ISSN: 2148-4724
E-ISSN: 2548-0030

TMSJ

TURKISH MEDICAL STUDENT JOURNAL

Volume: 6 Issue: 1 Feb 2019

http://tmsj.trakya.edu.tr/

TMSJ

TURKISH MEDICAL STUDENT JOURNAL

THE OFFICIAL JOURNAL OF
TRAKYA UNIVERSITY FACULTY OF MEDICINE

Citation Abbreviation: Turkish Med. Stud. J.

VOLUME 6 - ISSUE 1 - FEB 2019

Published three times a year

Free access to the Journal's web site: *http://tmsj.trakya.edu.tr*

Manuscript Submission*: tmsj@trakya.edu.tr*

Editorial Office
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Printing at: Trakya Üniversitesi Basımevi
Edirne Teknik Bilimler M.Y.O Sarayiçi Yerleşkesi, 22020 Yeni İmaret, Edirne, Turkey
Phone: +90 (284) 224 02 83
Printing Date: February 2019
ISSN: 2148-4724 E-ISSN: 2548-0030

TMSJ
TURKISH MEDICAL STUDENT JOURNAL

Indexed In
Türk Medline
Science World Index
Science Library Index
Academic Keys

Publisher
Trakya University

TMSJ
TURKISH MEDICAL STUDENT JOURNAL

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Turkish Medical Student Journal (TMSJ) is an independent, non-profit, peer-reviewed, international, open access journal; which aims to publish articles of interest to both physicians and scientists. TMSJ is published three times a year, in February, June and October by Trakya University. The language of publication is English.

TMSJ publishes original researches, interesting case reports and reviews regarding all fields of medicine. All of the published articles are open-access and reachable in our website. The primary aim of the journal is to publish original articles with high scientific and ethical quality and serve as a good example of medical publications for stimulating students, doctors, researchers. Our mission is to feature quality publications that will contribute to the progress of medical sciences as well as encourage medical students to think critically and share their hypotheses and research results internationally.

The Editorial Board and the Publisher adheres to the principles of International Council of Medical Journal Editors (ICMJE), Committee on Publication Ethics (COPE).

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All manuscripts submitted for publication are reviewed for their originality, methodology, importance, quality, ethical nature and suitability for the journal by the editorial board and briefly revised by the advisory board whose members are respected academicians in their fields. Well-constructed scheme is used for the evaluation process. All manuscripts are reviewed by two different members of the editorial board, followed by peer revision from at least two professors, belonging to different institutions, who are experts in their areas. The editors assist authors to improve the quality of their papers. The editor-in-chief has full authority over the editorial, scientific content and the timing of publication.

ETHICS

Turkish Medical Student Journal depends on publication ethics to ensure all articles published in TMSJ are acceptable in terms of scientific ethical standards and do not include any kind of plagiarism. TMSJ expects authors and editorial board to adhere the principles of Committee on Publication Ethics (COPE). To reach the highest standards, TMSJ has an advisory board member who is a professional in ethics.

All original articles submitted to the TMSJ have to be approved by an ethical committee and include the name of ethics committee(s) or institutional review board(s), the number/ID of the approval(s). Additionally, informed consent documents obtained from patients involving case reports is required for the submission.

All received manuscripts are screened by a plagiarism software (iThenticate). Similarity percentage more than 25 (or more than 5 for one paper) and six consecutive words cited from an another published paper in the same order are the causes of immediate rejection.

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All opinions, reports and results within the articles that are published in the TMSJ are the personal opinions of the authors. The Editorial board, the editorial advisory board, the publisher and the owner of the TMSJ do not accept any responsibility for these articles.

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CATEGORIES OF ARTICLES

The Journal publishes the following types of articles:

Original Research Articles: Original prospective or retrospective studies of basic or clinical investigations in areas relevant to medicine.

Content:

- Abstract (average 400 words; the structured abstract contain the following sections: aims, methods, results, conclusion)
- Introduction
- Material and Methods
- Results
- Discussion
- References

Review Articles: The authors may be invited to write or may submit a review article. Reviews including the latest medical literature may be prepared on all medical topics.

Content:

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- Titles on related topics
- References

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Content:

- Abstract (average 200 words; the structured abstract contain the following sections: introduction, case report, conclusion)
- Introduction
- Case presentation
- Discussion
- References

Editorial Commentary/Discussion: Evaluation of the original research article is done by the specialists of the field (except the authors of the research article) and it is published at the end of the related article.

Letters to the Editor: These are the letters that include different views, experiments and questions of the readers about the manuscripts that were published in this journal in the recent year and should be no more than 500 words.

Content:

- There's no title and abstract.
- The number of references should not exceed 5.
- Submitted letters should include a note indicating the attribution to an article (with the number and date) and the name, affiliation and address of the author(s) at the end.
- The answer to the letter is given by the editor or the author(s) of the manuscript and is published in the journal.

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- Titles on related topics
- References

What is Your Diagnosis? : These articles are related with diseases that are seen rarely and
show differences in diagnosis and treatment, and they are prepared as questions-answers.

Content:

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- References

MANUSCRIPT PREPARATION

Authors are encouraged to follow the following principles before submitting their material.

-The article should be written in IBM compatible computers with Microsoft Word.

ABBREVIATIONS: All abbreviations in the text must be defined the first time they are used, and the abbreviations should be displayed in parentheses after the definition. Authors should avoid abbreviations in the title, abstract and at the beginning of the first sentences of the paragraphs.

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Scientific or Technical Report: Smith P. Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX) Dept. of Health and Human Services (US). Office of Evaluation and Inspections: 1994 Oct. Report No: HHSIGOE 169200860.

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Manuscripts published in electronic format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) l995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL:http://www.cdc.gov/ncidodlElD/cid.htm

EDITORIAL

Dear readers,

On the behalf of our team, I am very proud of publishing the first issue of the year 2019. This issue consists of four original articles, one case report, one review, and one letter-to-the-editor.

Investigating new approaches in the treatment of cancer is one of the contemporary topics of the medical research world, as great progress has been covered in the treatment of many cancer types owing to newly discovered anti-cancer substances. Özkan et al. found out that spider web extract causes a strong antineoplastic effect in HeLa cell line. Avul et al. presented a case, who was diagnosed with malignant castration-resistant prostate cancer and was applied Lutetium - 177 (a prostate-specific membrane antigen). They also reported that the tumor has regressed, which was evaluated by Gallium - 68 prostate-specific membrane antigen PET scan, pointing out the success of a new treatment approach in the treatment of malignant castration-resistant prostate cancer. We appreciate their work and are proud to be able to share their findings with our precious readers.

Beginning the New Year, new members have started to work in our editorial board: Kaan ÇİFCİBAŞI from Ludwig Maximilian University Faculty of Medicine, Alperen ELİBOL from Acıbadem University Faculty of Medicine, Nazlıcan KÜKÜRTÇÜ from Akdeniz University Faculty of Medicine, Berra KURTOĞLU from TOBB Economics and Technology University Faculty of Medicine; and four editors from Trakya University School of Medicine: Alperen Taha CERTEL, Arda Ulaş MUTLU, Beliz KOÇYİĞİT, Irmak İrem ÖZYİĞİT. I personally appreciate their motivation, being sure about the effort they will give to increase the quality of our journal.

The year 2019 has started with the news of great medical student congresses around the world. I would like to announce the medical congresses: 4th SaMED (7-10 February 2019, Sarajevo, BOSNIA-HERZEGOVINA), BIMCO 2019 (2-5 April 2019, Chernivtsi, UKRAİNE), ICMS (9-12 May 2019 Sofia, BULGARIA). We are also going to be in those congresses to represent our journal, so we kindly invite our readers to be part of this amazing scientific atmosphere.

