroprevalence of 16.7±9.6% (3 out of 18 people) men, and 5.6±5.4% (1 out of 18 people) in women. The highest age-related seroprevalence was registered in patients aged 30-39 years old – 11.2±7.4% (2 people out of 18). Depending on the geographical distribution, the association of these markers was detected in 18.8±9.8% of cases (3 people out of 16) from the Central area of the country, in 50.0±35.3% of cases (1 person out of 2) from the Southern area, whereas no cases have been reported in the northern area.

DISCUSSIONS

Viral hepatitis remains a challenging public health problem. Many studies show that TB patients are at higher risk of becoming infected with viral hepatitis viruses, including viral hepatitis E (3, 15).

Patients suffering from pulmonary tuberculosis on the underlying viral hepatitis are becoming increasingly sensitive to the hepatotoxic effect of anti-tuberculosis drugs. This is mostly due to the fact that hepatitis infection leads to liver damage, making the organ more susceptible to drug damage (6, 10).

A study conducted by Kim et al. reported that 13.7% of the studied patients treated with anti-TB drugs developed drug-induced liver injury (6). In patients with liver disease, TB treatment may increase the chance of developing liver failure. This means a delayed treatment of tuberculosis in patients suffering from acute hepatitis. However, one study found that patients who received antiviral drugs shortly after being diagnosed with TB had a lower risk of liver damage caused by anti-TB drug hepatotoxicity (3). Based on the risks listed above, a series of worldwide studies set out similar tasks as in the present study to establish the prevalence rates of viral hepatitis in TB patients in order to assess their potential risk (1, 2, 11, 16-19).

In this context, the results obtained by Feleke B.E. et al. (18) found a prevalence of viral hepatitis B of 15.1% (95% CI: 13.92-16.28%) in a sample of 3537 TB patients, which is similar to the present study, which found an HBsAg seroprevalence of 13.5±2.4%.

As regarding the prevalence of hepatitis C virus infection in TB patients, a reference meta-analysis study including 21 studies found a prevalence of 2% to 27%. Based on a random effect model, the overall prevalence was 7% (95% CI: 6-9%) (17). The result of 9.0±2.0% HCV marker seroprevalence obtained in the present study falls within this range. On the other hand, the above- mentioned meta-analysis (17) found that the prevalence of hepatitis C virus in men with tuberculosis is about 10% (95% CI: 14-16%) compared to women, where this rate was significantly lower, namely by 2% (95% CI: 1-4%), indicating a statistically significant difference (p=0.0672). This finding showed that men have a higher risk of developing HCV than women (odds ratio, OR = 2.02 (95% CI: 1.28–3.18). The present study also found higher levels of anti-HCV marker seroprevalence in men compared to women viz. 9.2±2.5%

vs. 8.6±3.4%, though not to the same extent.

Moreover, the seroprevalence of the anti-HVE IgG marker found in the present study, namely, 12.0±2.3% is almost similar to the results obtained in a study, which was conducted in Italy (2013) showing an anti-HEV prevalence of 11.6% (113/973) (16).

Our results suggest that the levels of seroprevalence of viral hepatitis B, C and E markers in patients with tuberculosis from our country are quite high, however, they fit into the global trend related to this area. Thus, special attention should be given to testing and carrying out preventive measures against these infections in order to avoid liver injury during treatment with anti-tuberculosis drugs, as well as their possible association with viral hepatitis.

CONCLUSIONS

1. The TB patients are at higher risk of developing viral hepatitis, being proved by the high seroprevalence of the following markers: HBsAg – 13.5±2.4%; anti-HVC – 9.0±2.0% and anti-HVE IgG – 12.0±2.3%.
2. The simultaneous presence of the anti-HVE IgG marker associated with anti-HCV was detected in 22.2±9.8% of cases, predominantly in men – 16.7±9.6% and among patients aged 30-39 years old in 11.2 ±7.4% of cases.
3. The seroprevalence of the anti-HVE IgG marker associated with HBsAg was found in 25.9±5.1% of cases, predominantly in men – 22.2±8.0% and among patients aged 40-49 years in 24.0±5.8 % of cases.

CONFLICT OF INTERESTS

No conflicts of interest.

ETHICAL APPROVAL

Study Protocol was approved by Medical Ethics Committee of National Center of Disease Control and Public Health (N2018-055; 24.12.2018).

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PREGNANCY-RELATED ANXIETY LEVEL AND ASSOCIATED FACTORS IN EXPECTANT FATHERS: CASE-CONTROL STUDY IN TURKEY

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Keywords: *expectant fathers, pregnancy, anxiety, childbirth, state-trait anxiety inventory.*

Introduction. *Perinatal behavior and involvement of fathers during pregnancy are known to affect maternal health, fetal outcomes, and the emotions and behavior of the infant after birth.* ***Material and methods.*** *This present paper is a case-control study performed on 232 men with pregnant wives (expectant fathers) with no known chronic or psychiatric illnesses and 316 with non-pregnant wives. Two questionnaires STAI forms and a questionnaire developed by the researcher were applied to the participants.* ***Results.*** *The frequency of anxiety in the EF was 9.9% for the STAI-1 and 10.7% for STAI-2. The frequency of anxiety in the control groups was 5.3% for the STAI-1 form and 2.5% for the STAI-2 form. In our study, the mean STAI-1 score of the EFs was 41.2±7.5, the mean STAI-1 score of the controls was 38.0±7.3, and there was a statistically significant difference between each other (p<0.001). The mean STAI-2 score of the EFs was 41.2±7.5, the mean STAI-2 score of the controls was 38.2±6.8, and there was a statistically significant difference between the two groups (p<0.001). The mean STAI-2 score of the EF group was 43.8±7.3 in the first trimester, 41.6±7.7 in the second trimester, and 39.6±7.0 in the third trimester. It was determined that spouses in the 1st trimester had higher anxiety levels than those in the second and third trimesters, according to the STAI-2 form (p=0.008). EF who had a postnatal babysitter, good sleep quality, did not experience a bad event, and were compatible with wives had a significantly lower level of anxiety (respectively p=0.008, p=0.019, p<0.001, p=0.01).* ***Conclusions.*** *Particularly in first pregnancies and the first trimester of pregnancy, father candidates may experience anxiety disorders more frequently and intensely. EF may need psychological support during pregnancy.*

Cuvinte cheie: *viitorii taţi, sarcină, anxietate, naştere, inventar de stări de anxietate.*

NIVELUL DE ANXIETATE ATESTAT ÎN PERIOADA SARCINII ŞI FACTORI ASOCIAŢI LA VIITORII TAŢI: STUDIU DE CAZ REALIZAT ÎN TURCIA

Introducere. *Comportamentul perinatal şi implicarea taţilor în timpul graviditătii afectează considerabil sănătatea mamei, starea fătului, emoţiile şi comportamentul sugarului după naştere.* ***Material si metode.*** *Cercetarea noastră este un studiu de caz realizat pe un lot de 232 de bărbaţi cu soţii însărcinate (eventuali taţi), fără boli cronice sau psihiatrice cunoscute şi pe un grup de control de 316 bărbaţi cu soţii care nu sunt însărcinate. Participanţii au completat Formular STAI şi un chestionar elaborat de cercetător.* ***Rezultate.*** *Frecvenţa anxietăţii la EF este de 9,9% pentru forma STAI-1 şi 10,7% pentru STAI-2. Frecvenţa anxietăţii la controale este de 5,3% pentru forma STAI-1 şi de 2,5% pentru forma STAI-2. În studiul nostru, scorul mediu STAI-1 al EF a fost 41,2±7,5, scorul mediu STAI-1 al controalelor a fost 38,0±7,3 şi a existat o diferenţă semnificativă statistic între ele (p<0,001). Scorul mediu STAI-2 al EF a fost de 41,2±7,5, scorul mediu STAI-2 al controalelor a fost 38,2±6,8 şi a existat o diferenţă semnificativă statistic între ele (p<0,001). Scorul mediu STAI-2 al grupului EF a fost de 43,8±7,3 în primul trimestru, 41,6±7,7 în al doilea trimestru şi 39,6±7,0 în al treilea trimestru. S-a stabilit că soţii din trimestrul I au niveluri de anxietate mai mari decât cei din trimestrul II şi al treilea, conform formularului STAI-2 (p=0,008). EF care au avut o babysitter postnatală, somn de bună calitate, nu au experimentat un eveniment rău şi au fost compatibili cu soţiile au manifestat un nivel de anxietate semnificativ mai scăzut (respectiv, p=0,008, p=0,019, p<0,001, p=0,01).* ***Concluzii.*** *În special în perioada primei sarcini şi în primul trimestru de sarcină, candidaţii la rolul de tată pot prezenta, mai frecvent şi mai intens, tulburări de anxietate. EF pot avea nevoie de suport psihologic în timpul sarcinii.*

INTRODUCTION

Anxiety disorders are unreasonable fears and anxiety that may be accompanied by physical symptoms. Anxiety is a normal response to stress, sadness, or a threat, but if it becomes very intense when faced with a stimulus, it increases rather than decreases over time, and, most importantly, it impairs the person's functionality, thus, anxiety should be considered a pathological disorder (1).

The lifetime prevalence of anxiety disorders has been reported to vary between 13.6% and 28.8%. The 12-month prevalence of anxiety disorders was found to be 6.7% in the Turkish Mental Health Profile (2).

The loss of functionality or decreased quality of life caused by anxiety disorders is equivalent to that of chronic diseases. Although they are familiar and cause significant loss of functionality, the probability of being diagnosed is low, since most of these patients refer to other departments of general medicine rather than psychiatry and undiagnosed anxiety disorder cases lead to high health expenditures (1).

The role of the father during pregnancy is not considered crucial in society. In the prenatal period, however, fathers have a significant influence on the well-being of their children. Perinatal behavior and involvement of fathers during pregnancy are known to affect maternal health, fetal outcomes, and the emotions and behavior of the infant after birth (3, 4).

It is expected that the father-to-be will support his wife during pregnancy and protect the financial and moral peace of the family. Along with these expectations, the obligations arising from the child may cause anxiety in the father (5, 6). *The aim of this study* is to reveal the effects of pregnancy on the anxiety level of expectant fathers and the factors affecting the anxiety level.

MATERIAL AND METHODS

This present study is a case-control study conducted on the spouses of pregnant women who were followed up within the health care institutions in Turkey between March-June 2016. In our study, the spouses of 742 women who applied to Kahramanmaras Sutcu Imam University Health Practice and Research Hospital, Necip Fazıl City Hospital, and Family Health Centers Polyclinics were informed about the study, and 569 participants agreed to take part in the study. Participants were randomly selected for the study. The informed consent was obtained from the patients under study. Twenty-one patients were excluded from the study because they did not submit the forms or receive psychiatric treatment. As a result, 548 individuals were included, 232 expectant fathers (cases) and 316 men whose spouses were not pregnant (controls).

Data collection tools

STAI (State-Trait Anxiety Inventory 1-2) forms and a questionnaire developed by the researcher were applied to the father candidates. The questionnaire questioned the demographic characteristics of expectant fathers, using of cigarettes and other tobacco products (Maras powder), infertility treatment, sleep quality, experiencing bad events, couple compatibility, and marital and pregnancy characteristics. Maras powder is a smokeless tobacco product used sublingually in Turkey.

