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Betimi i Hipokratit

Në çastin kur po hy në radhët e anëtarëve të profesionit mjekësor premtoj solemnisht se jetën time do ta vë në shërbim të humanitetit. Ndaj mësuesve do ta ruaj mirënjohjen dhe respektin e duhur. Profesionin tim do ta ushtroj me ndërgjegje e me dinjitet. Shëndeti i pacientit tim do të jetë brenga ime më e madhe. Do t'i respektoj e do t'i ruaj fshehtësitë e atij që do të më rrëfehet. Do ta ruaj me të gjitha forcat e mia nderin e traditës fisnike të profesionit të mjekësisë.

Kolegët e mi do t'i konsideroj si vëllezër të mi.

Në ushtrimin e profesionit ndaj të sëmurit tek unë nuk do të ndikojë përkatësia e besimit, e nacionalitetit, e racës, e politikës, apo përkatësia klasore. Që nga fillimi do ta ruaj jetën e njeriut në mënyrë apsolute. As në kushtet e kërcënimit nuk do të lejoj të keqpërdoren njohuritë e mia mjekësore që do të ishin në kundërshtim me ligjet e humanitetit. Këtë premtim po e jap në mënyrë solemne e të lirë, duke u mbështetur në nderin tim personal.

The Oath of Hippocrates

Upon having conferred on me the high calling of physician and entering medical practice, I do solemnly pledge myself to consecrate my life to the service of humanity. I will give my teachers the respect and gratitude which is their due. I will practice my profession with conscience and dignity. The health of my patient will be my first consideration. I will respect the secrets which are confided in me, even after the patient has died. I will maintain by all the means in my power, the honor and the noble traditions of the medical profession.

My colleagues will be my brothers.

I will not permit considerations of religion, nationality, race, party politics or social standing to intervene between my duty and my patient. I will maintain the utmost respect for human life from its beginning even under threat and I will not use my medical knowledge contrary to the laws of humanity. I make these promises solemnly, freely and upon my honor

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Original scientific paper

EFFICACY OF TRICHLOROACETIC ACID IN TREATMENT OF HPV RELATED INFECTIONS OF THE UTERINE CERVIX

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ABSTRACT

Objective: To establish the efficacy of single topical 85% trichloroacetic acid (TCA) treatment of the subclinical Human papillomavirus (HPV) infections of the uterine cervix.

Methods: This is a retrospective study including patients with HPV infection of the uterine cervix established by HPV DNA PCR assay. All the patients were treated with 85% TCA, applied topical and treatment response was followed up by HPV DNA PCR assay in various groups of patients, two, three, four, six and twelve months after the treatment with TCA. Remission was defined as complete clearance of HPV.

Results: In total, 173 patients with HPV specific type were included in the study. Unfortunately 70 patients didn't return for following check up after the treatment and in 103 patients follow up HPV DNA PCR was made, which showed that 70 of them had complete HPV clearance. In addition, 68% and 33 patients were confirmed as positive, 32% and 10 of the HPV positive group still had the same type of HPV, 30% and 23 of them were typed with new type of HPV, 70%.

Conclusion: A single treatment of topical TCA for subclinical HPV infections is associated with high HPV clearance, especially two, three and four months after the treatment. The HPV clearance six and twelve months after the treatment, decreases progressively. This gives us the right to think of TCA as an effective agent for subclinical HPV infection treatment.

Key words: #human papillomavirus #trichloroacetic acid #cervical intraepithelial neoplasia #cervical cancer

INTRODUCTION

Cervical cancer remains the third most common cancer in women worldwide. There are 604 237 new diagnosed cases, representing 6.5% of all female cancers and 341843 deaths in 2020 year, 90% of whom were in less developed countries. Incidences of cervical cancer are disproportionally distributed between developed and less developed countries. Developed countries have progressively declined the incidence of cervical cancer by providing cancer screening programs and HPV vaccination programs. According to Global Cancer Observatory, our country, North Macedonia is among the countries with middle high age standardized rate of 7,5 (1).Statistical evaluation or annual review of Institute of public health of North Macedonia, showed that in average

there are 150 new diagnosed cases of cervical cancer per year in our country, and one third of them died (2).