With this issue, my work as the editor-in-chief in the journal has come to an end, after publishing four issues since November 2017. In this period, we have published 16 original articles, 6 case reports, 2 reviews, and 2 letters-to-the-editor. We have changed our editorial policy, as we gathered editors from different institutions. As of February 2019, our editorial board consists of 9 members (2 of them being international editors) from different institutions among the total of 24 members (37.5%). Owing to that, we achieved the opportunity to introduce our journal in wider areas, to raise the recognition level of our journal, and more importantly to be able to evaluate the articles with editors from different institutions. Moreover, we started to publish our issues in DergiPark as well, the open journal system of Turkey. All the achievements that Turkish Medical Student Journal accomplished, are the results of the great effort of every editorial board member. I entrust my duty in the journal as an editor-in-chief to Nur Gülce İŞKAN believing, without any doubt, she and her team are going to give all their efforts to increase the impact and quality of the journal even further.

Hope to meet you again in the next issue.

Koray DEMİRCİ
Editor-in-Chief

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TMSJ
Turkish Med. Stud. J. 2018; 5: 50-3 DOI: 10.4274/tmsj.galenos.2019.06.01.01
Received: 11.06.2018 - Accepted: 11.01.2019

AN INVESTIGATION ON THE ANTICANCER EFFECT OF SPIDER WEB IN HUMAN CERVICAL CELL LINE

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ABSTRACT

Aims: The aim of this study is to investigate the anti-cancer and anti-proliferative effect of spider web extract, and mitochondrial apoptosis signaling response via gene expression levels belonging to caspase-3 depended apoptosis in both healthy Astrocyte and cancerous HeLa cell lines.

Methods: We used HeLa and C8-D1A Astrocyte type I clone cell lines as the human cervical cancer and healthy cell line, respectively. We treated the cells with spider web extract and performed MTT assay. IC50 was calculated by probit analysis. Molecular fluorescence staining and TALI cytometer measurements were also applied. The response of the mitochondrial apoptosis pathway to spider web extract treatment was analyzed by the qRT-PCR assay.

Results: The spider web extract administered in different concentrations to the HeLa cell line reduced cell viability at a statistically significant level after 24 hours of application. In the astrocyte cell line, the spider web extract did not cause statistically significant cell death. In the analysis of gene expressions, a strong mitochondrial apoptosis signaling was observed.

Conclusion: While spider web extract does not cause any cytotoxic effects on healthy cell lines, it causes a strong fatal effect in cancer cell line. In addition, IC50 dose of the spider web extract is satisfying compared to many other natural products in the market which have anti-cancer effects. Spider web extract causes a programmed cell death by following death signaling pathways.

Keywords: Spider, in vitro, cervical cancer

INTRODUCTION

Cervical cancer is the fourth most common type of cancer, estimated to affect one million women worldwide (1). Cervical cancers constitute 13.4% of deaths caused by female genital tract cancers (2). When it is detected early, there is a chance of complete recovery. However, if it is treated, the prognosis is poor. Rapid proliferation, resistance to treatment, early and late metastases as well as poor diagnosis are the causes of its lethal course. The need for new or supportive treatment methods has risen due to problems such as the long-term toxic effects of the current drugs. This reduces the chance of success in the treatment. The biggest problem encountered with chemotherapeutic agents is the rapid development of irreversible drug resistance by cancer cells (3, 4).

According to recent studies, spider venom and its derivatives such as peptides may have anti-cancer effects by inducing cell cycle arrest and apoptosis, inhibiting invasion, metastasis, angiogenesis, also blocking specific transmembrane channels. The researchers studied with spider venom or the spider itself (5-7). However, there is no study about anti-cancer effects of spider web in cancer cell lines to the best of our knowledge.

The spider web is a natural product that is being used in the Thrace Region and Bulgaria for the purpose of treatment of late-healing or deep wounds. It has been thought to have certain substances which play a role in controlling cell proliferation and inflammatory processes (8). Therefore, we extracted the spider web with

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different methods and analyzed it using liquid chromatography– tandem mass spectrometry (Lc-MS/MS). According to our previous study, there are various phenolic substances such as gallic acid, trans caffeic acid, naringin and melatonin at a very high level in the spider web and they are preserved despite natural events such as sunlight, wind or rain (TurkPatent, 2017/19916) (Table 1) (8). These substances play active roles in breaking the drug resistance in cancer cell lines that show multi-drug resistance and also, naringenin and quercetin are the most known members of the flavonoid family (9). Therefore, we hypothesized that spider web extract (SWE) can be an apoptosis activator and have anti-proliferative effects in cervical cancer cell lines. Additionally, it may be an anti-oxidant for healthy cells.

The aim of this study is to investigate the anti-proliferative, anti-cancer and apoptotic effects of the SWE on HeLa cell lines and to find the IC50 dose. In addition, we also studied endogenous apoptosis genes at the gene expression level and the mechanism by which the possible apoptotic effect of the extract induced. Finally, we applied the determined doses to healthy astrocyte cell lines and investigated its cytotoxicity.

Table 1: Phenolic substances, catecholamines and plant stress hormones detected in spider web extract with different alcohols.

Polyphenolic Contents of Spider Web			
Phenolic Substance	***RT****	***Response***	***Concentration (ng/ml)***
Gallic Acid	0.534	1345	41.3478
Catechin	0.539	171	10.5757
Protocatechuic Acid	0.556	48444	790.1062
2,5-Dihydroxybenzoic Acid	0.564	13569	606.5906
Trans-Caffeic Acid	0.607	9409	89.1363
Trans-p-Coumaric Acid	0.607	292	103.6793
Naringin	3.983	407	41.4550
Quercetin	4.130	27798	361.0320
Trans-Sinapic Acid	4.138	38	195.4869
Catecholamines and Hormones	***RT****	***Response***	***Concentration (ng/ml)***
Melatonin	4.186	81	3.9153
Salicylic Acid	3.745	2109	42.2579
Abscisic Acid	4.197	75	2.4156
Jasmonic Acid	4.373	14	1.5531
Indole-3-Acetic Acid	3.805	17	23.7142

RT: Response Time

MATERIAL AND METHODS

The extraction of spider web

In the study, we extracted 478 mg of spider web with a 3:1.5:1.5 v/v isopropyl alcohol (Sigma W292907), ultra-pure water, Acetonitrile (Sigma 271004) in a heating magnetic stirrer at 50 °C for 45 minutes. We centrifuged the final volume of 40 ml of this extract at 5000 rpm for 10 minutes, after the supernatant was passed through a syringe tip (45 µm) to obtain a final volume of 12.5 ml of the extract. We blew off the extract completely, using a hotplate and stocked the obtained product with water. This stock was used in all studies within 1-2 days of storage at 4 ° C in the dark, while the other part was divided into two equal solutions and stored at -20 °C and -80 °C for possible future use.

Culture and passage of the cells

In the study, we used HeLa (ATCC® CCL2 ™) cell line and brain astrocyte C8-D1A Astrocytic type I clone (ATCC® CRL-2541 ™) cell line as the human cervical cancer cell line and healthy cell line, respectively. Cell lines were cultured in the laboratories of Medical Biology Department in Trakya University. We cultured the cells grown in DMEM: F-12 Medium (ATCC® 30-2006 ™) containing heat-inactivated 10% FBS (Gibco Life Technologies), 2 mM L-glutamine (Gibco-Life Technologies) and 1% penicillin/streptomycin (Invitrogen, Life Technologies) in sterile incubators at 37 °C and the presence of 5% CO2. All the studies started from the 5th passage of the cell lines and the study was terminated at 20th passage maximum.

MTT assay and determination of IC50 dose

In this study, we seeded HeLa cell line and the C8-D1A cell line with multi-pipettes at 10,000 to 15,000 well during application in 96-well culture plates. At the end of one night (about 16 hours), we applied spider web extracts, and left them for incubation for 24 and 48 hours. In the MTT viability analyzes, the groups were composed of 6 wells, and in these groups, there were control groups containing solvent. After the first dose of SWE was made with 100-fold dilution, we used it in 9 different concentrations by serial dilution with solvent by 50% percent. We performed MTT assay after incubation for cell survival (viability) analysis. For this analysis, we pipetted the "Yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide)" test solution, prepared at a dose of 5 mg/ml, equally to all wells as 20 µl/well. Then, we incubated the plates for 2-4 hours. After the incubation, we completely removed the medium in the wells with a multi-pipet so as not to damage the cell line adhered to the base. We added 200 µl of ultra-pure DMSO to each medium and incubated for 2 hours. Plates from the incubator were read spectrophotometrically at 492 and 570 nm wavelength with a Multiskan GO microplate reader. The value obtained from the control group was determined as 100% viability and the comparative mortality rate was determined based on this value. IC50 dose was calculated by probit analysis.