During the study, we found it appropriate to apply the STAI-1 (State Anxiety Inventory) and STAI-2 (Trait Anxiety Inventory) forms due to their widespread use, high item homogeneity, high internal consistency, and high reliability over time (7, 8). The State-Trait Anxiety Inventory was developed by Spielberger et al. It was adapted and standardized in Turkey by Oner and Le Compte in 1977, as well as its validity and reliability obtained. Cronbach Alpha (α) value was found between 0.94 and 0.96 for the State Anxiety Inventory and between 0.83 and 0.87 for the Trait Anxiety Inventory. The State and Trait Anxiety Inventory is a self-evaluation questionnaire consisting of short statements. The State Anxiety Inventory requires a person to evaluate how they feel at a particular moment and under certain conditions and to respond by considering their feelings about their conditions. The Trait Anxiety Scale, on the other hand, requires the person to evaluate and respond to how they usually feel. These two scales, consisting of twenty questions per each, were applied to the participants on two separate pages and were scored from 1 to 4. Expressed emotions or behaviors intensity is as follows: (1) Not at all, (2) A little, (3) A lot, and (

4) Completely, according to the degree of severity in the State Anxiety Inventory; On the other hand, in the Trait Anxiety Scale, according to the degree of frequency, it is (1) almost never, (2) sometimes, (3) a lot, and (4) almost always (9). Based on the obtained mean anxiety score and standard deviations, as well as considering the relevant literature, it was considered appropriate to have a cut-off score of ≥50 for the anxiety disorder.

Statistical analysis

The data analysis used numbers (n) and percentages to summarize categorical measurements, mean and standard deviation (SD) were used for continuous measurements with a normal distribution, and median (min-max) was used for non-normally distributed variables. While comparing STAI-1 and STAI-2 mean scores, the t-test was used to reveal the differences between the two groups, and the analysis of variance (ANOVA) was used to investigate the difference between groups of three and more. Sidak Pairwise Comparison Test was applied to examine the groups with different variant analyses. Statistically, $p<0.05$ was considered significant. Logistic regression analysis was used with a cut-off score of 50 and above (50≤) to investigate whether independent variables affecting STAI 1 and 2 scores were risk factors. While evaluating the findings obtained in the study, the IBM SPSS (Statistical Package for the Social Sciences) 20.0 statistical package program was used.

Ethics committee approval

Approval was obtained from Kahramanmaras Sutcu Imam University Faculty of Medicine Clinical Research Ethics Committee, based on decision nr. 14, dated 21.03.2016.

RESULTS

A total of 548 individuals were enrolled within the study, including 232 expectant fathers (EF) and 316 men with a non-pregnant partners (control). The mean age of the EFs was 33.7±6.0 (min=23, max=51), and the mean age of the controls was 36.5±7.1 (min=23, max=57). Nearly half of EFs (43.9%) and Controls (53.1%) had a university degree. The spouses of 80 EFs (34.8%) were employed; mean STAI-1 scores were 40.5±6.6, and mean STAI-2 scores were 41.0±6.2. The spouses of 107 (34.0%) of the controls were employed, their mean STAI-1 scores were 37.3±7.9, and their mean STAI-2 score was 38.0±7.3.

EF and control groups were similar regarding their spouses' education, residence, occupation, income status, social security, and employment status ($p>0.05$). Of the EFs, 96 (41.9%) come from nuclear families, and 133 (58.1%) from extended families. Of the controls, 199 (63.8%) had nuclear families (a couple and their dependent children), and 113 (36.2%) had extended families (a family that extends beyond the nuclear family). The family type of EFs differed significantly from the Controls frequency ($p<0.001$). However, there was no significant difference in anxiety scores in STAI-1 ($t=1.21$ and $p=0.23$) and STAI-2 ($t=1.91$ and $p=0.058$) forms between EFs and controls with extended family type. There was no difference revealed. The mean STAI-1 score was 40.0±7.5 in fathers who had a postnatal babysitter and 44.8±7.3 in fathers who did not, and the difference was statistically significant ($p=0.008$). When the EF and Control groups were compared regarding the number of children, the ratio of those with no children, one child, and two children was similar. Only the control group had a statistically higher number of children of 3 and above ($p=0.002$). The STAI-1 score of EFs with three or more children was 41.6±8.7, and the score of Controls was 38.6±7.7 ($t=0.35$ and $p=0.044$). There was no statistical difference in STAI-2 between EF and Control groups with three or more children ($p>0.05$). The STAI-2 score of EFs with children aged 0-1 was 47.7±9.1, and the score of Controls was 39.1±6.9 ($t=0.78$ and $p=0.021$). No statistical difference was found in the STAI- 1 score of EF and Controls with children aged 0-1 ($p>0.05$). No one in the control group was receiving infertility treatment. Only 28 (12.3%) people had received infertility treatment in EFs (tab. 1). In the EF group, the STAI-1 score for those who received infertility treatment was 41.4±6.3, and for those who did not – 41.2±7.6. There was no statistical difference between them ($p>0.05$). The STAI-2 score of those who received infertility treatment was 41.0±9.6, and those who did not receive it were 41.3±7.2. There was no statistical difference between them ($p>0.05$).

It was determined that the participants in the EF and Control groups showed similar sociodemographic data. It was revealed that dissimilar –

sociodemographic data (family type, having children aged 0-1, having more than three children) did not cause anxiety for the control group (tab. 1).

Table 1. Sociodemographic characteristics of the participants.

Indicators		EGE n (%)	Control n (%)	P*
Education status	Elementary and below	44 (20.5)	61 (21.0)	0.46
	Senior high school	76 (35.5)	75 (25.9)	
	University and above	94 (43.9)	154 (53.1)	
Residential area	Provincial center	181 (83.8)	232 (85.0)	0.65
	District	21 (9.7)	26 (9.5)	
	Village-Town	14 (6.5)	15 (5.5)	
Income status	Income less than expenses	33 (14.6)	64 (20.6)	0.12
	Income equals expenses	141 (62.4)	183 (58.8)	
	Income more than expenses	52 (23.0)	64 (20.6)	
Partner's employment status	Yes	80 (34.8)	107 (34.0)	0.84
	Not working-Housewife	150 (62.2)	208 (66.0)	
Status of having children	No child	42 (18.1)	39 (12.3)	0.002
	One child	75 (32.3)	76 (24.1)	
	Two child	75 (32.3)	109 (34.5)	
	Three and above child	40 (17.2)	92 (29.1)	
Number of children	Number of 0-1-year- old children	4 (1.7)	59 (18.7)	0.021
	Number of children aged 1-5	13 (5.6)	30 (12.9)	
Family type	Nuclear family	96 (41.9)	199 (63.8)	0.001
	Extended family	133 (58.1)	113 (36.2)	
Infertility Treatment	Yes	28 (12.3)	-	-
	No	199 (87.7)	-	
	Total	**232 (100.0)**	**316 (100.0)**	

EGE: Expectant fathers, n = frequency, % = column percentage * Chi-square test

Almost all the EFs (90.9%) evaluated themselves as a compatible couple. The mean STAI-1 scores of those with a compatible couple in the EFs were 40.8±7.1. It was determined that the mean STAI-1 scores of men with a compatible couple in the EFs were lower than those of men who were incompatible with their spouses and said they had no idea (t=2.58, p=0.01). The mean STAI-2 scores of the participants with good sleep quality in the EF group were 39.6±6.6. It was determined that those with good sleep quality had lower average STAI-1 scores than those with poor sleep quality (F=4.02, p=0.019). The mean STAI-1 score of those who experienced a bad event was 41.5±7.5, and the mean STAI-1 score of those who did not experience a bad event was 38.6±7.4. It was determined that the mean STAI-1 scores of those who experienced a bad event were higher than those who did not (t=3.76, p<0.001). About one out of every ten control group members rated themselves as a compatible couple. The mean STAI-1 scores of those with a compatible couple in the Controls were 38.2±7.2. In the control group, there was no statistical difference between the anxiety scores of the men who evaluated themselves as a compatible couple and those who evaluated themselves as incompatible or having no idea (t=1.32, p>0.05). The mean STAI-1 scores of the participants with good sleep quality in the Control group were 36.1±7.2. It was determined that those with good sleep quality had lower average STAI-1 scores than those with moderate or poor sleep quality (F=6.76, p=0.001). In the control group, the mean STAI-1 score of those who experienced a bad event was 40.7±6.7, and the mean STAI-1 score of those who did not experience a bad event was 37.1±7.3. It was determined that the mean STAI-1 scores of those who experienced a bad event were higher than those who did not (t=3.76, p<0.001). The results of the participants' other risk factors are given in Table 2.

Table 2. Risk factors of participants.

		EF n (%)	Control n (%)
Smoker	Yes	132 (56.9)	129 (40.8)
	No	100 (43.1)	187 (59.2)
No Caregiver After Birth	Yes	43 (18.5)	-
	No	189 (87.9)	-
Using smokeless tobacco (Maras powder)	Yes	28 (12.1)	47 (14.9)
	No	204 (87.9)	269 (85.1)
Social security	Yes	220 (94.8)	296 (93.7)
	No	12 (5.2)	20 (6.3)
Compatible couple	Yes	211 (90.9)	282 (89.2)
	No-No İdea	21 (9.1)	34 (10.7)
Bad event	Yes	47 (20.3)	76 (24.1)
	No	180 (78.9)	239 (75.9)
Good sleep quality	Good	89 (38.4)	117 (37.0)
	Medium	119 (51.3)	159 (50.3)
	Bad	24 (10.3)	40 (12.7)

EF: Expectant fathers, n = frequency, % = column percentage

Based on the STAI-1 form, 7.2% of participants scored 50 and above. Based on the STAI-2 form, 6.0% of participants scored 50 and above. The frequency of anxiety in the EF is 9.9% for STAI-1 form and 10.7% for STAI-2. The frequency of anxiety in the controls was 5.3% for STAI-1 form and 2.5% for STAI-2 form. Therefore, the spouse's pregnancy can cause anxiety disorders in men. The results obtained are given in Table 3.

Table 3. Comparing the EF and Control groups, based on their form 1 and form 2 scores.

Score	Group	n	Average	SD	Min-max	t	p*
STAI-1 SCORE	EF	232	41.2	7.50	25-71	5.02	0.01
	Control	316	38.0	7.38	20-55		
STAI-2 SCORE	EF	232	41.2	7.53	24-75	4.90	0.01
	Control	316	38.2	6.84	22-54		

EF: Expectant fathers, STAI: State-Trait Anxiety Inventory, * Student's t-test

In the study, variance analysis was performed to compare the trimester scores of the EF group with form 1 and form 2. The Sidak pair-wise comparison test was used to determine the different groups. The obtained results are shown in Table 4 and Figure 1. For expectant fathers, it was determined that having spouses of Trimester 1, 2, or 3 affected STAI-2 scores (F=4.98, p=0.008). It was determined that the difference was because fathers with spouses in the 1st trimester have higher STAI-2 scores than those in the 2nd and 3rd trimesters.

Table 4. Comparing the trimester and form 1 and 2 scores for the EF group.

Group	Score	Trimester	n	Average	SD	F	p	Dual comparison
EF	STAI-1 Score	0-3. months	46	41.5	7.43	1.47	0.23	
		4-6. months	95	42.0	7.76			
		7-9. months	90	40.2	7.25			
		Total	**231**	**41.2**	**7.51**			
	STAI-2 Score	0-3. months	46	43.8	7.37	4.98	0.008	0-3. months> 4-6 months and 7-9. months
		4-6. months	95	41.6	7.78			
		7-9. months	90	39.6	7.03			
		Total	**231**	**41.3**	**7.54**			

EF: Expectant fathers, STAI: State-Trait Anxiety Inventory, *Oneway ANOVA

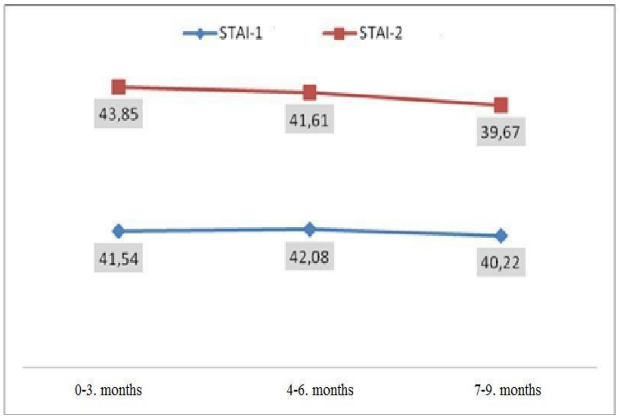

Figure 1. Comparison of STAI form 1 and 2 scores by trimester for the EGE group.