HPV is the cause of almost all cervical cancers and is responsible for a substantial fraction of other anogenital cancers an oropharyngeal cancers (3). It is one of the most common causes of sexually transmitted disease in men and women worldwide. Papillomaviruses are ubiquitous and more than 200 types were recognized by DNA sequence data, showing genomic differences. 40 of them show anogenital tropism. Based on their association with cervical cancer and precursor lesions, HPVs can also be grouped to high risk and low risk HPV types. Low risk types include types 6, 11, 42, 43 and 44. High risk HPV types include types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70 (4).

The HPV genome encodes DNA sequences for six early (E) proteins associated with viral gene regulation and cell transformation, two late (L) proteins which form the shell of the virus and a region of regulatory DNA sequences. Most important HPV proteins in the pathogenesis of malignant disease are E6 and E7 (5). They act in a cooperative manner to immortalize epithelial cells through binding with two intracellular proteins p53 and retinoblastoma (Rb). In normal cells p53 protein is a negative regulator of cell growth and also functions as a tumor suppressor protein by halting cell growth after chromosomal damage and allowing DNA repair enzymes to function. Binding of E6 to p53 allows unregulated cellular cycling, promoting the antiapoptotic effect, permitting accumulation of chromosomal mutations without DNA repair. This leads to chromosomal instability in the HPV containing cells. The Rb protein inhibits the effect of positive growth regulation and induces cell apoptosis in response to DNA damage (6,7,8,9). E7 interacts with Rb protein via an E2F/Rb protein complex which allows cyclin A to promote cell cycling. This results with unchecked cell growth in the presence of genomic instability that may lead to malignant change (10, 11, 12, 13). Cooperative interaction between E6 and E7 enhances immortalization efficiency. High risk HPV E6 and E7 expressing cells have a decreased ability to maintain genomic integrity as they act as mitotic mutators and induce mitotic abnormalities, including anaphase bridges, unaligned or lagging chromosomes and multipolar mitoses, which are histopathological hallmark of high risk HPV associated cervical lesions and cervical cancer (5,14).

The infectious cycle of HPV is tailored to the differentiation program of the target cells, keratinocytes, from basal cell to terminally differentiated superficial squames. The time from infection to virus release takes about 3 weeks, the time needed for the basal keratinocytes to migrate through the epithelium and undergo complete differentiation and desquamation. The period between infection and appearance of lesion ranges from weeks to months, suggesting that the virus effectively evades host defenses. There is no cytolysis or cytopathic death as a consequence of virus replication and therefore no inflammation. There appears to be little or no release of proinflamatory cytokines. As an exclusively intracellular pathogen, HPV doesn't induce blood born viremic phase of the life cycle and only minimal amount of virus is exposed to immune defenses, so the virus is practically invisible to the host defense (15, 16). Its invisibility is due to limiting expression of viral protein until later stages of epithelial differentiation, infecting only cells of basal layer of the cervical epithelium and virally mediated suppression of the proinflamatory proteins that activate cytotoxic T lymphocytes, which assist in killing infected cells. Although most women clear the infection within a few months, those who do not are at risk for development of cervical precancer and cancer (16, 17).

Factors that contribute to development of cervical dysplasia are age older than 55, as 50% of high risk infections persist in women older than 55. Duration of infection is well known as predisposing factor for appearance of cervical precancerous lesion and high oncogenic HPV DNA types are more related to cervical lesions. If the HPV remains in an episomal nonintegrated state, it results with low grade lesion and if virus becomes integrated into the human genome, high grade lesions and cancer may develop (18). Low grade lesion is cervical intraepithelial neoplasia 1 (CIN1) and it refers to mildly atypical cellular changes in the lower third of the epithelium. High grade cervical lesions are cervical intraepithelial dysplasia 2 (CIN2) and 3 (CIN3). CIN 2 refers to moderately atypical cellular changes confined at the basal two thirds of the epithelium and CIN 3 refers to severely atypical cellular changes encompassing greater than two thirds of the epithelial thickness. If the lesion breaks through the basal membrane than microinvasive carcinoma is diagnosed (19).