Molecular fluorescence staining

In the study, we used the NucBlue® Live ReadyProbes® to monitor apoptosis induction and nuclear morphology, CM-H2DCFDA staining for oxidative stress, and CellEvent ™ Caspase-3/7 Green molecular probe for caspase-dependent apoptosis detection. For fluorescence staining, we plated HeLa cells to 24-well plate as 5x104 cells/well. The following day, we treated IC50 dose of the SWE to all cells except for the control group. After 24 hours of incubation, we labeled the first cell group with 5 µl CellEvent ™ Caspase-3/7 Green Detection Reagent, the second group with four drops of NucBlue® Live ReadyProbes® Reactant (Thermo Scientific ready-made probe guides) and 10 µl CM-H2DCFDA and incubated the plate for 30 minutes at 37 °C. Images were obtained with 40X magnification on a Zeiss Axio Vert. A1 fluorescence microscope which has a DAPI filter for NucBlue® and FITC filter for both caspase 3/7 and CM-H2DCF-DA. We performed each staining experiment on living cells.

TALI cytometer measurements

We also determined the apoptotic properties of SWE on human cervical cancer via Annexin V/PI staining with image-based cytometer. For this purpose, cells were cultured 25 cm^2 cell culture flask and then incubated them for 24 hours. We used Tali® Image Cytometer (Life Technologies, USA) to observe the apoptotic and cell cycle effects of the SWE. At the end of the treatment, we removed the mediums in the flasks. We incubated these flasks for 5 minutes with 3 ml of trypsin EDTA and harvested the cells by automatic pipetting into Eppendorf tubes and centrifuged the tubes at 1000 rpm for 2.5 minutes. We completely withdrew the upper trypsin and supernatant and used the subcellular cells for analysis. The experiments to determine the apoptosis level by Tali cytometry were done by using Tali® Apoptosis Kit Annexin V Alexa Fluor® 488 and Propidium Iodide (Life Technologies). According to the kit procedure, we suspended the cells remaining at the bottom of the tube in 1ml 1X Annexin binding buffer, 300 µl of this mixture was taken and vortexed by adding 5 µl of Annexin V Alexa Fluor® 488 (Component A) per 100 µl. Afterwards, we incubated the mixture at room temperature for 20 minutes in the dark. After centrifugation at 1000 rpm for 2.5 min, we discarded the supernatant and we suspended the cells at the bottom again with 100 µL of ABB. We mixed this mixture with 1 µL of Tali® Propidium Iodide (PI, component B) solution and incubated for 1-5 minutes in the dark. After the incubation, we took out 25 µl of the mixture in the tubes to the slides in the prepared chitin, and Tali apoptosis analysis option for apoptosis and PI cell cycle analysis option for cell cycle were selected and read with the instrument software. In the analysis with Tali, each group was read by counting 20 different areas and each group was studied as 5 repetitions and 5 different slides for each repetition.

Gene expression studies (For mitochondrial apoptosis pathway)

RNA isolation

We used total RNA PureLink® RNA Mini Kit (Life Sciences, USA) for isolation of total RNA from cell lines treated with SWE according to the kit protocol. We removed the cells from the flasks using 3 ml trypsin, centrifuged and removed the supernatant. Then, we treated them with lysis buffer + 1% 2-mercaptoethanol, prepared in 1 ml kit, and transferred to the spin column. After centrifugation at 12000 G for 15 seconds at 25 °C, we removed the bottom part from the spin column. We added 700 microliters of wash buffer I and centrifuged at 25 °C for 12 seconds at 12000 G. We followed the same procedure after adding 500 microliters of wash buffer II. After the lower columns were discarded, we centrifuged the cells at 12000 G for 2 minutes and then, we passed the cells through special columns and collected the RNAs attached to the columns into the Eppendorf tube with purified water after washing the columns with the washing solution provided in the kit and cleaning the other compounds. We measured RNA quantity isolated from cell lines by using Qubit® Fluorometer (Invitrogen, USA). We equalized all RNA quantities with DNA, RNA-free ultra-pure water (Sigma, USA). We performed cDNA synthesis by the equalized RNAs by using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) with PCR conditions as follows: Step 1: 25 °C, 10 min; Step 2: 37 °C, 120 min; Step 3: 85 °C, 5 min. The resulting cDNAs were stored at -20 ° C for future analysis.

qRT-PCR Analysis

In our study, we studied the intrinsic apoptosis signaling (Mitochondrial pathway): Caspase 3, Caspase 8, BAX, BCL-2, PUMA, NOXA, P53, Cytochrome C and Apaf 1 genes. These cDNAs were amplified in qPCR according to SYBR Green qPCR Mastermix protocol using ABI Step One Plus real-time PCR using the specific primers and PCR conditions given in Table 1 in 96-well plates. The Ct values of the peaks which were obtained during the amplification were used to determine the gene expressions and were calculated by the ΔΔCt method. GAPDH, 18 S, β-actin specific control primers were used as endogenous control.

Table 2: Specific primers gene sequences and PCR conditions of the study.

P53	F	5'	CACGAGCGCTGCTCAGATAGC	3'	PCR conditions
	R	5'	ACAGGCACAAACACGCACAAA	3'	
PUMA	F	5'	CAGACTGTGAATCCTGTGCT	3'	
	R	5'	ACAGTATCTTACAGGCTGGG	3'	
NOXA	F	5'	GTGCCCTTGGAAACGGAAGA	3'	
	R	5'	CCAGCCGCCCAGTCTAATCA	3'	
BAX	F	5'	TTCATCCAGGATCGAGCAGA	3'	1 cycle of 2 min at 50°C and 10 min at 95°C followed by 48 cycles of denaturation at 95°C for 15 sec, annealing and extension at 60°C for 1 min
	R	5'	GCAAAGTAGAAGGCAACG	3'	
BCL2	F	5'	ATGTGTGTGGAGAGCGTCAA	3'	
	R	5'	ACAGTTCCACAAAGGCATCC	3'	
Cyt-C	F	5'	AGTGGCTAGAGTGGTCATTCATTTACA	3'	
	R	5'	TCATGATCTGAATTCTGGTGTATGAGA	3'	
APAF-1	F	5'	GATATGGAATGTCTTCAGATGGCC	3'	
	R	5'	GGTCTGTGAGGACTCCCCA	3'	
CASPASE 3	F	5'	GGTATTGAGACAGACAGTGG	3'	
	R	5'	CATGGGATCTGTTTCTTTGC	3'	
CASPASE 8	F	5'	AGAGTCTGTGCCCAAATCAAC	3'	
	R	5'	GCTGCTTCTCTCTTTGCTGAA	3'	

Statistical analysis

The differences between the cell numbers determined by Tali and the averages of the expression values obtained by the ΔΔCt method in qRT-PCR array were determined by one-way ANOVA and Student's T-test, and the groups of means were determined by the Duncan's test according to the status of the study data. Mean ± standard deviation, numbers and percentages were used as descriptive statistics. We used SPSS 20 statistical package program in the study. A p-value of <0.05 was evaluated as statistically significant.

RESULTS

The SWE administered with different concentrations in the HeLa cell line reduced cell viability at a statistically significant level after 24 hours of application (FHeLa = 28.44, sd = 9-40, p <0.0001) (Figure 1). We determined the highest viable cell ratio as 65.99 ± 1.76% for 3.125 µg/ml-SWE after 24 hours, while only 45.36 ± 3.18% of the cells remained alive at 400 µg/ml concentration. We determined that the cell death was dependent on the dosage, and the correlation coefficient was -0.635 and the significance level was p = 0.049.