According to Table 5, smoking, having no postnatal caregiver, no social security, and Maras powder were independent risk factors affecting the STAI-1 scale. If the significant variables are interpreted, smoking increases the probability of anxiety 2.5 times, not having a caregiver after birth 1.6 times, using Maras powder 1.5 times, and not having social security 1.2 times.

According to Table 6, variables like smoking, not having a caregiver after birth, and using Maras powder were independent risk factors affecting the STAI-2 scale. If the significant variables are interpreted, smoking status increases the probability of anxiety by 2.3 times, not having a caregiver after birth 1.6 times, and using Maras powder 1.8 times.

Table 5. STAI-1 and risk factors.

MODEL	Wald	p	Odds Ratio	95% GA Lower Limit	95% GA Upper Limit
Smoker	8.2	0.01	2.5	1.5	3.3
No Caregiver After Birth	6.9	0.01	1.6	1.2	2.1
Maras powder	2.6	0.02	1.5	0.3	1.9
No Social Security	5.9	0.01	1.2	0.6	1.5

ModelX^2: 30.13; Success rate = 90%, Cox & Snell R^2= 0.401

Table 6. STAI-2 and risk factors.

MODEL	Wald	p	Odds Ratio	95% GA Lower Limit	95% GA Upper Limit
Smoker	6.6	0.01	2.3	1.4	3.3
No Caregiver After Birth	5.9	0.01	1.6	1.0	2.1
Maras powder	2.9	0.02	1.8	0.9	2.4

ModelX2: 26.29; Success rate = 88%, Cox & Snell R^2= 0.362

DISCUSSIONS

Anxiety disorders are among the most common psychiatric disorders and are an important public health problem. According to the Epidemiologic Catchment Area Program (ECA) data, the one-year incidence of anxiety disorders is 10.1%, while its lifetime prevalence is 14.6% (10, 11). While there are many studies on depression, common during pregnancy, in the world and our country, the number of studies on anxiety disorders is very few. Still, we could not find studies on anxiety disorders in men whose spouses are pregnant because of our literature review.

In the compilation of Paulson and Bazemore, the 12-month prevalence of depression in fathers was found to be 10.4% on average, considering the prenatal period (12). In the study carried out by Wynter et al., in which depression and anxiety disorders were evaluated in fathers together, the frequency of anxiety disorders was 12.2% that was higher than depression (13). In a study conducted by Leach et al. in 2016, the prevalence of men's anxiety was between 4.1% and 16.0% in the prenatal period (14). In the qualitative study conducted by Serçekuş et al. in Turkey, it was emphasized that prospective fathers are afraid of complications that may develop during childbirth (15).

In the study by Keeton et al. USA, using the STAI-1 scale, the rates of anxiety disorders in expec-

tant fathers were found to be 16% in the third trimester, 12% in the first month postpartum, 16% in the fourth month postpartum, and 18% in the sixth month postpartum (16). In the study of Figueiredo and Conde from Portugal using the STAI-1 scale, the rate of anxiety disorders in expectant fathers was 10.1% in the first trimester, 8.0% in the second trimester, 7.8% in the third trimester, 8.5% between 1 and 3 days after delivery, 4.4% in the third postpartum month (17). In the study of Tohotoa et al. from Australia using the HADS-A scale in expectant fathers, the rate of anxiety disorders was found to be 4.1% during pregnancy and 2.4% in the 6th month after delivery, which was the lowest rate in all studies (18). Therefore, the rate of anxiety disorders during pregnancy was found to be high in all studies of expectant fathers. While the rate of expectant fathers' anxiety disorders in the postpartum period was lower in most studies, Keeton et al. and Condon et al. maintain their rates during pregnancy (16, 19).

Based on the STAI-1 form in our study, 7.2% of participants scored 50 and above. Based on the STAI-2 form, 6.0% of participants scored 50 and above. The frequency of anxiety in the EF was 9.9% for STAI-1 form and 10.7% for STAI-2. Our study found similar rates to those in specialized literature worldwide (16-18).

Considering men with no children and whose spouses are pregnant with their first child, 10.1% of participants scored 50 and above in the STAI-1 form. Based on men with no children and whose spouses are pregnant with their first child, 11.3% of the participants scored 50 and above in the STAI-2 form. Similar rates were found in the study of Quinlivan and Condon conducted during pregnancy in men whose spouses were pregnant with their first child. Thus, the frequency of anxiety disorders is especially higher in men whose spouses are pregnant with their first child. For this reason, as emphasized in Bergström's study on 812 men, more attention should be paid to anxiety disorders, and the necessary social support should be given to young and first-time fathers (20).

Studies on anxiety disorders in fathers have gained more importance and frequency, especially in recent years worldwide. When the average scores obtained from the STAI-1 forms in the literature worldwide are examined, these vary between 28.8-37.5. Unlike other studies, the average STAI-1 scores found by Latifses are 45.3 and 50.2 (14). Different anxiety scores were obtained in studies. The difference may be due to different study designs (STAI or HADS-A), sociocultural structures, and trimester periods.

Ekelin et al. (21) found the mean STAI-1 score to be 28.8±8.7 in the 2nd trimester and 29.4±8.6 in the 3rd trimester in the study performed on the spouses of 652 pregnant women who came for examination and had expected ultrasonography results. In the survey conducted by Johnson and Baker on men whose spouses were pregnant, the mean STAI-1 score was found to be 37.5±9.0 (22). In the study of Field et al. in the second trimester of men whose spouses were pregnant, the mean STAI-1 score of depressed fathers (n=106) was 44.2±8.7, and the mean STAI-1 score of non-depressed fathers (n=50) was 34.9±7.7, and the mean STAI-1 score of father candidates was 36.3±17.9 detected (23).

In our study, the mean STAI-1 score of the EFs was 41.2±7.5, the mean STAI-1 score of the controls was 38.0±7.3, and there was a statistically significant difference between each other ($t=5.02$, $p<0.001$). The mean STAI-2 score of the EFs was 41.2±7.5, the mean STAI-2 score of the Controls was 38.2±6.8, and there was a statistically significant difference between each other ($t=4.90$, $p<0.001$).

In our study, the mean STAI-1 score of the EF group was 41.5±7.4 in the first trimester, 42.0±7.7 in the second trimester, and 40.2±7.2 in the third trimester. It was found that there was no statistically significant effect of trimesters on the mean anxiety score according to the STAI-1 form. The mean STAI-2 score of the EF group was 43.8±7.3 in the first trimester, 41.6±7.7 in the second trimester, and 39.6±7.0 in the third trimester. It was determined that spouses in the 1st trimester have higher anxiety levels than those in the second and third trimesters, according to the STAI-2 form ($F=4.98$, $p=0.008$). In our study, anxiety scores were significantly higher in the first trimester compared to STAI-2 scores. Health risks that may arise in the first trimester, such as nausea and vomiting disorders, and lifestyle changes that occur during pregnancy, may have affected first-trimester anxiety scores.

There may have been a decrease in anxiety scores in the 2nd and 3rd trimesters with the adaptation to the lifestyle changes brought about by pregnancy. According to this result, while state anxiety was not significant in the first trimester, trait anxiety was significant.

In the study conducted by Bergström et al. (20), it was found that the risk of depressive symptoms increased in prospective fathers with bad partner relationships. It was determined that the mean STAI-1 scores of men with a compatible couple in the EFs were lower than those of men who were incompatible with their spouses and said they had no idea (p=0.01). The study carried out by Field et al. (23), expectant fathers' anxiety, anger, and daily difficulty scores were found to be higher. In our study, it was determined that the mean STAI-1 scores of those who experienced a bad event were higher than those who did not (p<0.001). In addition, fathers who were postpartum caregivers had significantly lower STAI-1 anxiety levels (p=0.008). Tohotoa et al. (18) found that the anxiety level of fathers who were told about sleep problem-solving strategies decreased. In our study, father candidates with poor sleep were found to have significantly higher levels of anxiety (p=0.019). Improving the socioeconomic status of expectant fathers may contribute to reducing their anxiety levels of expectant fathers.

In the prospective study of Alibekova et al., it has been determined that smoker father candidates show more anxiety symptoms before and after birth than non-smoking fathers (24). When we evaluated our study, not assigning a babysitter after birth, smoking, and using Maraş powder were risk factors for anxiety disorders.

It was proved that the average STAI-1 scores in studies conducted abroad ranged from 28.8-37.5. Our averages were higher than those of the other studies conducted abroad. However, in the handbook of Öner and Le Compte, the mean scores assessed on most Turkish adults using the STAI form were similar to the mean scores obtained in our study (7). Today, in many studies conducted in Turkey using the STAI form, the mean STAI scores of individuals vary between 36 and 53 (25, 26). The difference between the Turkish and international mean scores is probably due to a more emotional structure of Turkish society. In our culture, men are expected to be strong and protective. For this reason, men are more introverted and may not express themselves as openly as women (5). Considering all these, as Öner and Le Compte emphasized, each population should be evaluated within itself and compared with the average scores obtained from healthy individuals (7).

There were some limitations to our study. The clinical interview is the gold standard for diagnosing anxiety disorder. However, the STAI form is the most common anxiety scale preferred worldwide, which is sufficient for self-evaluation survey studies with its high sensitivity. However, since the association between depression and anxiety is common, more useful results can be obtained using another questionnaire that also assesses depression. Another limitation is that since there is no study on the subject in Turkey, comparisons are made with studies abroad, not in Turkish society. In our study, the rate of university graduates was higher. The extended family type was more frequent in EFs. This frequency may be because the caring parents began to live together with the family. Larger field studies are needed to reflect society more accurately.

CONCLUSIONS

1. Our study found that the frequency and severity of anxiety disorders increased in men whose spouses were pregnant. This difference was particularly evident in young fathers whose spouses were pregnant with their first child. Simultaneously, it was observed that fathers had higher anxiety levels, particularly during the first trimester of pregnancy. Our results show that anxiety disorders during pregnancy are not unique only to women, and expectant fathers also suffer from anxiety disorders.
2. The expectant fathers who had a postnatal babysitter, good sleep quality, and did not experience a bad event, were compatible with wives, and used tobacco derivatives had a significantly lower level of anxiety. Improving the socioeconomic status of expectant fathers may contribute to reducing their anxiety levels of expectant fathers.

3. Patients should also be evaluated psychologically, regardless of the reason for admission, to avoid anxiety disorders, which is a significant public health problem. Expectant fathers may need psychological support during pregnancy. Physicians should evaluate the mother and the father-to-be from a providing psychological perspective.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest regarding the content of this article. The Authors report no financial support regarding the content of this article.

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ETHICAL APPROVAL

Approval was obtained from Kahramanmaras Sutcu Imam University Faculty of Medicine Clinical Research Ethics Committee with decision number 14 dated 21.03.2016, indicating that there was no ethical and scientific objection to the study.