The detection of HPV is facilitated by advances in molecular biology and molecular detection of HPV DNA is the golden standard for identification of HPV. Many studies showed high sensitivity and specificity for detecting HPV. Three categories of molecular assays

are available for detection of HPV infection in tissue and exfoliated cell samples, all of which are based on the detection of DNA of HPV and include non amplified hybridization, hybrid capture assays and polymerase chain reaction. Detection of HPV E6/E7 mRNA and the presence of oncogenic activity in cervical specimens can be performed by reverse transcriptase PCR or nucleic acid sequence based amplification (NASBA). These tests indicate similar sensitivity as HPV DNA tests with slightly higher specificity for detecting high level lesions. Nowadays three DNA based and one RNA based assay have been approved by the US Food and Drug Administration (FDA). Although there are a lot of tests in development, S5 test is taken for accurate and early detection of HPV. S5 test is a type of a test that measures DNA methylation of the most common high risk HPV DNA types 16, 18, 31, 33 and EPB41L3 gene expression from cervical smear samples and urine samples. Than a score is generated, which is proportional to the risk of cervical lesion. The HPV E7 protein disrupts cell cycling, leading to an increase in cellular p16 protein expression. Studies are in progress to determine the role of p16 as a possible diagnostic marker. Recently, scientists have developed nanoparticle assisted PCR assay for detection of HPV 16 an 18 DNA. More studies are needed to confirm the utility of these tests in clinical practice. Noon of them is FDA approved yet (20, 21, 22, 23, 24).

According to Sexually transmitted infections treatment guidelines 2021 by CDC, the treatment of HPV is directed to the macroscopic or pathologic precancerous lesions caused by HPV. Subclinical HPV infection typically clears spontaneously, therefore antiviral therapy is not recommended to eradicate HPV infections (26). But in our experience, a huge amount of HPV infections, especially those caused by high risk HPV types, are persistent and high risk for premalignant or malignant lesions development, more often in patients older than 30. It is very important in HPV infected patients to achieve remission. By remission we obtain complete clearance of the virus. This decreases the viral load of the epithelial cells and postpone the possibility of malignant alteration of the cells. Performing a peeling of the cervical transformational zone denudes the cervix from the infected cells and provides the means to reepitelize with new HPV noninfected cells.

The treatment involves using 85% trichloroacetic acid which is traditionally used for medical and cosmetic skin peeling.It is a small molecule, approved by FDA as an active substance in 2016. It can be found in concentration from 10-90%. It is primarily used in cosmetics as a chemical peeling for acne scars, melasma, xanthelasmas and in treatment of anogenital condylomas and warts. It makes precipitation and denaturation of the cell proteins, so the epithelium desquamates to the basal membrane. There are no registered cases of acute intoxication after topical use. Systemic levels of trichloroacetic acid which are reached after topical administration for clinical indication, cannot reach the systemic levels of TCA needed for mutagenicity. These features make the TCA perfect agent for cervical peeling and lowering the epithelial burden with HPV (27, 28).

MATERIALS AND METHODS

We have first started practicing 85% TCA as an agent for treatment of HPV related cervical infection since April 2017 in a private general hospital Remedika, Skopje, North Macedonia, associated with Medical Faculty of University Goce Delcev Stip, North Macedonia, as a response to the great results from the Austrian scientists who had applied it as an agent for treatment of cervical intraepithelial neoplasia. The study encomphases173 patients, from April 2017 to December 2021. All of them confirmed with HPV DNA PCR assay for presence of HPV that persisted for minimum of 6 months. TCA is a clinically applied treatment and it was used in 85% concentration, provided by General Hospital Remedika. Inclusive criteria are: patient with HPV confirmed with HPV DNA PCR assay, persistent at least for 6 months, who voluntarily by signing informed consent, agreed to take part in the study. Exclusive criteria are: known allergy to TCA, patients at an age younger than 21, patients with biopsy confirmed cervical intraepithelial neoplasia, patients with cervical operative procedures and laser procedures for treatment of cervical dysplasia or malignancy and pregnant women. The treatment was provided only by specialist in gynecology and obstetrics.