In the astrocyte cell line, the SWE did not cause any statistically significant cell death (FAstrocyte = 1.305; standard deviation (sd) = 9-50, p = 0.258) (Figure 1).

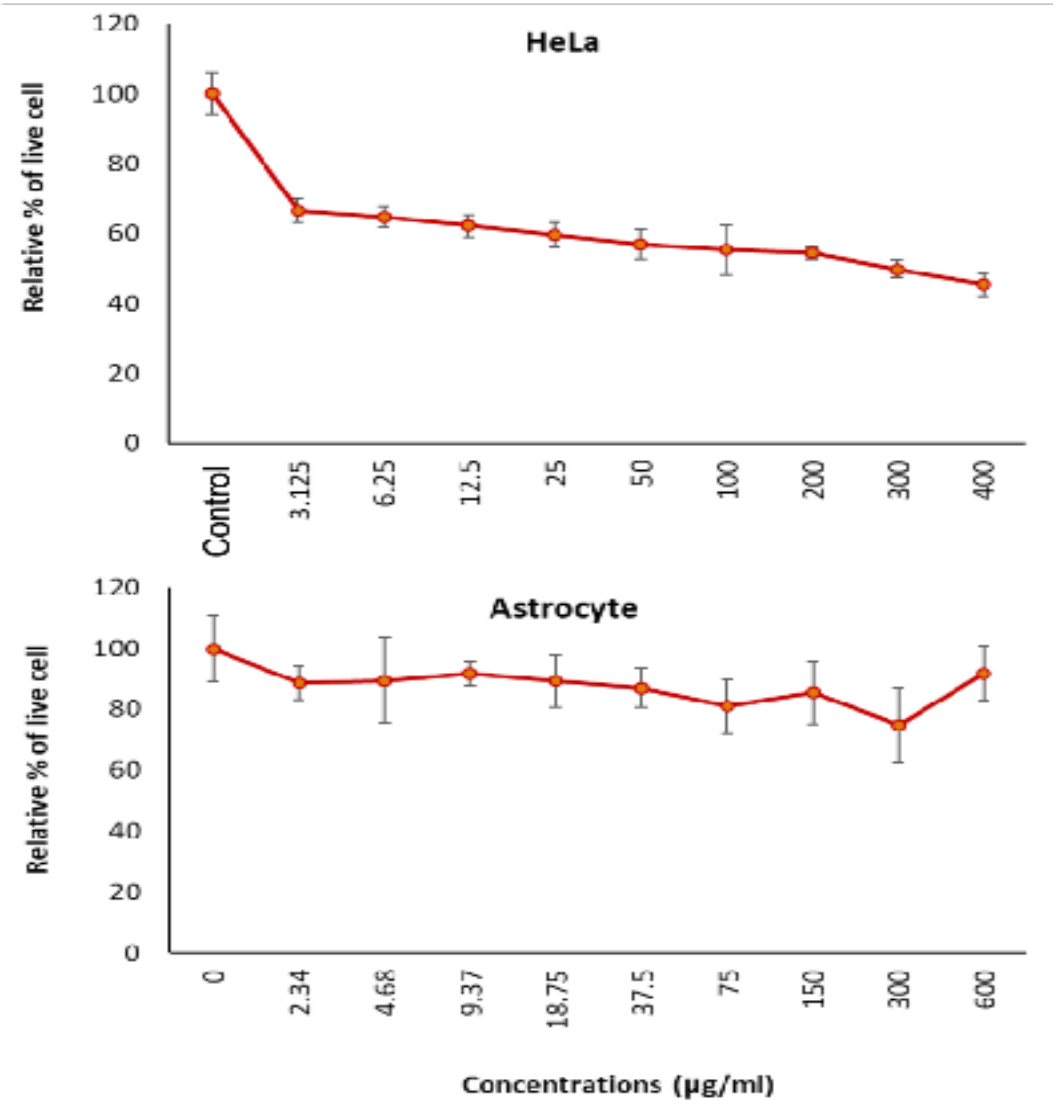

Figure 1: The effect of application of SWE for 24 hours on cell viability in HeLa and healthy astrocyte cell lines MTT test, data % Viability mean ± standard deviation.

The IC50 value for the spider web extract was determined as 291.471 µg/ml and this dose was used in the following sections of the study.

As a result of the MTT test on HeLa cell lines, we determined only the living and dead cell ratios of the SWE and solvent (control) application in the study. We also performed TALI image-based cytometer analysis to determine whether the death of the cell lines by SWE were based on apoptosis.

Among the cells which were stained by using Annexin V-Alexa Fluor® 488, Propidium Iodide and Tali® Apoptosis Test Kit, dead or late apoptotic cells were red or yellow fluorescence, apoptotic cells were green fluorescence. We performed the analysis using the instrument software and the results are given in Figure 2.

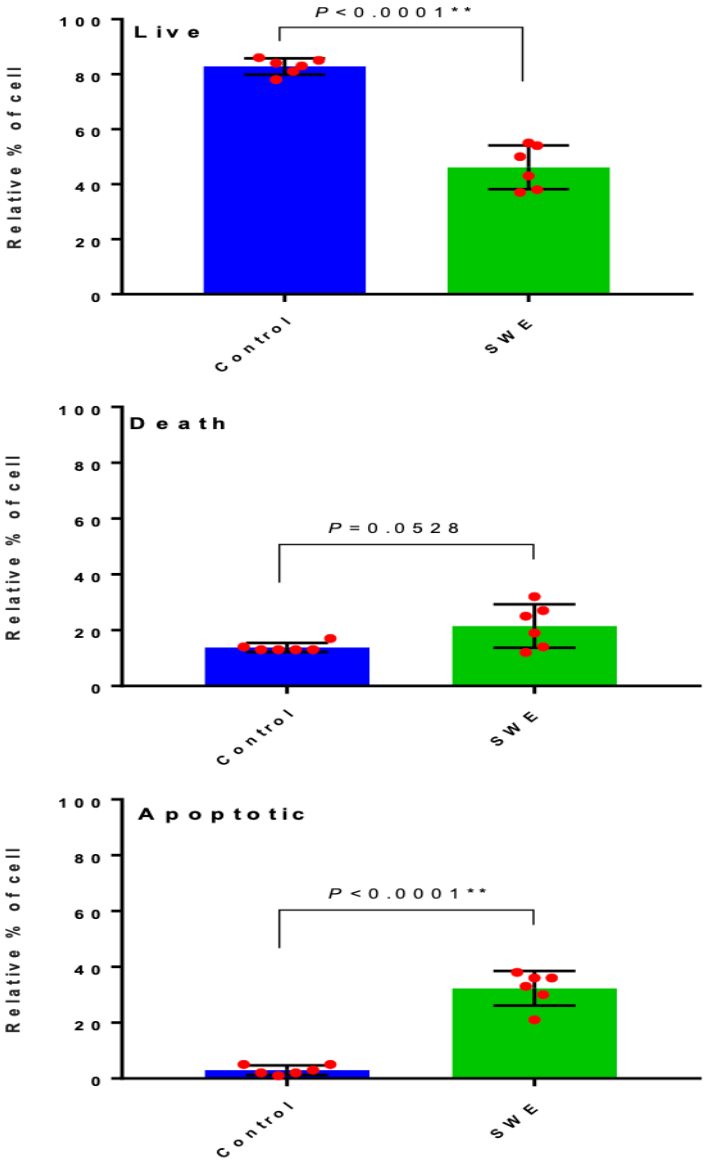

Figure 2: The percentages of living, dead and apoptotic cells detected in the HeLa peripheral cervical cancer cell line in a TALI image-based cytometry analysis. All data are given as the mean values of the percentages for each group ± sd. n = 6; Student's T-test p≤0.05. (SWE: Spider web extract)

As a result, spider web application decreased the number of live cells in HeLa cells by a statistically significant ($p<0.0001$, Figure 2) level compared to control group (82.83%) and the alive cell ratio was found to be 46.16% after 24 hours. Propidium iodide positive cells showed dead cells and the dead cell ratio was determined as 13.83% in the control group, to 21.5% in the SW group. However, no statistically significant difference was found between the groups. SWE increased the number of apoptotic cells at a statistically significant ($p<0.0001$, Figure 2) level compared to the control, and the apoptotic cell ratio was 3% at the control, whereas 32.33% of the SWE cells showed apoptosis.