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DETECTION OF MUTATIONS AND FUSIONS IN LUNG ADENOCARCINOMA USING ION TORRENT SEQUENCING TECHNOLOGY

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Keywords: *sequencing, mutations, fusions, targeted therapy, gene panel.*

Introduction. *Adenocarcinomas are the most common lung tumors and are often diagnosed in advanced stages when the tumor has a polyclonal form with a wide variety of genetic alterations and activated mutational processes. Comprehensive analysis of mutational status from FFPE tissue samples in such patients can provide a therapeutic perspective and contribute considerably to clinical decisions thereby increasing the overall survival rate.* ***Material and methods.*** *22 genes were analyzed for mutations and 4 genes for fusion transcripts using two Ion AmpliSeq panels. DNA was isolated from paraffin-embedded tissue sections while RNA analysis was performed using two types of samples: paraffin blocks and fresh tumor tissue. Key variant detection and data analysis was performed using next platforms: Ion Reporter, ONCOMINE, R language.* ***Results.*** *The study of 30 tumor samples allowed the detection of 147 mutations and 4 fusions in 19 genes, and the therapeutically actionable variants were associated with different drugs clinically approved or in the phase of clinical trials. The most genetic variants were identified in the TP53, EGFR and NOTCH1 genes with a prevalence of over 50% in the TP53 gene, while all 4 detected fusions (one fusion per sample) represent the association of the ALK gene with other partners: EML4(13)-ALK(20) – present in 2 samples; EML4(6)-ALK(20); and an ALK fusion with an unknown partner gene.* ***Conclusions.*** *Analyzing the mutational status of tumor samples from patients with lung adenocarcinoma it has been ascertained the therapeutic utility of gene panel sequencing covering point mutations, INDELs and SNVs, as well as gene fusions, using FFPE tissue as primary material. This is valid for both targeted monotherapies and combined therapies.*

Cuvinte cheie: *secvențiere, mutații, fuziuni, terapie țintită, panel de gene.*

DETECȚIA MUTAȚIILOR ȘI A FUZIUNILOR ÎN ADENOCARCINOMUL PULMONAR FOLOSIND TEHNOLOGIA DE SECVENȚIERE *ION TORRENT*

Introducere. *Adenocarcinoamele sunt cel mai frecvent atestate tumori pulmonare și adesea sunt diagnosticate în stadiile avansate, când tumoarea are formă policlonală, cu o varietate mare de alterații genetice și procese mutaționale activate. Analiza comprehensivă a statutului mutațional din probe de țesut FFPE la astfel de pacienți poate oferi o perspectivă terapeutică și contribuie considerabil la luarea deciziilor clinice, astfel determinându-se creșterea ratei de supraviețuire globală.* ***Material și metode.*** *Au fost analizate 22 gene pentru determinarea mutațiilor și 4 gene pentru identificarea transcripților de fuziune în baza a două paneluri Ion AmpliSeq. ADN-ul a fost izolat din secțiuni de țesut parafinat, în timp ce analiza ARN-ului a fost realizată utilizându-se două tipuri de probe: blocuri de parafină și țesut tumoral proaspăt. Detectarea variantelor cheie și analiza datelor a fost efectuată cu ajutorul platformelor Ion Reporter, ONCOMINE și limbajul R.* ***Rezultate.*** *Studierea a 30 de probe tumorale a permis detectarea a 147 mutații și 4 fuziuni în 19 gene, iar variantele care pot fi acționate terapeutic au fost asociate cu diferiți agenți medicamentoși aprobați clinic sau aflați în faza de studii clinice. Cele mai multe variante genetice au fost identificate în genele TP53, EGFR și NOTCH1 cu o prevalență de peste 50% în gena TP53, iar cele 4 fuziuni (câte o fuziune per probă) reprezintă în totalitate asocierea genei ALK cu alți parteneri: EML4(13)-ALK(20) – prezentă în 2 probe; EML4(6)-ALK(20); și o fuziune ALK cu o genă partener necunoscută.* ***Concluzii.*** *Analizând statutul mutațional al probelor tumorale de la pacienții cu adenocarcinom pulmonar, s-a constatat utilitatea secvențierii panelurilor de gene care acoperă atât mutațiile punctiforme, INDEL-urile (inserții și deleții mici) și SNV-urilor (variațiile unui nucleotid), cât și a fuziunilor genice, cu utilizarea ca material primar a țesutului FFPE, pentru selectarea celor mai potrivite terapii atât din perspectiva aplicării monoterapiilor țintite, cât și a terapiilor combinate.*

INTRODUCTION

Although lung adenocarcinomas (LUAD) are the most common lung tumors, they are often diagnosed late when local tissue invasion is well advanced and metastasis is present. Genetic alterations such as somatic mutations or gene fusions are often associated with the development of these tumors and molecular profiling allows to approach the correct treatment strategy. According to The Cancer Genome Atlas (1), patients with this type of tumors show mutations in the tumor suppressor TP53 overlapping with other oncogenic driver alterations such as KRAS, EGFR, BRAF, ERBB2 mutations or ALK, RET, ROS1 gene fusions all of which have potential therapeutic implications. The complete genetic profile of LUAD is not simple to establish in routine clinical practice since this often involves the use of invasive techniques, the availability of a well-equipped molecular biology laboratory with qualified personnel and, last but not least, involves high costs when performing the analyses. Although a few years ago experts in the field recommended only EGFR testing in advanced cancers for this type of tumor, more recent recommendations include testing for EML4-ALK fusions. However, advances in molecular profiling suggest the need to increase the number of molecular targets that need to be tested in any stage of lung adenocarcinoma (2). This will provide a more comprehensive genetic picture of the tumor and, respectively, a more effective treatment strategy.

Aim: in our study we proposed the use of Ion Torrent sequencing technology for the detection of mutations and fusions in the tumors of patients with lung adenocarcinomas, and for fusion transcripts – two types of RNA: the first, isolated from FFPE samples (Formalin-fixed, paraffin-embedded), and the second – from fresh tumor tissue. The research was carried out in the Cancer Biology Scientific Laboratory within the Oncological Institute of Moldova during the years 2021-2022.

MATERIAL AND METHODS

The study analyzed 30 tumors taken from 30 patients with the histological diagnosis of lung adenocarcinoma with a content of more than 20% tumor cells per sample. DNA was isolated from paraffin-embedded tumor tissue sections (3 10-µm sections), and RNA from both paraffin-embedded sections (4 15-µm sections) and fresh tissue. Purification of nucleic acids was performed using the *RecoverAll Total Nucleic Acid Isolation Kit (Invitrogen, ThermoFisher Scientific)*. The *Ion AmpliSeq Colon and Lung Cancer Research Panel v2* was used for mutation detection, which includes 22 genes (KRAS, EGFR, BRAF, PIK3CA, AKT1, ERBB2, PTEN, NRAS, STK11, MAP2K1, ALK, DDR2, CTNNB1, MET, TP53, SMAD4, FBX7, FGFR3, NOTCH1, ERBB4, FGFR1, FGFR2) and for the identification of fusion transcripts – the *Ion AmpliSeq RNA Fusion Lung Cancer Research Panel*, which allows the determination of more than 70 transcripts in the ALK, RET and ROS1 genes in only 1% of RNA used. The performance of the genomic investigations was evaluated by assessing the number of reads, the average coverage of the bases (Coverage) in the regions of interest, as well as the imbalance of the expression of the 3'/5' amplicons in the case of translocations. For all samples, a maximum performance was obtained, the number of reads exceeding 100,000 and the average base coverage being over 500x. To determine the fusions, the 3'/5' imbalance thresholds recommended by *Ion Reporter* (3, 4) were respected. The average charge density of the chip as well as the ISP (Ion Sphere Particles) details of the experiment are shown in Figure 1.

Detection of key variants and data analysis was performed using Ion Reporter platforms (AmpliSeq Colon and Lung Cancer v2 single sample and AmpliSeq RNA Lung Fusion – w1.2 – Single Sample workflows), ONCOMINE (5), R language (6, 7).

RESULTS

Following the isolation of the RNA samples, a mean concentration of 20.92 ng/µL (non-normal distribution, SD=13.73; Median=15.90; IQR= 12.80) was obtained for the paraffin-embedded tissue samples and a mean 11.17 ng/µL (non-normal distribution, SD=7.12; Median=10.20; IQR=4.64) for fresh tissue. The mean DNA concentration was 7.57 ng/µL (non-normal distribution, SD=7.26; Median=4.40; IQR=8.38). Regarding the average read length, this was 69 nucleotides (non-normal distribution, SD=15.95; Median=72; IQR=29) for the RNA extracted from FFPE sections, 111 nucleotides (non-normal distribution normal, SD=25.54; Median=102;

IQR=36) for RNA from fresh tissue and 117 for DNA (non-normal distribution (fig. 2), SD=4.11; Median=118; IQR= 2.75).

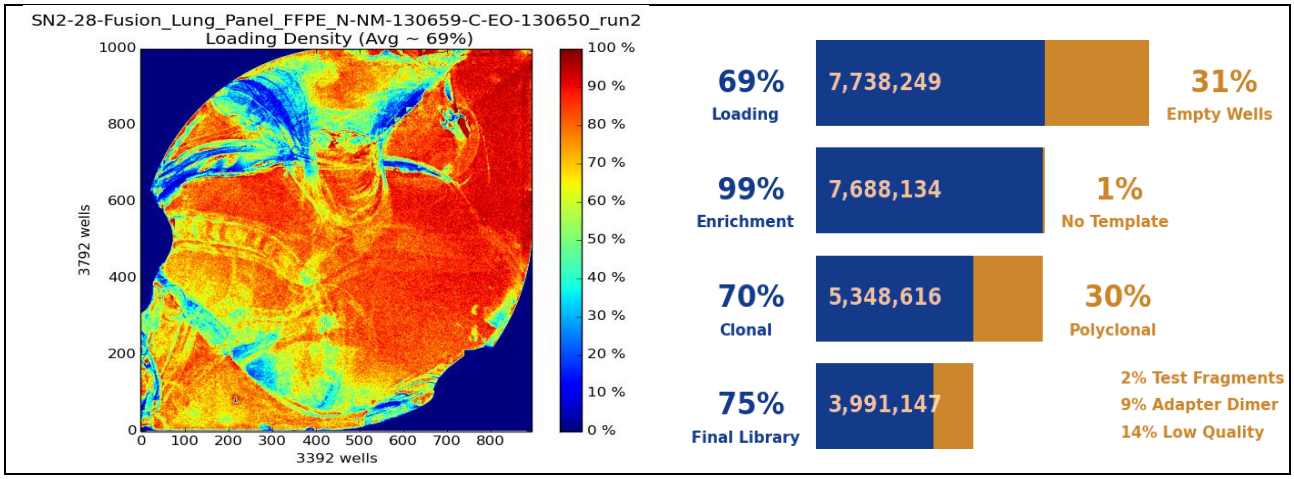

Figure 1. Details of the sequencing experiment (left – average chip load density, right – ISP details).

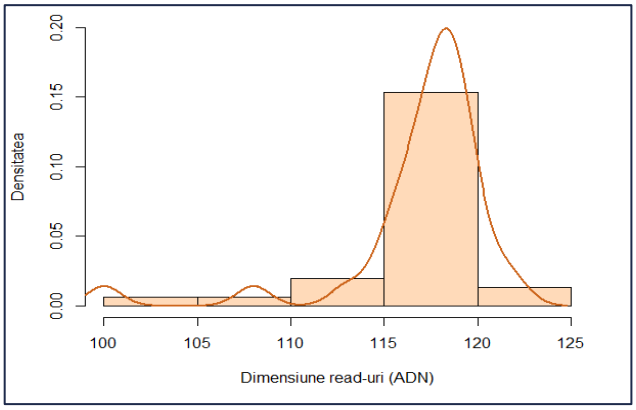

Figure 2. Data distribution with reference to size of reads (DNA).

Thus, 4 samples were identified with a 3'/5' imbalance score indicating the presence of 4 fusions (one fusion per sample): EML4(13)-AK(20) – present in 2 samples; EML4(6)-ALK(20); and an ALK fusion with an unknown gene. Only one sample had an equivocal score for RET gene translocation. Regarding DNA sequencing, of the 22 genes included in the panel, mutations were detected in 18 (EGFR, TP53, NOTCH1, CTNNB1, MET, BRAF, KRAS, STK11, DDR2, SMAD4, NRAS, ERBB4, PTEN, AKT1, PIK3CA, FGFR3, FGFR2, FGFR1) and in 13 (EGFR, TP53, NOTCH1, CTNNB1, MET, BRAF, KRAS, STK11, ERBB4, PTEN, AKT1, PIK3CA, FGFR3) – the presence of so-called key variants. Key variants in cancer are biologically relevant mutations that induce cell proliferation. A summary of the identified mutations is presented in Table 1.