After reviling the cervix with a speculum, about 2ml of 85% trichloroacetic acid was topically applied to the ectocervix and TZ and a small amount of the solution that was left in the syringe was inserted into the internal os of the cervix to treat the caudal part of the endocervix. Furthermore, the TZ turned white, indicating denaturation and precipitation of proteins. TCA has low viscosity, therefore care was taken because it can easily drop onto normal tissue, which can also become chemically coagulated. Patients were advised not to have

sexual intercourse for 2 weeks, to choose showering instead of bathing for 4 weeks and to use sanitary pads rather than tampons during menstruation. The follow up was scheduled for 8 weeks after the TCA treatment. On the follow up visit we found that reepithelization of the cervical surface was assessed. Moreover, an endocervical and ectocervical smear was taken for HPV DNA PCR assay for confirmation of the HPV infection. We had five groups of patients and in some of them HPV DNA PCR assay was made at the second, third, fourth, sixth and twelfth month, respectively. To asses side effects patients were asked for vaginal bleeding or discharge, signs of pelvic inflammatory disease, postcoital bleeding, need for medical treatment and using pain reliefs and were asked to report for any other treatment related symptoms. Patients with complete HPV clearance were recommended to have their next PAPtest in 12 months and in case of HPV persistence, repeated HPV DNA PCR test was recommended as well.

The material was statistically analyzed using the methods of descriptive statistics.

RESULTS

In total 173 patients with HPV specific type were included in the study. The patients were divided in four age groups, 21-30 years old (79 patients), 31-40 years old (65 patients), 41-50 years old (28 patients) and 51 years old, as well as older patients group (1 patient). 28 types of HPV were identified and one group with mixed HPV infection was identified, too. Most common HPV type among young patients in the age group from 21 to 30 years old, was HPV type 16 with 11% and mixed infection with 13%. HPV type 31 was most frequent in the age groups of 31 - 40 and 41-50 years old , 13% and 9% respectively.

Type of HPV	21-30	31-40	41-50	51-	Total
type 06	1				1
type 16	9	6	3		18
type 17	2		1		3
type 18	7	2	2		11
type 19	1				1
type 30		1			1
type 31	6	9	6		21
type 32		1	1		2
type 33	1	1	2		4
type 34		2			2
type 35	6	5	2		13
type 39	3	2	1		6
type 40	2	1			3
type 45	4	2	1		7
type 46	1		1		2
type 51	2	2	1		5
type 52	4	4		1	9
type 53	3	4	2		9
type 56	2	4	1		7
type 58	3	1	1		5
type 59	2	3			5
type 62	1	1			2
type 63	1				1
type 66	4	3	1		8
type 68	1	1	1		3
type 73	1	1			2
type 82	1	1	1		3
type 86		1			1
Mixed infection	11	7			18
Total	79	65	28	1	173

Table 1. Type specific Human papillomavirus by age groups

Unfortunately 70 patients didn't return for follow check up after the treatment and in 103 patients follow up HPV DNA PCR was made and showed that 70 of them had a complete HPV clearance and 33 patients were confirmed as positive, thus 68 % and 32% in the given order.

1. Follow diagram representing Human papillomavirus status

10 of the HPV positive group still had the same type of HPV and 23 of them were diagnosed with a new type of HPV, 30 % and 70% respectively.

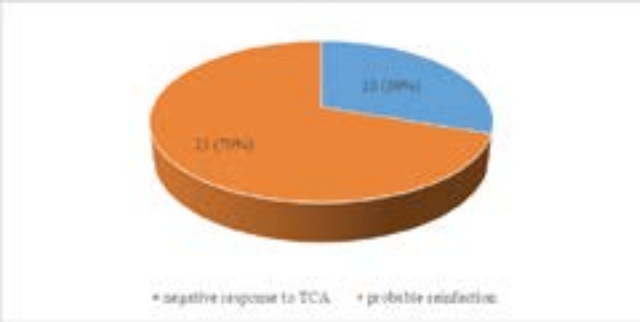

Chart 1. Number of Human papillomavirus positive patients after treatment with TCA

Remission rate among the groups divided by age, 21-30 years (34 %), 31-40 years (21%), 41-50 years old (12%) and patients older than 50 (100%) . HPV infection rate after the treatment, among groups divided by age were 55%, 30% and 15% respectively. Two months after the treatment with TCA, 18 patients were HPV positive (40%), and 27 were negative (60%). After three months, HPV positive were 4 patients (19%) and 17 were negative (81%). Four months after the procedure, HPV negative were 19