In the analysis of TALI cytometer, SWE caused a strongly programmed cell death. In order to support this analysis, the cells were stained with different substances and images were given in Figure 3 and 4.

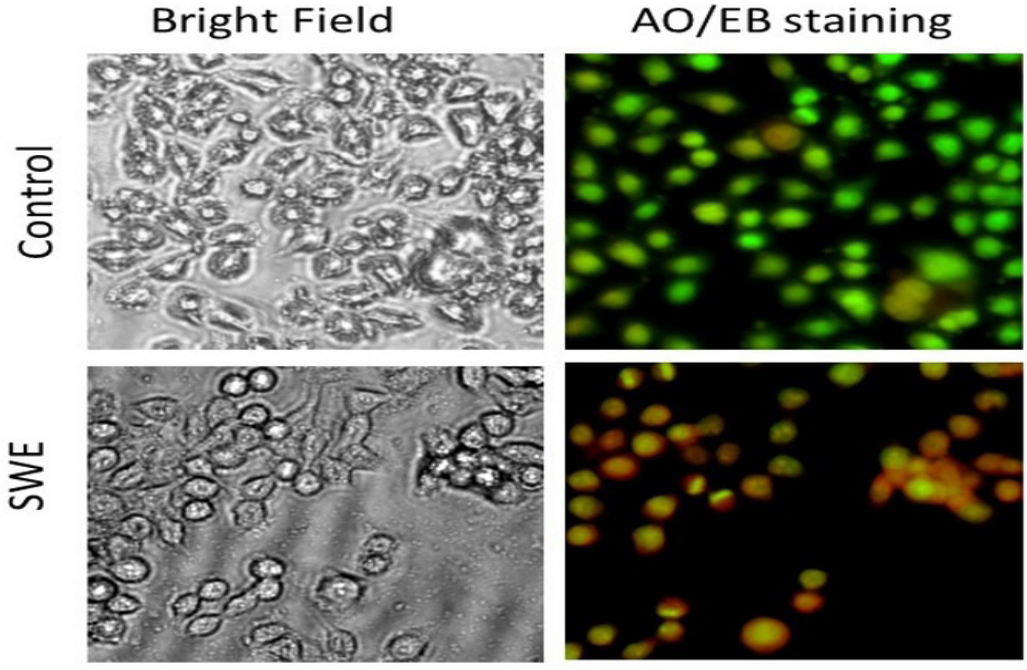

Figure 3: Cell morphology obtained by light field microscopy after incubation for 24 hours in HeLa cell line with IC50 dose of SWE and solvent (Control), apoptosis image obtained with Acridine orange/Ethidium Bromide staining FITC and Texas Red filter.

As Figure 3 shows, cell morphologies were different between the control and SWE groups. Especially in the SWE group, not only the density of the cells was decreased but also the shape of cells differed and became smaller and gemmiferous. In AO/EB staining, alive and healthy cells were green fluorescence while necrotic cells were only red fluorescence, apoptotic cells were green and red or orange fluorescence. As it is seen in Figure 3, SWE caused a greater number of apoptotic cells by comparison to the control group in HeLa cell line. The same phenomenon was identified in nuclear staining, especially after the SWE application, nuclear morphologies of HeLa cells were impaired and apoptotic bodies became clear to see. Cell Event Caspase 3-7 molecular probes specifically bound to Caspase 3 and Caspase 7 proteins were detected extensively in cell lines subjected to SWE application and showed a dense apoptosis (Figure 4).

We also investigated the effects of SWE on HeLa cells in mitochondrial apoptosis pathways by gene expressions. The results were given in Figure 5 and 6.

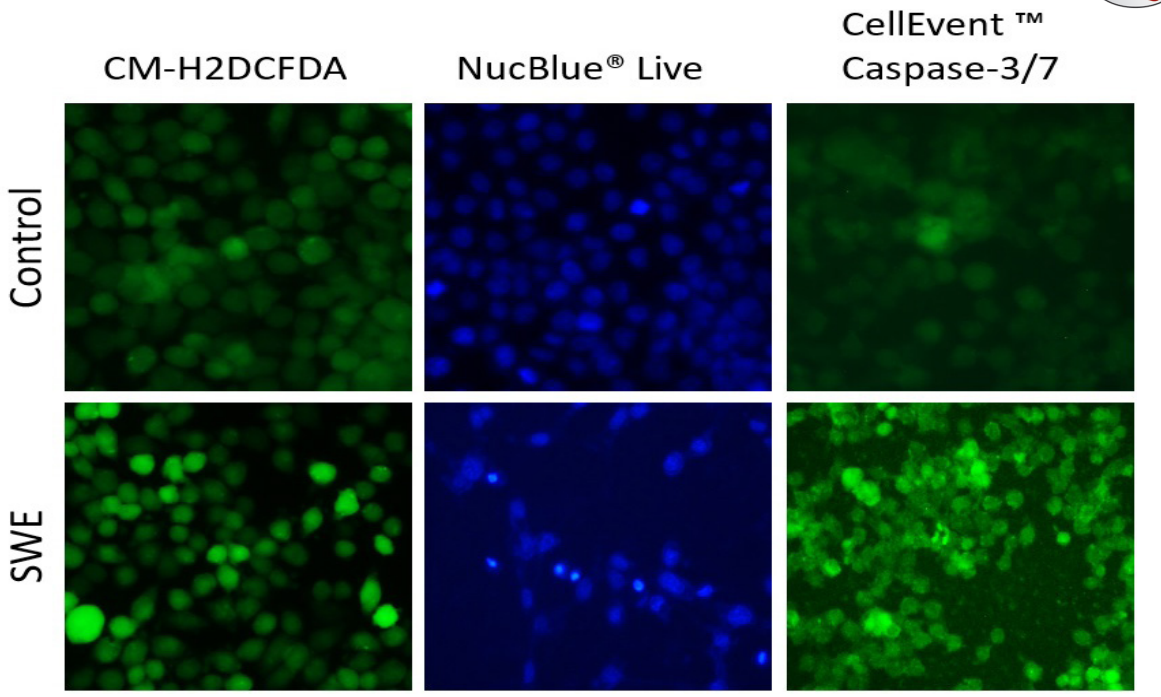

Figure 4: Images obtained by fluorescent staining with oxidative stress (CM-H2DCFDA), nuclear apoptosis (NucBlue® Live ReadyProbes®) and caspase-dependent apoptosis (CellEvent ™ Caspase-3/7 Green Detection Reagent) molecular probes in HeLa cell lines after the application of SWE in the IC50 dose.

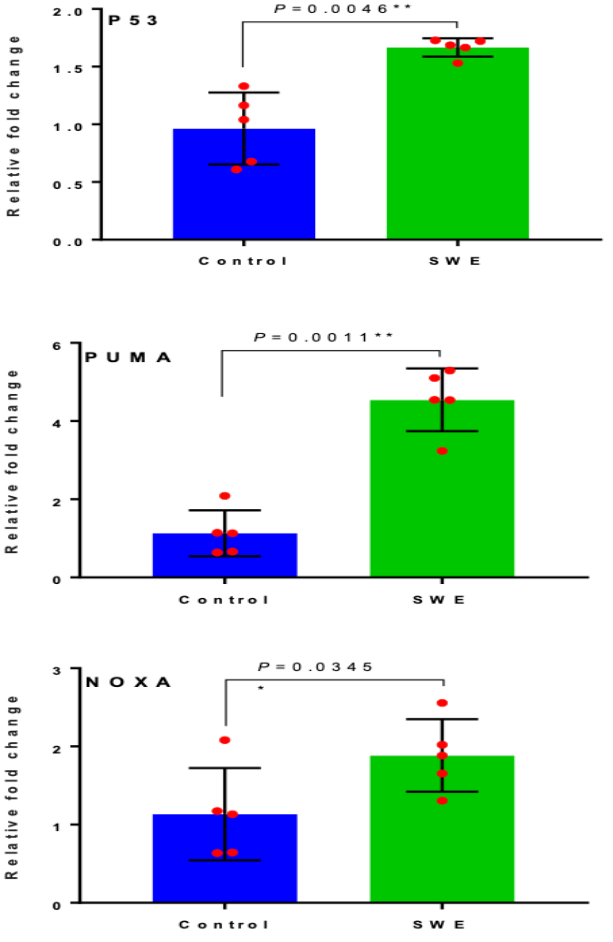

*Figure 5: The effect of SWE on IC50 dose and solvent (control) application for 24 hours in HeLa cell line on P53, PUMA, and NOXA gene expressions. Data: mean ± standard error; relative fold increase was normalized by Actin-β gene expression. *Data is statistically significant P≤0.05, ** Data is statistically significant P≤0.01, Student's T-test.*