Table 1. Summary of variants identified in samples following DNA sequencing.

The total number of genetic variants	147
Types of variants detected	91
Key variants	77
Non-key variants	14
Maximum number of variants detected per sample	28
Minimum number of variants detected per sample	2
Average number of variants detected per sample	4.9

Most variants were identified in the TP53 gene with 51% of the total number of mutations detected. The EGFR gene follows with 17.7% and NOTCH1 with 7.5% (fig. 3).

The top of the key variants by the number of samples in which they appear is shown in Figure 4.

The *NOTCH1 V1578del* deletion that is the most frequent key variant, according to the ONCOMINE platform, is not associated with relevant therapies both in lung adenocarcinoma and in other types of cancer, however, from the results obtained, a major frequency of this mutation is observed with tumors positive for ALK fusions, *NOTCH1 V1578del* being present in 3 of 4 ALK positive samples.

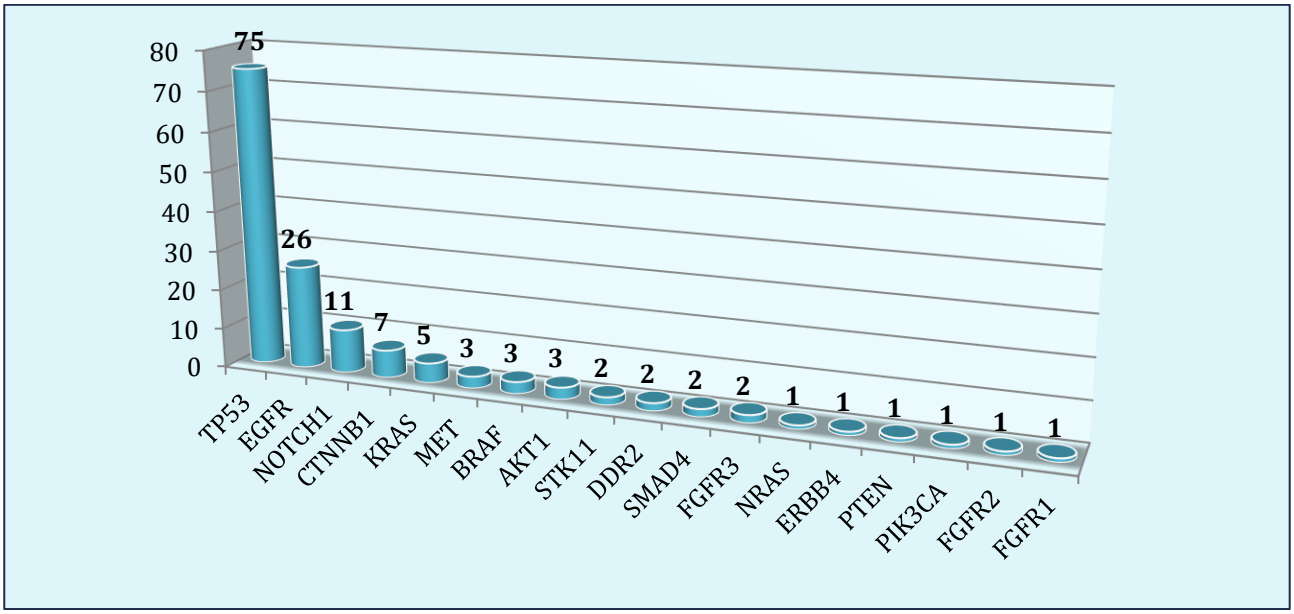

Figure 3. Number of mutations identified per gene.

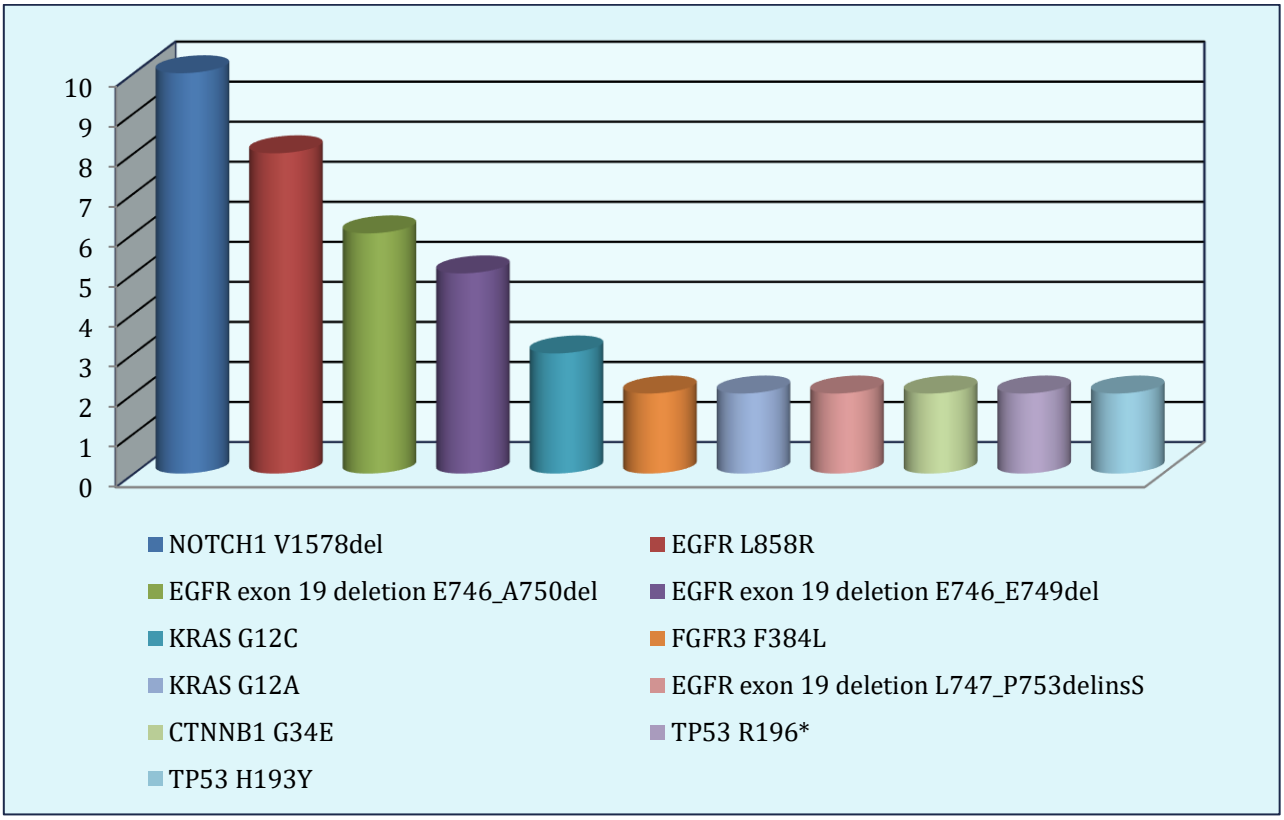

Figure 4. Top of key variants by number of samples.

DISCUSSIONS

The shorter length of reading units in the case of RNA from paraffin-embedded tissue is explained by its molecular degradation during the process of obtaining paraffin blocks. However, following sequencing, reproducibility of results for fusion transcripts could be observed in both types of RNA samples (FFPE and fresh tissue). According to the specialized literature (8, 9) gene fusions

EML4(13)-ALK(20) and EML4(6)-ALK(20) are the most common EML4-ALK variants that appear in non-small cell lung cancer and constitute around of 75-80% of all translocation variants. Treatment options for ALK-positive lung adenocarcinomas are: Crizotinib (Xalkori or Crizalk), Ceritinib (Zykadia), Alectinib (Alecensa), Brigatinib (Alunbrig), and Lorlatinib (Lorbrena).

Regarding the *L858R* substitution and deletions in EGFR, they are associated with the following therapies: afatinib, bevacizumab + erlotinib, dacomitinib, erlotinib, erlotinib + ramucirumab, gefitinib, osimertinib, atezolizumab + bevacizumab + chemotherapy, bevacizumab+gefitinib, gefitinib + chemotherapy, Osimertinib + chemotherapy. *KRAS G12C* and *KRAS G12A* genetic variants associate with cabozantinib in other cancers, and only KRAS G12C can be targeted with sotorasib in non-small cell lung cancer (5).

The research carried out allowed the evaluation of the most important gene mutations and fusions in 30 samples of lung adenocarcinoma as well as their association with different drugs clinically approved or in the phase of clinical trials (5). Analysis of RNA from two types of samples (FFPE and fresh tissue) was performed and the same results were obtained. We can mention that Ion Torrent sequencing is a suitable technique for evaluating genetic alterations in lung adenocarcinoma and can contribute to the best clinical decisions. Advances in tumor genome sequencing and the identification of druggable molecular targets favor the selection of the most effective therapies in the treatment of cancer, however there are some cost barriers to the widespread translation of these technologies into clinical practice. In order to obtain maximum benefits from the exploration of the tumor genome, well-equipped molecular biology laboratories are needed, with qualified personnel both in terms of laboratory analysis and for the use of bioinformatics algorithms so that the molecular data can be effectively interpreted, integrated with the clinical profile and reflected in clinical reports.

CONCLUSIONS

1. Research allowed reproducibility of results for fusion transcripts in both types of RNA samples (FFPE and fresh tissue).
2. The obtained results confirm the need for clinical testing for EML4-ALK fusions in patients with lung adenocarcinoma.
3. As KRAS mutations are among the top key variants after EGFR mutations, testing for actionable genetic variants of the KRAS gene in patients with lung adenocarcinoma is of increased interest.
4. Further studies are needed to confirm or refute a possible relationship between ALK gene fusions and the NOTCH1 V1578del mutation.

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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ETHICAL APPROVAL

This study was carried out with the approval of the National Ethical Expertise Committee of Clinical Study No. 1086/28.04.2021 (2 SOFT/1.2/207 LUNGNEX-RD) and of the Ethics/Bioethics Committee no. 14, 05-18/21/01.02.2021 of PMSI Oncological Institute, Republic of Moldova (State Program 20.80009.8007.02).