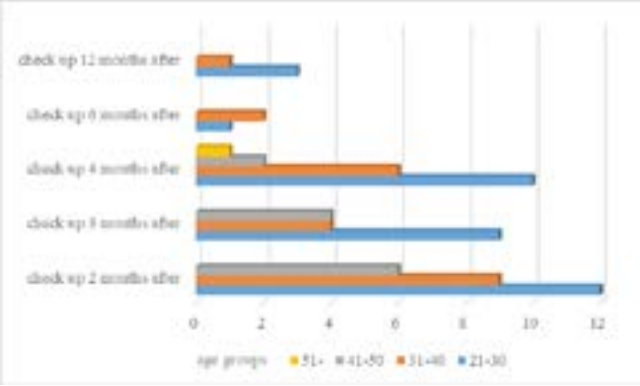

Chart 2. Number of Human papillomavirus negative patients by age groups after the treatment with TCA

This also applies to the HPV infected group.

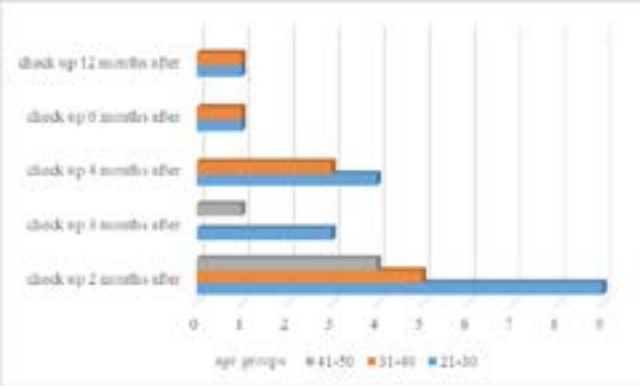

Chart 3. Number of Human papillomavirus positive patients by age groups after the treatment with TCA

There were no side effects observed during the treatment and follow up, such as heavy bleeding, pain, heavy vaginal discharge, postcoital bleeding or pain and vasovagal symptoms. At the follow up visit, reepithelisation of the cervix was complete in all patients.

DISCUSSION

TCA is a potential treatment for subclinical HPV infections. The acid is cheap, the technique does not require special training to perform, anesthesia is not needed because the treatment causes little or no pain at all. Moreover, it can be preformed outpatient without hospitalization and the patient can be discharged home immediately after the treatment, advised not to have sexual intercourses for two weeks, to take showers instead of a bath and to use sanitary towels instead of menstrual tampons (29, 30, 31). In the study of Geisler et al. 2016, the efficacy of TCA after a single topical application was established, with no major differences between high grade and low grade CIN and with remission rates of 80,3% and 82,3%respectively (31). Based on the Suwartono and Andrijono's (2020) study, there is no significant difference between application of 85% TCA compared with cryotherapy for treatment of patients with positive visual inspection with acetic acid (VIA) result. So the TCA treatment should be favored unlike the cryotherapy, which represents an invasive technique requiring special professional and logistic support (32). The ongoing prospective study, TRICIN, which is sponsored by Krankenhaus Barmherzige Schwestern Linz and is planned to end in December 2022, expects high remission and regression rates after single topical use of 85% TCA for CIN I and II, of 70% or even higher (33). Our study demonstrated that by a chemical coagulation of proteins, a single treatment of topical 85% TCA for subclinical HPV infections is associated with high HPV clearance, especially two, three and four months after the treatment. The HPV clearance six and twelve months after the treatment, decreases progressively. This study confirms the future perspective of TCA as an effective agent for subclinical HPV infection treatment. It seemed that the type of HPV does not affect the efficacy of the treatment so it could be applied to all HPV types and mixed infections. The limitation of this study is that it can not answer the question why the efficacy of the treatment decreases progressively six months later. There are three theories according to whom eather it comes to a reinfection due to change of sexual partner, it may comes to a reinfection due to an infected vaginal epithelium

which is in direct contact with the cervical epithelium, or HPV persists in latent form in some of the basal epithelial cells. Nevertheless, we established that the cervical epithelium is safe from HPV infection at least 6 months and more, so the time necessary for subclinical infection to evolve to cervical lesion is postponed. More studies are needed to establish the long term outcome of the treatment with TCA. It can be considered reapplication of TCA for treatment of reinfections or persistent HPV infection, but further studies are needed to prove the safety of the reuse of TCA, six months after the primer treatment. Therefore, the treatment with TCA indirectly can affect the preterm birth rate, preventing from the invasive surgical techniques, required for treatment of high grade cervical lesions.

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