The spider web extract application increased tumor suppressor p53 gene expression (1.6 fold) at a statistically significant level (p=0.0046, Figure 5) after 24 hours. Similarly, Puma (4.54 fold) and Noxa (1.88 fold) gene expressions associated with p53 gene expression increased at a statistically significant level (p=0.0011, p=0.0345 respectively) (Figure 5). However, there was no statistically significant increase in BCL 2 gene expression compared to the control. On the other hand, pro-apoptotic BAX (6.10 fold) gene expression was increased at a significant level (p=0.0025, Figure 6) compared to the control. BCL2/BAX ratio declined in the favor of BAX. Cyt-c (1.38 fold) expression was not significantly different from control, whereas Apaf 1 was overexpressed (14.83 fold). At the same time, caspase 3 (1.61 fold) and caspase 8 (1.76 fold) gene expressions were also increased in a statistically significant level (p= 0.0078, p=0.0114 respectively) compared to the control (Figure 6). A strong mitochondrial apoptosis signaling was observed.

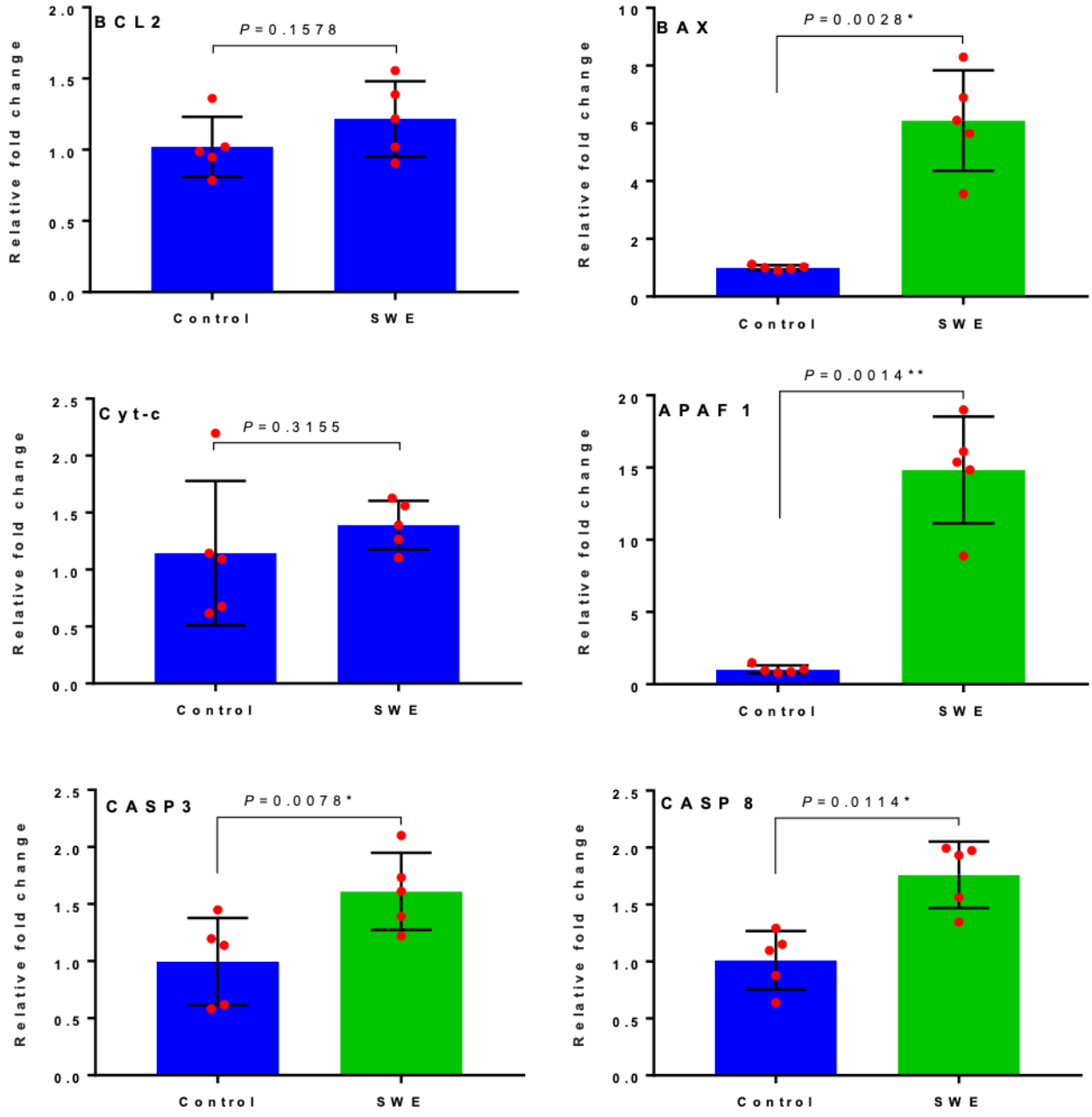

*Figure 6: The effect of SWE on the IC50 dose and solvent (control) in HeLa cell line for 24 hours on BAX, Cyt-C, APAF1, Caspase 3 and Caspase 8 gene expression in the mitochondrial apoptosis signaling pathway. Data: mean ± standard error; relative fold increase was normalized by Actin-β gene expression *Data was statistically significant P≤0.05, ** Data was statistically significant P≤0.01, Student's T-test.*

DISCUSSION

Since the existence of mankind and the development of civilization, people have benefited from many living things and their products in order to maintain a long and healthy life and to avoid diseases. With the development of modern medicine, these materials have been lost in importance for a long time, and they have become popular in search for different solutions for serious side effects of drugs. The SWE was one of those natural materials which has been used for different purposes such as healing wounds in some areas of Balkan peninsula throughout history (8). It is also used in the area of medicine as a biomaterial because of its endurance and elasticity (7, 8). In addition, there are various phenolic substances in spider web as mentioned before.

Naringenin and Quercetin are among the substances that are found in the spider web and they are the most known members of the flavonoid family (3). It has been reported that they have anti-cancer effect in different cancer cell lines (1, 10-22). Gallic acid, one of the natural phenolic compounds which is found in mostly plants that are used in Chinese alternative medicine, has dose-dependent effects of viability, proliferation and induces invasion in HeLa and other cervical cancer cell lines. In addition, it suppresses the angiogenesis (18, 19). Trans Caffeic Acid is also another substance that is reported to induce apoptosis and has anti-cancer effects (21, 22). Additionally, there are some studies which suggest that even if melatonin is a hormone in the human body, it also has anti-oxidant effects (23). All of these materials were present in the spider web which is found in our prestudy. Therefore, it can be said that spider web may be an apoptosis activator and has anti-proliferative effects. Thus, we applied SWE to HeLa cervical cancer cell line and healthy astrocyte cells in order to detect its effects.