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BACTERIUM WITH LEUCONOSTOC PSEUDOMESENTEROIDES

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Keywords: *Leukonostoc pseudomesenteroides, congenital malformation, bacteremia, Vancomycin resistant, Amoxicillin clavulanate.*

Introduction. *Leukonostoc pseudomesenteroides until recently was classified as a commensal microorganism, but from 1985 until now several sporadic cases of infection with this pathogen have been described in humans. Bacteremia with Leuconostoc spp. are increasingly reported. Unique cases of infection of other sites have also been described. Most often, these cases have been reported in patients with cancer, and are often considered opportunistic for acquired immunodeficiencies. However, several cases have also been reported in immunocompetent patients.*

Material and methods. *An analysis of the literature and the clinical case are presented here: an 11-month-old child with congenital malformation – rabbit lip, who had pneumonia 2 months before his admission to the Toma Ciorba Infectious Diseases Clinical Hospital.*

Results*. Meningococcemia and meningitis were clinically established upon admission. A few days later the blood culture result was with Leuconostoc pseudomesenteroides. The etiological treatment was corrected and the sterile result of the blood culture was obtained. The child was released in a satisfactory condition.*

Conclusions. *The clinical case presented is the first case of infection with Leuconstoc spp. described in the Republic of Moldova. The correct choice of the etiological treatment allows the eradication of this pathogen.*

Cuvinte cheie: *Leuconostoc pseudomesenteroides, malformație congenitală, bacteriemie, Rezistent la Vancomycin, Amoxicilina Clavulanat.*

BACTERIEMIE CU LEUCONOSTOC PSEUDOMESENTEROIDES

Introducere. *Leuconostoc pseudomesenteroides până nu demult a fost catalogat drept microorganism comensal, însă din 1985 până în prezent au fost descrise câteva cazuri sporadice de infecție cu acest agent patogen la om. Bacteriemiile cu Leuconostoc spp. sunt tot mai frecvent raportate. Au fost descrise cazuri unice de infectare și ale altor situsuri. Cel mai des, aceste cazuri au fost atestate la pacienții cu stări canceroase, fiind deseori apreciate drept oportuniste imunodeficiențelor dobândite. Însă, și la imunocompetenți au fost descrise câteva cazuri.*

Material și metode. *Este prezentat o analiză a literaturii și un caz clinic al unui copil de 11 luni cu malformație congenitală – cheloschizis (buza de iepure), suportând pneumonie cu 2 luni înainte de internare.*

Rezultate. *La internare au fost stabilite clinic meningococcemia și meningita. Peste câteva zile rezultatul hemoculturii a permis identificarea de Leuconostoc pseudomesenteroides. A fost corectat tratamentul etiologic și obținut rezultatul steril al hemoculturii. Copilul a fost externat în stare satisfăcătoare.*

Concluzii. *Cazul clinic prezentat este primul caz de infecție cu Leuconostoc spp. descris în Republica Moldova. Alegerea corectă a tratamentului etiologic permite eradicarea acestui agent patogen.*

L. – *Leuconostoc*; **spp.** – species; **CNS** – Central Nervous System; **PMSI** – Public Medical Sanitary Institution; **IDCH** – Infectious Diseases Clinical Hospital; **SpO_2** – peripheral blood oxygen saturation; **ESR** – erythrocyte sedimentation rate, **ALT** – alanine aminotransferase; **AST** – aspartate aminotransferase, **Anti-CMV-IgG** – Antibodies Immunoglobulin G to *Cytomegalovirus*, **Anti-CMV IgM** – Antibodies Immunoglobulin M to *Cytomegalovirus*, **HBsAg** – Hepatitis B surface antigen, **anti-HCVsum** – summary antibodies to the hepatitis C virus, **B/P** – blood pressure, **CSF** – cerebrospinal fluid.

INTRODUCTION

Streptococcus-like bacteria of the genus L. *(Leuconostoc)* were isolated in humans for the first time by Handwerger in the mid-80s of the last century. *Leuconostoc spp*. are facultative-anaerobic, gram-positive, catalase-negative, non-spore-forming, motile, heterofermentative cocci widely distributed in the environment, including soil and plants (1).

The genus Leuconostoc includes the "typical representative" *L. mesenteroides* and 8 "minor" species, including *L. pseudomesenteroides*. Members of the genus *Leuconostoc* are more of an economic importance, as they are used in the manufacture of dairy products and wine. The first suspected case of human infection with *Leuconostoc spp*. was recorded in 1985 (2). Until then, *Leuconostoc* species were considered non-pathogenic for humans. Since 1985, infections caused by *Leuconostoc* have been reported more frequently (3-5), becoming more and more important as opportunistic pathogens of immunodeficiency states (6-8). For example, a South Korean study describes 6 cases of bacteremia with *Leuconostoc* in patients over 60 years old, with aggravating underlying diseases. Within 30 days of the identification of the bacteria, four of these 6 patients died (9).

At the same time, cases have also been described in immunocompetent patients. For example, secondary infection with *Leuconostoc spp*. following the application of a patch with plant components on the wound of a patient with amputation (10), or purulent meningitis caused by *Leuconostoc spp*. in a previously healthy patient (4), or bacteremia with the same pathogen in an immunocompetent patient suffering from Chagas disease (11), etc.

Leuconostoc infections often occur in patients whose underlying diseases are treated with vancomycin (12, 14). There are described cases of isolation of *Leuconostoc spp*. from the blood of patients with malignant neoplasms and long-term catheterization, as well as from the removal of infected wounds, in postoperative infections and in odontogenic abscesses (4, 12, 15-17). Pulmonary infection with *Leuconostoc* in a patient with lymphoma is also described (18). Xinfeng Lin and co-authors (19) in his paper associated hemophagocytic syndrome with *Leuconostoc pseudomesenteroides* infection in an adult patient.

Central Nervous System (CNS) infections caused by *Leuconostoc spp*. are extremely rare. Most often they are associated with a deficient immune field, such as, for example, purulent meningitis with *Leuconostoc spp*. in newborns (20, 21). A case of nosocomial meningitis caused by *Leuconostoc spp*. has been described in connection with the use of a catheter with an extraventricular drainage system due to the presence of a thalamic hematoma with ventricular extension (2). Ventriculitis and brain abscess have also been described in CNS infections with *Leuconostoc spp* (22, 23).

Several cases of *L. mesenteroides* bacteremia associated with the use of oral dietary supplements for children have been reported (3). Other additional factors that predispose to infection are: prolonged treatment in the hospital, the use of devices that violate the integrity of the skin and mucous membranes (venous catheters, gastro- or tracheostomy, drainage fistulas), extensive surgical interventions, decreased intestinal barrier function, antibiotic therapy with drugs that are not sufficiently active against *Leuconostoc spp.* (3, 5-7, 24).

It must be recognized that infectious diseases caused by *Leuconostoc spp*. are rare. This is also due to the lack of practice of its isolation. *Leuconostoc spp*. can be taken into consideration only in the case of isolation from obviously sterile sources (blood, cerebrospinal fluid, peritoneal and joint fluids), ob serving all the rules of asepsis. If *Leuconostoc* is found in material with a high risk of external contamination (for example, in the wound exudate), its presence is significant

only if no other more virulent microorganisms are found or if *Leuconostoc* is isolated in large quantities in several analysis of the sampled material (5, 6).

The complexity of identifying the microorganism is associated with the similarity of some of its properties with other, more frequently detected bacteria – *pneumococci, streptococci and lactobacilli.* These similarities can cause misidentification (25). The natural resistance of *Leuconostoc spp*. to vancomycin allows its rapid differentiation from most other streptococcal bacteria, except *Pediococcus spp*. and certain vancomycin-resistant strains of Enterococci. At the same time, carrying out tests using leucine aminopeptidase and pyrrodonylarylamidase allows the differentiation of Leuconostoc spp. with Pediococcus spp. and vancomycin-resistant *Enterococci* (26). In recent years, automated microbiological analyzers have been successfully used to confirm *Leuconostoc* bacteremia (27).

Leuconostoc is one of the few Gram-positive bacteria, including *Pediococcus spp*., *Lactobacillus spp*., *Erysipelothrix*, *enterococci*, naturally resistant to glycopeptide antibiotics – vancomycin and teicoplanin. However, despite resistance to glycopeptides, *Leuconostoc spp*. is sensitive to most antibiotics active against *streptococci*. Thus, *Leuconostoc* is usually sensitive to penicillin, ampicillin, clindamycin, erythromycin and fosfomycin. Moderate activity is shown by imipenem, cephalosporins, tetracyclines and chloramphenicol (6, 25). Clinical data, extremely limited in number, indicate that apparently the antibiotics of choice for the treatment of infections caused by *Leuconostoc spp*. are penicillin and ampicillin (5, 25).

CASE PRESENTATION

Patient V.D., 11 months old, was admitted to PMSI IDCH "Toma Ciorba" on 05.01.2022. When the patient was admitted, the following anamnestic data were found:

- Heredo-collateral antecedents: born with a congenital malformation – rabbit lip with a defect of the hard and soft palate, at 39 weeks, weighing 2970 gr.; was abandoned by parents (disenfranchised).
- Medical history: underwent 2 facial surgeries, performed at PMSI "E. Cotaga"; in November-December 2021 – pneumonia; until the age of 9 months showing retardation in physical development, not sitting on their own.
- Living conditions: being adopted, he lives in a family that takes care of him; sufficient nutrition (quantitative and qualitative).

Epidemiological anamnesis: in the adoptive family there are children who previously (December 2021 – January 2022) showed signs of acute respiratory infection with cough, rhinorrhea and pain in the pharynx.

The history of the disease: considered sick since 03.01.2022, when fever up to 39.0°C appeared, liquid stool 3 times a day, general weakness, lack of appetite. The child caregiver administered oral antipyretics. On 04.01.22 the caregiver noticed the appearance of a punctate rash on the left buttock. The liquid stool did not recur, but the fever remained up to 39.5°C. On 05.01.22 multiple punctate eruptions appeared on the lower limbs, the fever continued to persist. The child caregiver requested Urgent Medical Assistance, after which the child was hospitalized with suspicion of meningococcal infection at PMSI Ungheni District Hospital, where he was administered sol. Levomycetin 250 mg and Ringer infusion i/v 100 ml. On the same day, the child was transported to PMSI IDCH "Toma Ciorba" for diagnostic evaluation and treatment.

Clinical examination: serious general condition, body temperature – 37.4°C, drinks very little, with difficulty; punctate and stellate hemorrhagic eruptions located on the lower limbs and in the lower abdomen, two elements – on the buttocks in the resorption phase; oral cavity – rabbit lip with hard and soft palate defect, slightly hyperemic oropharynx; clear consciousness, tracks objects, sleeps intermittently, suspects occipital stiffness; breathing rate – 26/min, SpO_2 – 97%, heart rate = 120/min, T/A = 95/65 mmHg.

The presumptive diagnosis was established: Meningococcal infection. Meningococcemia. Meningococcal meningitis. Lumbar puncture was performed with cerebrospinal fluid sampling. Blood, urine, cerebrospinal fluid, pharyngeal smears and eruptive elements were taken for bacteriological investigations.

The antibiotic treatment was continued with ceftriaxone, the pathogenetic one included dexamethasone. In continuation, the improvement

of the general condition of the child, the normal ization of the body temperature was confirmed.

Nonspecific paraclinical investigations during treatment were as follows (tab. 1, 2):

Table 1. Blood count in dynamics.

Indicators / data	06.01.22	10.01.22	14.01.22
Hemoglobin (g/L)	94↓	114	108↓
Erythrocytes (10^{12}/L)	4.21	5.23↑	4.81
Platelets (10^9/L)	285	521↑	586↑
Leukocytes (10^9/L)	25.74↑	20.57↑	15.96↑
Unsegmented (%)	48↑	4	1
Segmented (%)	21	27	10
Eosinophils (%)	0↓	0↓	1
Lymphocytes (%)	25↓	54	65
Monocytes (%)	2.9	11.5	15↑
ESR (mm/h)	22↑	5	10

Table 2. Biochemical analysis of blood in dynamics.

Indicators / data	06.01.22	10.01.22	14.01.22
ALT (U/L)	251.9↑	101.8↑	78.7↑
AST (U/L)	78.7↑	38.3	50.0↑
Urea (mmol/L)	4.81	5.20	
Creatinine (mcmol/L)	36↑	59↑	73↑
Glucose (mmol/L)	8.61↑	5.43	4.45
Amylase (U/L)	13↓		
Prothrombin index (%)	97		

CSF from 06.01.22: slightly cloudy; Pandy's reaction – positive (+); cytosis – 37 cells/mcl, neutrophils – 55%, lymphocytes – 45%, protein – 0.33 g/L; unchanged erythrocytes – moderate amount; chlorides – 128.8 mmol/L; glucose – 5.80 mmol/L.

Chest radiograph from 06.01.22: lung pattern accentuated bilateral bronchovascular with bilateral perihilar peribronchovascular infiltrate. Reactive, structured pulmonary hila. Free pleural sinuses. Cord – normal.

Specific immunological paraclinical investigations (10.01.22): Anti-CMV-IgG – positive; Anti CMV IgM – positive; HBsAg – negative; anti-HCVsum – negative.

The bacterioscopic and bacteriological results of the cerebrospinal fluid, from the eruptions, from the pharynx, on the background of antibacterial therapy, were negative.

Bacteriological investigation of urine from 10.01.22: *Candida albicans* (susceptible to clotrimoxazole, fluconazole, ketokonazol, nystatin, etc.).

On 12.01.22, the preventive result of the bacteriological investigation of the blood, taken on 06.01.22, was received, with the finding of an increase in the broth of gram-positive, catalase-negative cocci flora. To determine the germ, the automatic microbiological analyzer Vitek 2 was used: identified *Leuconostoc pseudomesenteroides*, sensitive to ampicillin, azithromycin, cefepime, chloramphenicol, clarithromycin, erythromycin, norfloxacin, tetracycline; intermediate sensitivity to ciprofloxacin, levofloxacin; resistant to cefotaxime, clindamycin, cotrimoxazole, vancomycin.

The final diagnosis was established: Acute meningococcemia and meningococcal meningitis. Septicemia with *Leuconostoc mesenteroides*. *Cytomegalovirus* infection. Cytomegaloviral hepatitis. *Candida albicans* urinary tract infection. Rabbit lip with cleft palate, bilateral. Hemophagocytic anemia.

Considering the result of the blood culture and anti-CMV-IgM – positive, the decision was made to change the etiotropic treatment, namely: cancellation of treatment with Ceftriaxone, indicating Amoxicillin clavulanate and Viferon. In continuation, the child remained afebrile, became active, with improvement in appetite and sleep,

without finding deviations from normal during the objective examination. On 14.01.22, blood was taken for the repeated investigation of sterility: the result – negative. On the same date, the patient was discharged home in satisfactory condition.

DISCUSSIONS

Infections caused by *Leuconostoc spp*. are poorly studied. Available literature data allows reconsideration of its relationship with the human body. Thus, at present, *Leuconsotoc spp*. can be considered pathogenic for humans. The clinical case presented is of a child, presumed immunocompromised, confirmed with bacteremia with *Leuconostoc pseudomesenteroides*, which was associated with meningococcal infection with meningococcemia and meningococcal meningitis, on the background of *Cytomegalovirus* infection in reactivation and immunosuppression. The timely choice of etiological treatment in the case of identification of *Leuconostoc spp*., is a necessary condition for obtaining a positive clinical result.

CONCLUSIONS

1. The presented clinical case is the first case of infection with *Leuconostoc spp*. described in the Republic of Moldova.
2. The correct choice of etiological treatment allows the eradication of infection with *Leuconostoc spp.*

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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OLGA BURDUNIUC - UN SPIRIT LABORIOS ŞI INOVATIV

Lupta pentru mai bine
şi către mai bine este cheia succesului.

(Mircea Albulescu)

Zig Ziglar spunea: „Succesul este utilizarea la maximum a capacităţilor pe care le posezi". Acest dicton i se potriveşte perfect conferenţiarului universitar Olga Burduniuc, originară din satul Mândreşti, raionul Teleneşti. Munca şi perseverenţa sunt calităţile definitorii ale dnei Olga Burduniuc hărăzite de la naştere.

Olga Burduniuc a absolvit Şcoala medicală din oraşul Orhei (1993) şi Facultatea de Medicină Preventivă a Universităţii de Stat de Medicină şi Farmacie „Nicolae Testemiţanu" (2003). În 2005 obţine diploma de licenţă, iar în 2014 susţine cu succes teza de doctor în ştiinţe medicale. În 2016 devine Master în Managementul Sănătăţii Publice, iar în 2017 obţine titlul de conferenţiar universitar.

Olga Burduniuc este fondatoarea Asociaţiei de Biosiguranţă şi Biosecuritate din Republica Moldova (2017) şi a revistei ştiinţifice One Health & Risk Management (2019).

Este autor şi coautor a 218 lucrări ştiinţifice, metodico-didactice, inclusiv o monografie monoautor, două monografii colective, coautor a două manuale, 10 indicaţii/recomandări metodice şi 14 ghiduri practice. A participat la peste 100 de manifestări ştiinţifice în ţară şi peste hotare. Este autor a 5 certificate de inovator şi 14 brevete de invenţie. A participat în şase proiecte naţionale şi cinci internaţionale. A obţinut 15 medalii de aur, 4 de argint şi 3 de bronz la diverse expoziţii de invenţii, naţionale şi internaţionale. A avut un aport considerabil la dezvoltarea Sistemului Naţional de Supraveghere a rezistenţei la antimicrobiene (RAM), inclusiv prin prisma conceptului „One Health/O singură sănătate". Reprezintă Republica Moldova în domeniul standardizării metodologiei de testare RAM EUCAST (European Committee on Antimicrobial usceptibility Testing) în calitate de preşedinte al Comitetului Naţional. A fost nominalizată ca Punct Focal Naţional pentru CAESAR (eng. Central Asian and European Surveillance of Antimicrobial Resistance) şi GLASS (eng. Global Antimicrobial Resistance and Use Surveillance System).

Din 2014, Doamna Olga Burduniuc a deţinut funcţia de Şef Laborator Microbiologic, iar din 2022 – Şef Direcţia Diagnostic de Laborator în Sănătate Publică. Aceste ascensiuni profesionale constituie rezultatul efortului şi al dedicaţiei investite în cariera sa profesională.

Pentru merite deosebite în dezvoltarea medicinei şi rezultate remarcabile în activitatea profesională, Doamnei Olga Burduniuc i-au fost înmânate distincţii internaţionale (IFBA's 2019 Biosafety Hero, oferită de către Federaţia Internaţională a Asociaţiilor de Biosiguranţă (IFBA) şi naţionale de stat: Diploma Ministerului Sănătăţii (2018, 2020, 2022), Diploma de onoare a Ministerului Educaţiei, Culturii şi Cercetării (2021) şi Medalia „Nicolae Milescu Spătaru" (2022).

Doamna conferenţiar Olga Burduniuc este un Om al cetăţii, care face totul cu pasiune, cu generozitate şi dedicaţie pentru cei din jur, fiind mereu implicată în diverse activităţi sociale, ştiinţifice şi de voluntariat, o persoană receptivă la nevoile tuturor.

Mulţi ani prosperi, Doamna Olga Burduniuc!

Cu profund şi deosebit respect,
colegii consiliul de redacţie al Revistei ştiinţifice *One Health & Risk Management*

LARISA SPINEI – CERCETĂTOARE ȘTIINȚIFICĂ DE SUCCES

Numitorul comun pentru toți
cei care au avut succes este munca.

(John D. Rockefeller)

Profesorul Larisa Spinei este absolventă a Facultăţii Medicină Generală a Universităţii de Stat de Medicină şi farmacie „Nicolae Testemiţanu” din Chişinău, Republica Moldova. După susţinerea tezei de doctor în ştiinţe medicale (1989), a început să studieze unele aspecte ale dizabilităţii la copii în Republica Moldova. Rezultatele obţinute au fost oglindite în teza de doctor habilitat „Aspecte medico-sociale şi organizatorice ale invalidităţii la copii în Republica Moldova”, care a fost susţinută cu succes în cadrul USMF „Nicolae Testemiţanu” în anul 1997, consultat ştiinţific fiind profesorul Eugen Popuşoi.

Sub conducerea profesorului Larisa Spinei au fost susţinute 3 teze de doctor habilitat şi 14 teze de doctor la specialităţile: medicină socială şi management, obstetrică şi ginecologie, traumatologie pediatrică, psihiatrie şi farmacie.

Experienţa profesională a domniei sale a fost extinsă şi în activităţile cu diverse organizaţii internaţionale (OMS, UNICEF, PNUD), în proiecte ştiinţifice naţionale şi internaţionale.

Domnia sa este titularul cursurilor „Metodologia cercetării ştiinţifice” şi „Medicina bazată pe dovezi” în cadrul Şcolii doctorale în domeniul „Ştiinţe medicale”. Trebuie de menţionat contribuţia semnificativă adusă la elaborarea Protocolului de cercetare în calitate de membru al Comisiei de verificare a materialului primar.

Profesorul Larisa Spinei este membru al WAMS (the World Academy of Medical Sciences), International Board of Biostatistics, Netherlands, expert al ANACEC, al colegiului de redacţie la revistele „Sănătate Publică, Economie şi Management în Medicină” şi „One Health & Risk Management”.

Rezultatele activităţii ştiinţifice şi didactice au fost expuse în 195 de lucrări ştiinţifice, inclusiv 3 manuale, 5 monografii, 3 ghiduri. A participat cu comunicări la diferite forumuri ştiinţifice naţionale şi internaţionale.

Activitatea multilaterală, contribuţia semnificativă la dezvoltarea ştiinţei şi promovarea rezultatelor remarcabile obţinute de profesorul Larisa Spinei au fost înalt apreciate, Domnia sa fiind decorată cu Diploma de Onoare a Guvernului Republicii Moldova, cu Diploma de Recunoştinţă a Academiei de Ştiinţe a Moldovei şi cu Medalia „Nicolae Testemiţanu”.

Mulţi ani prosperi, Doamna Larisa SPINEI!

Cu profund şi deosebit respect,
colegii consiliul de redacţie al Revistei ştiinţifice *One Health & Risk Management*

REQUIREMENTS FOR AUTHORS

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The manuscript (written in English and French) should be in accordance with the guidelines published in: *Uniform Requirements for Manuscripts Submitted to Biomedical Journal (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). The manuscripts should be written in font Cambria, size 11 points, spaced at 1.0, fully justified alignment, fields 2 cm on all sides. All pages must be numbered consecutively (in the right bottom corner) and continuously. Abbreviations should be explained at first occurrence in the text and should not be excessively used. The manuscripts must not exceed the number of words (without the title, affiliation, abstract and references): review articles – 4,500 words; research articles – 3,000 words; expert opinions – 2,500 words; case presentation – 1,700 words; experimental and clinical notes – 1,300 words; book reviews and presentations – 2,000 words; teaching articles – 4,000 words. The volume of tables and figures should not exceed ⅓ from the volume of the manuscript. The journal reserves the right to make any other formatting changes. Rejected manuscripts are not returned.

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The manuscript should comprise the following subheadings (capitalized):

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The **summary** should contain 1,600 signs with spaces:

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The summary should not include tables, charts, and bibliographic notes; information not included in the article.

Figures. The text included in figures should be written in font Cambria, 10 point. Each figure should be accompanied by a heading and legend. They should be numbered with Arabic numerals and placed in parentheses (*e.g.:* fig. 1). Both the title (*e.g.* Figure 1) and legend are centred, below the figure.

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Pregătirea manuscrisului (elaborat în limbile engleză și franceză) va fi în conformitate cu instrucțiunile publicate în: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Manuscrisele trebuie să fie cu font Cambria, dimensiune 11 puncte, spațiat la interval 1,0, aliniere justificată, câmpurile 2 cm pe toate laturile. Toate paginile trebuie să fie numerotate consecutiv (în colțul de jos, în partea dreaptă) și să includă nume-rotarea continuă a paginilor. Abrevierile trebuie să fie explicate la prima apariție în text și nu trebuie utilizate excesiv. Manuscrisele nu trebuie să depășească (fără a număra titlul, afilierea, rezumatul și refe-rințele): pentru articole de sinteză/referate – 4500 de cuvinte; pentru articole de cercetare – 3000 de cuvinte; pentru opinii ale experților – 2500 de cuvinte; prezentare de caz și imagini din practica clinică/laborator – 1700 de cuvinte; note experimentale și clinice – 1300 de cuvinte; recenzii și prezentări de carte – 2000 de cuvinte; articole didactice – 4000 de cuvinte. Volumul tabelelor și figurilor nu trebuie să depășească ⅓ din volumul manuscrisului. Revista își rezervă dreptul de a face orice alte modificări de formatare. Manuscrisele respinse nu sunt returnate.