Even if anti-neoplastic agents such as 5-fluorouracil, cisplatin, which are known as conventional in today's cancer therapies, are being used intensively, the researchers are trying to develop molecules which have specific targets, prefer specific signal pathways and effect the cellular interaction in there. While these types of molecules cause significant death effects in cancer cells, the effects on healthy cells remain at minimum (24). Almost all of the cancer drugs developed until today are derived from natural products and they are among the most studied topics (25). For this reason, we investigated the effects the effects of SWE at its IC50 dose on mitochondrial apoptosis pathway which is one of the most effective pathways in cancer cells and causes a programmed cell death, by MTT test and fluorescence staining methods. As we have mentioned before, SWE increased the expression of tumor suppressor or pro-apoptotic genes such as p53, Puma, Noxa and BAX. Therefore, it can be said that SWE caused a strong mitochondrial apoptosis signal.

As a conclusion, while spider web extract does not cause any cytotoxic effects on healthy cell lines, it causes a strong death effect in cancer cell line. In addition, we thought that IC50 dose of the spider web extract is satisfying compared to many other natural products in the market which have anti-cancer effects.

The mechanism of death effects of spider web extract on HeLa cancer cell line is not based on general toxicity. It rather causes a programmed cell death by following death signaling pathways. We determined that the resulting cell death is due to a mitochondrial apoptosis pathway in cervical cancer (HeLa). We thought that the spider web extract is a natural product worthy of further studies since it is rich in phenolic substance content.

Ethics Committee Approval: N/A
Informed Consent: N/A
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: ANÖ, OD. Design: ANÖ, OD. Supervision: ANÖ, OD. Resources: ANÖ, OD. Materials: ANÖ, OD. Data collection and/or processing: ANÖ, OD. Analysis and/or Interpretation: ANÖ, OD. Literature Search: ANÖ, OD. Writing Manuscript: ANÖ, OD. Critical Review: ANÖ, OD.
Financial disclosure: This study was financialy supported by the Scientific and Technological Research Council of Turkey, 2016/2 1919B011700745.

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Turkish Med. Stud. J. 2018; 5: 54-7 DOI: 10.4274/tmsj.galenos.2019.06.01.02
Received: 11.12.2018 - Accepted: 28.12.2018

SYSTEMIC CANNABIDIOL DOES NOT REDUCE COMPOUND 48/80-INDUCED ITCHING BEHAVIOR IN MICE

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ABSTRACT

Aims: Cannabinoids are chemical compounds including natural cannabinoids found in the Cannabis plant, their synthetic counterparts, and endocannabinoids. Cannabidiol, a phytocannabinoid derived from the Cannabis plant, exerts anticonvulsant, anxiolytic, anti-inflammatory, neuroprotective, analgesic effects. Although there are many similarities between the pathophysiological mechanisms of pain and itch, researches that investigate the effect of cannabinoids on itching are insufficient. Here, we aimed to examine the antipruritic effect of cannabidiol and the contribution of spinal cannabinoid receptors.

Methods: Male Balb/c mice, weighing 20-30 g, were used. Itching behavior was produced by intradermal injection of compound 48/80 (100 µg/50 µl); cannabidiol (1, 3, 10 mg/kg, ip) was administered 30 minutes before compound 48/80 injections. Then, scratching of the injected site by the hind paws was videotaped for 30 minutes. Locomotor performances were assessed using a rotarod apparatus.

Results: Cannabidiol had no effect on compound 48/80-induced itching behavior at any dose given; moreover, cannabidiol did not produce any impairment on motor function. AM-251, a cannabinoid receptor type 1 antagonist, and AM-630, a cannabinoid receptor type 2 antagonist were administered intrathecally to observe the contribution of spinal cannabinoid receptors to the antipruritic action of cannabidiol. We observed that cannabidol did not possess any effect on itching behaviour.

Conclusion: Our results indicate that systemic administration of cannabidiol does not attenuate compound 48/80 induced itching behavior in mice.

Keywords: Cannabidiol, compound 48-80, pruritus

INTRODUCTION

Pruritus can be described as an unpleasant and strange sensation of irritation, which may also involve tingling, biting or burning that initiates itching in the skin in related areas. Many similarities between the neuronal pathways and the pathophysiological mechanisms of pain and itching have been proposed; itching sensation is transmitted to spinal cord's dorsal horn by primary afferent C fibers, and then to the brain by spinothalamic pathways (1, 2). Glutamate is suggested as the principal excitatory neurotransmitter in the spinal cord not only for pain but also for itching; similarly, descending inhibition is involved in the development of both pain and itching sensations (3, 4). Similar symptoms to allodynia, hyperalgesia and abnormal pain also occur in pathological itching conditions (2, 5). Taken together, the spinal cord seems to be an attractive target for developing new drugs against pruritus (6-8).

Cannabinoids are chemical compounds including natural cannabinoids found in the Cannabis plant (phytocannabinoid), their synthetic counterparts, and substances that make up the endogenous cannabinoid system (endocannabinoid) synthesized in the body (9). Cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2) receptors are the primary targets of all cannabinoids. Cannabis has been used for medical purposes, especially to treat pain, until the Marihuana Tax Act of 1937 which decreased its use rapidly. In recent years, there have been changes in policies which led to an increase in the use of medical cannabis in many countries (10, 11).

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This study was presented in Union of Thrace Universities 2nd International Health Sciences Congress, Tekirdağ, TURKEY, 15-17 November 2018.

In addition to pain states, cannabinoids have been proposed as potential antipruritic drugs. Cannabinoid receptor agonists have been shown to reduce histamine-induced scratches, whereas cannabinoid receptor antagonists provoked pruritic responses via CB1 receptors (12, 13). Attenuation of itching replies by augmenting endocannabinoid tonus via the inhibition of the endocannabinoid degradative enzymes, such as fatty acid amide hydrolase and monoacylglycerol lipase which is a different promising method for treating pruritus (14-16). Our research group also indicated that the synthetic cannabinoid agonist WIN 55,212-2 exerts dose-dependent antipruritic effects and this effect is partially mediated by spinal cannabinoid receptors CB1 (17, 18).

Unlike most of the other cannabinoids, the non-psychoactive phytocannabinoid cannabidiol exhibits little or no orthosteric binding potential at cannabinoid CB1 and CB2 receptors (19). However, cannabidiol has extensive therapeutic properties, including anticonvulsant, anxiolytic, anti-inflammatory, neuroprotective, analgesic effects, etc (20). Thus, the purpose of this study is to investigate the antipruritic effect of cannabidiol in compound 48/80-induced itching behavior in mice and whether spinal cannabinoid receptors are involved in this action.

MATERIAL AND METHODS

The investigations were approved by the institutional ethics committee of Trakya University. This experiment was carried out in young male Balb/c mice (obtained from Center of the Laboratory Animals, Trakya University), weighing 20-30 g (n=8 for each group). Animals were maintained under a 12-12 h light/dark cycle at a constant temperature of 21±2 °C with food and water ad libitum. Mice were housed in a group of 8 per cage and the experiments were conducted in a quiet room between 10:00 and 17:00. Animals were allowed to acclimate to laboratory conditions for one week before the experiments were performed; each mouse was tested only once. All procedures involving mice were carried out in strict accordance with "Guide for the Care and Use of Laboratory Animals" published by National Academy of Sciences (21).

Itching behavior was produced by intradermal injection of compound 48/80 (100 µg/50 µl); compound 48/80 is a well-known histamine releasing agent which produce scratches subsequent to mast cell degranulation. Scratching injected site by the hind paws was accepted as the itching behavior; mice scratched several times after compound 48/80 injection, and this reaction is counted as one bout of scratching. Scratches were video recorded in a quiet room, and then counted for 30 min. Testing was accomplished according to previously described procedures (22-24).

Locomotor performances were assessed using a rotarod apparatus (Commat, Ankara, Turkey). The animals were acclimatized to the apparatus before the assessments. Then, mice were placed on the drum rotating at 16 rpm and the performance time until the mice fell from the drum. 180 seconds cut-off frequency was adjusted before the experiments.

Groups of eight mice each received increasing doses of cannabidiol (1, 3, 10 mg/kg, ip). Cannabidiol was administered 30 min before compound 48/80 injections. Then, the cannabinoid CB1 receptor antagonist AM-251 (1g/mouse) and the cannabinoid CB2 receptor antagonist AM-630 (4g/mouse) were given intrathecally 10 min prior to cannabidiol administration in order to determine whether spinal cannabinoid receptors are involved in the effect of cannabidiol on itching behavior.