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- **Concluzii**
- **Cuvinte cheie**: 3-5 cuvinte

În rezumat nu vor fi incluse tabele, grafice și note bibliografice; informații care nu sunt prezentate în studiu.

Figuri. Textul inclus în figuri trebuie să fie scris cu font Cambria, dimensiune 10 puncte. Fiecare figură trebuie să fie însoțită de titlu și legendă. Ele vor fi numerotate cu cifre arabe și vor fi menționate în text în paranteze (*ex:* fig. 1). Titlul (*ex:* Figura 1) și legenda figurii trebuie să fie scrisă centrat, sub figură.

Tabele. Textul inclus în tabele trebuie să fie scris cu font Cambria, dimensiune 10 puncte. Fiecare tabel trebuie să fie însoțită de titlu. Tabelele vor fi inserate în text, fără a depăși lățimea unei pagini. Ele vor fi numerotate cu cifre arabe și vor fi menționate în text în paranteze (*ex:* tab. 1). Titlul tabelului va fi poziționat deasupra tabelului centrat (*ex:* Tabelul 1).

Referințele trebuie să fie numerotate în ordinea apariției în text. Citarea sursei de referință va fi conform stilului *AMA*, plasată la sfârșitul articolului și va include doar referințele citate în text (menționând numărul de referință în paranteză rotundă). Dacă aceeași referință este citată de mai multe ori, ea va fi trecută în text cu același număr ca la prima citare. Numărul total de referințe nu va depăși 50 de surse. Acuratețea datelor ține de responsabilitatea autorului.

Pentru mai multe informații consultații: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

EXIGENCES POUR LES AUTEURS

Normes de rédaction
La préparation des manuscrits (rédigés en anglais et français) sera conforme aux instructions publiées dans *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2 ; 1-4* (www.icmje.org). Les manuscrits doivent être en police Cambria, taille 11 points, espacés à l'intervalle 1,0, alignement justifié, champs 2 cm de tous les côtés. Toutes les pages doivent être numérotées consécutivement (dans le coin inférieur droit) et inclure une numérotation continue des pages. Les abréviations doivent être expliquées lors de la première apparition dans le texte et ne doivent pas être utilisées de manière excessive. Les manuscrits ne doivent pas dépasser (sans mentionner le titre, l'affiliation, le résumé et la bibliographie) le vo-lume suivant: pour articles de synthèse/rapports – 4500 mots; pour les articles de recherche – 3000 mots; pour les opinions d'experts – 2500 mots; présentation de cas et photos de la pratique clinique/de laboratoire – 1700 mots; notes expérimentales et cliniques – 1300 mots; commentaires et présentations de livres – 2000 mots; articles pédagogiques – 4000 mots. Le volume des tableaux et des figures ne doit pas dépasser ⅓ du volume du manuscrit. La revue se réserve le droit d'apporter toute autre modification de formatage. Les manuscrits rejetés ne sont pas retournés.

Tous les manuscrits à publier doivent être accompagnés par deux résumés: dans la langue originale et en anglais.

Titre et auteurs
Le titre doit être le plus court que possible (maximum – 120 signes avec espaces), éloquent pour le contenu du manuscrit. Les noms des auteurs seront écrits complets: prénom, nom (*ex:* Albert LEBRUN). Quant à l'affiliation, on devra indiquer: Section/ Département/Chaire, Université/Hôpital, Ville, Pays – pour chaque auteur. Les données de l'auteur correspondant et les coordonnées – adresse e-mail (*ex:* auteur correspondant: Albert Lebrun, e-mail: albert.lebrun@gmail.com*)* seront obligatoires ci-dessous.

Structure du manuscrit
Le manuscrit comprendra les sous-titres suivants (avec lettres majuscules):

- **RÉSUMÉ** (voir les exigences ci-dessous)
- **INTRODUCTION** (reflétera l'actualité et la présentation générale du problème étudié, le but et l'hypothèse de l'étude)
- **METHODES**
- **RESULTATS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLIT D'INTERETS**
- **REMERCIEMENTS ET FINANCEMENT**
- **APPROBATION ÉTHIQUE** (préciser la présence ou l'absence d'avis favorable du comité d'éthique: no, date, institution et consentement éclairé)
- **REFERENCES**

Le **résumé** contiendra 1600 signes avec espaces:

- **Introduction**
- **Méthodes**
- **Résultats**
- **Conclusions**
- **Mots clés**: 3-5mots.

Le résumé ne comprendra pas des tableaux, graphiques et des notes bibliographiques; des informations non présentées dans l'étude.

Figures. Le texte inclus dans les figures doit être écrit avec police Cambria, taille 10 points. Chaque figure doit être accompagné par un titre et une légende. Ceux-ci seront numérotés avec des chiffres arabes et mentionnés dans le texte entre parenthèses (*ex:* fig. 1). Le titre (*ex:* Figure 1) et la légende de la figure doivent être centrés, au-dessous de la figure.

Tableaux. Le texte inclus dans les tableaux doit être écrit avec police Cambria, taille 10 points. Chaque tableau doit être accompagné par un titre. Les tableaux seront numérotés avec des chiffres arabes, mentionnés dans le texte entre parenthèses (*ex:* tab. 1), et seront insérés dans le texte, sans dépasser la largeur d'une page. Le titre du tableau sera placé au-dessus du tableau, centré (*ex:* Tableau 1).

Les **références** doivent être numérotées dans l'ordre où elles apparaissent dans le texte. La citation de la source de référence sera de style *AMA*, placée à la fin de l'article et n'inclura que des références citées dans le texte (mentionnant le numéro de référence entre parenthèses rondes). Si la même référence est citée plusieurs fois, elle sera transmise dans le texte avec le même numéro que celui de la première citation. Le nombre total de références ne dépassera pas 50 sources. La responsabilité pour l'exactitude des données est à la charge de l'auteur. Il faut indiquer dans le manuscrit seulement les références vraiment consultées par les auteurs. Les composants des sources de référence doivent être rédigés strictement selon les exigences.

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ТРЕБОВАНИЯ ДЛЯ АВТОРОВ

Правила составления

Подготовка рукописи (разработанной на английском и французском языках) будет осуществляться в соответствии с инструкциями, опубликованными в: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Авторы должны использовать шрифт Cambria, размер 11 точек, с интервалом 1,0, выравнивание по ширине, поля 2 см со всех сторон. Все страницы должны быть пронуме-рованы последовательно (в правом нижнем углу) и включать непрерывную нумерацию страниц. Сокращения должны быть объяснены при первом появлении в тексте и не должны использоваться чрезмерно. Объем рукописей не должен превышать (без названия, принадлеж-ности, резюме и литературы): для обзорных статей/рефератов – 4500 слов; для научных ста-тей – 3000 слов; для экспертных заключений – 2500 слов; для презентации случаев из клини-ческой/лабораторной практики – 1700 слов; для экспериментальных и клинических заметок – 1300 слов; для рецензий и презентаций книг – 2000 слов; для учебных статей – 4000 слов. Объем таблиц и рисунков не должен превышать ⅓ от объема рукописи. Журнал оставляет за собой право вносить любые другие изменения форматирования. Отклоненные рукописи не возвращаются.

Все рукописи, представленные для публикации, должны сопровождаться двумя резюме: на языке оригинала статьи и на английском языке.

Название и авторы

Название должно быть как можно короче (максимум – 120 знаков с пробелами), но дос-таточно информативным для содержания рукописи. Фамилии авторов будут написаны полностью: имя, фамилия (*например:* Иван ИВАНОВ). Принадлежность будет включать: Отделение/ Департамент/Кафедра, Университет /Больница, Город, Страна для каждого автора. Данные соответствующего автора и контактная инфор-мация – адрес электронной почты (*например:* контактная информация: Иван Иванов. e-mail: ivan.ivanov@gmail.com) будут обязательно ниже.

Структура Рукописи

Рукопись будет включать в себя следующие подзаголовки (они должны быть заглавными):

- **РЕЗЮМЕ** (см. требования ниже)
- **ВВЕДЕНИЕ** (будет отражать актуаль-ность и общее представление изучаемой проблемы, цель и гипотезу исследования)
- **МАТЕРИАЛЫ И МЕТОДЫ**
- **РЕЗУЛЬТАТЫ**
- **ДИСКУССИИ**
- **ВЫВОДЫ**
- **КОНФЛИКТ ИНТЕРЕСОВ**
- **БЛАГОДАРНОСТИ И ФИНАНСИРОВАНИЕ**
- **ЭТИЧЕСКОЕ ОДОБРЕНИЕ** (указать наличие или отсутствие одобрения со стороны комитета по этике: №, дата, учреждение и информированное согласие)
- **ЛИТЕРАТУРА**

Резюме должно содержать 1600 знаков с пробелами и будет включать в себя следующие подзаголовки:

- **Введение**
- **Материалы и методы**
- **Результаты**
- **Выводы**
- **Ключевые слова:** 3-5 слов

Резюме не должно включать таблицы, диаграммы и библиографические заметки, инфор-мацию, не представленную в исследовании.

Рисунки (графики, диаграммы). Текст, включенный в рисунки, должен быть написан в Cambria, размер 10 пунктов. Каждый рисунок должен сопровождаться заголовком и описанием. Название (*например:* Рисунок 1) и описание рисунка должны быть вписаны по центру, в низу рисунка. Они должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например:* рис. 1).

Таблицы. Текст, включенный в таблицы, должен быть написан в Cambria, размер 10 пунктов. Каждая таблица должна сопровождаться заголовком. Они должны вставляться в текст, не превышая ширину страницы. Должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например:* таб. 1). Название таблицы должно располагаться над таблицей в центре (*например:* Таблица 1).

Литература. Источники должны быть пронумерованы в порядке их появления в тексте. Ссылки на источники должны быть в стиле *AMA*, помещены в конце статьи и включать только источники, цитируемые в тексте (упоминание номера источника в круглых скобках). Если один и тот же источник цитируется несколько раз, он будет передан в тексте с тем же номером, что и первый раз. Общее количество источников не должно превышать 50. Ответственность за точ-ность данных лежит на авторе. Будут цитиро-ваться только те источники, с которыми ознакомились авторы рукописи. Компоненты справочных источников должны быть написаны строго в соответствии с требованиями.

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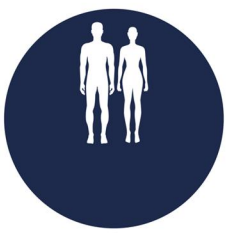
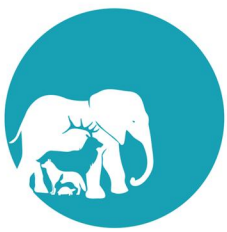
The *One Health* concept

Human health



The WHO defined health in 1946 as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity", with the later addition of "the capacity to lead a socially and economically productive life".

Animal health



The OIE defines animal welfare in 2008: an animal is in good condition if it is healthy, enjoys comfort, is well fed, is safe, is able to display its innate (natural) behavior and does not suffer from unpleasant conditions such as pain, fear and stress.

Plant and environmental health

Environmental health refers to those aspects of human health that include the quality of life determined by physical, biological, socio-economic and psycho-social factors in the environment. The interrelationships of people with the environment concern medicine, when an ecological system is in a state of equilibrium, the health of the population prevails.

Globally, the *One Health* concept is a worldwide strategy to expand interdisciplinary collaborations and communications in all aspects related to the health care of humans, domestic animals or wildlife, which can no longer be approached separately, but only jointly.

One Health addresses not only human and animal disease concerns, but also issues related to lifestyle, diet, exercise, the impact of different types of human-animal relationships, and environmental exposures that can affect both populations. In order to achieve the expected effects, it is also necessary to educate the population to make them aware of the risk factors and benefits of prevention, as well as communication and understanding between patients and healthcare providers.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