Cannabidiol was purchased from Tocris (UK), while compound 48/80, AM-251 and AM-630 from Sigma-Aldrich (St Louis, MO, USA. Compound 48/80 was dissolved in physiological saline, whereas cannabidiol, AM-251, and AM-630 were given in 20% dimethyl sulfoxide (DMSO), 1% Tween 80, 1% ethanol, and 78% saline. Cannabidiol was administered intraperitoneally in a volume of 0.05 ml/10 g body weight of mice, AM-251 and AM-630 were injected intrathecally (5l/mouse), and compound 48/80 was given intradermally (100 µg/50 µl). Previous studies guided doses and treatment (23-25).

Differences in the number of scratches in durations on the rotating rod were evaluated using analysis of variance and were followed by Bonferroni's multiple comparison tests. All data are expressed as mean SEM; $p<0.05$ was considered to be significant for all experiments.

RESULTS

Treatment with cannabidiol (1, 3, 10 mg/kg, ip) had no effect on compound 48/80-induced itching behavior at any dose given (Figure 1). 3mg/kg dose of cannabidiol seemed to reduce the number of scratches, but this reduction was not statistically significant (p=0.4499).

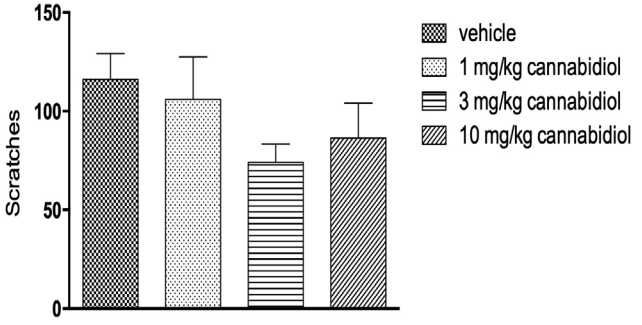

Figure 1: The effect of cannabidiol (1, 3, 10 mg/kg, ip) on the number of scratches.

The effect of cannabidiol (1, 3, 10 mg/kg, ip) on locomotion was evaluated in the rotarod test, where no significant change on motor function was observed (Figure 2).

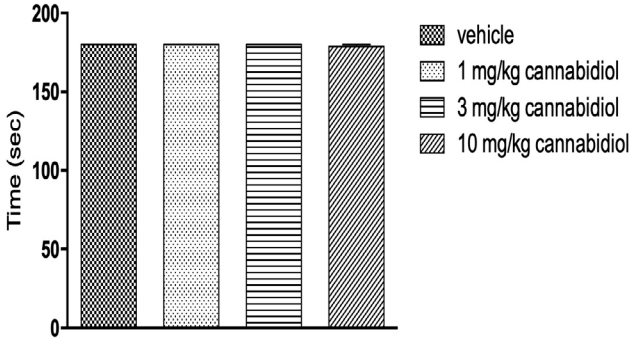

Figure 2: The effect of cannabidiol (1, 3, 10 mg/kg, ip) on locomotion.

AM-251, a CB1 antagonist; AM-630, a CB2 antagonist, were going to be administered intrathecally to observe the contribution of spinal cannabinoid receptors to the antipruritic action of cannabidiol, but they were not given when we observed cannabidiol did not exert any effect on itching behavior.

DISCUSSION

The non-psychoactive phytocannabinoid cannabidiol is a compound that does not produce typical subjective effects of marijuana. Different from the classical cannabinoids, including those found in the Cannabis plant, cannabidiol exhibits very low affinity with CB1 and CB2 (19). On the other hand, possible therapeutic uses of cannabidiol include analgesia, epilepsy, anxiety, schizophrenia, depression, and many more. Other than activity on cannabinoid receptors, there are multiple potential mechanisms underlying this wide spectrum of potential beneficial effects of cannabidiol. Firstly, recent findings also indicate that cannabidiol is a negative allosteric modulator of CB1 (26). Moreover, cannabidiol has been shown to be a transient receptor potential cation channel subfamily V member 1 (TRPV1) agonist which desensitize TRPV1 even at lower concentrations (27). Additionally, its pharmacological effects have been assigned to peroxisome proliferator-activated receptor (PPAR) agonism, intracellular calcium release and serotonin 1A receptor (5-HT1A) agonism (28, 29). Cannabidiol also appears to act via fatty acid amide hydrolase (FAAH) inhibition and augment endocannabinoid levels (28).

Considering similarities between pain and itching sensations and the potent analgesic effect of cannabidiol in different types of pain states, one would expect cannabidiol to show antipruritic action in mice (1-5). Its ineffectiveness in compound 48/80-induced itching behavior may be attributed to above-mentioned mechanisms unrelated to classical cannabinoid actions (26, 27, 29). Moreover, variations in physiological state, age, strain, and sex of the mouse, dose range and volume, route of administration and method of restraint are among the factors influencing this kind of behavioral research. Differences in assessment methods and existing animal models also seem to be important; for example, applying the pruritogens intradermally into the rostral part of the neck has been indicated not to discriminate pain and itching sensations but may give false positive results with analgesic drugs (23). Furthermore, the characteristics of the environment and the history of the subjects, such as exposure to stress, are suggested to interfere with the activity of cannabinoids in behavioral studies (30). Since cannabinoids have been proposed to excite circadian clock neurons and the activity of the endocannabi-

noid system is profoundly modulated by circadian rhythmicity, the ineffectiveness of cannabidiol in reducing scratches may have also resulted from the timing of drug administration (32, 33).

As we mentioned before, spinal cord appears promising for developing novel antipruritic drugs (6-8). In addition to the well-known involvement of spinal opioid receptor, gastrin-releasing peptide receptor and N-methyl-D-aspartate glutamate receptor in pruritus, serotonin, histamine, substance P and bradykinin receptors are among potential itching treatment targets (6, 7). It has also been demonstrated that blockade of spinal cannabinoid CB1 receptors partially reverse the antipruritic effect of synthetic cannabinoid WIN 55,212-2 (17). Here, we weren't able to investigate the contribution of spinal cannabinoid receptors since systemic cannabidiol had no effect on itching behavior.

Cannabidiol is used in some countries against pruritus; however, our findings suggest that systemic administration of cannabidiol does not diminish compound 48/80-induced itching behavior in mice. Recently, reduced pruritus and improved pain scores have been reported in patients with epidermolysis bullosa after combined treatment with tetrahydrocannabinol and cannabidiol (33). The effectiveness of cannabidiol in alleviating itching behavior should be investigated with further studies by using different itching models and experimental protocols.

Ethics Committee Approval: This study was approved by the Scientific Researches Committee of Trakya University School of Medicine.
Informed Consent: N/A
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: RDT, AU. Design: HD, EB, EÖÖ, RDT, AU. Supervision: RDT, AU. Resources: RDT, AU. Materials: HD, EB, EÖÖ, RDT, AU. Data collection and/or processing: HD, EB, EÖÖ, RDT, AU. Analysis and/or Interpretation: HD, EB, EÖÖ, RDT, AU. Literature Search: HD, EB, EÖÖ, RDT, AU. Writing Manuscript: RDT, AU. Critical Review: HD, EB, EÖÖ, RDT, AU.
Financial disclosure: This work was supported by a grant from Trakya University Research Council (TÜ-BAP-2017/44).

Acknowledgements: We thank K. Duvan Aydemir for her technical support for performing behavioral tests.

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Turkish Med. Stud. J. 2018; 5: 58-63 DOI: 10.4274/tmsj.galenos.2019.06.01.03
Received: 18.01.2019 - Accepted: 07.02.2019

EVALUATION OF MALNUTRITION STATUSES IN SYSTOLIC HEART FAILURE PATIENTS

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ABSTRACT

Aims: The aim of this study is to determine the malnutrition statuses of patients with systolic heart failure in Trakya University Hospital and Edirne Sultan 1st Murat State Hospital.

Methods: Mini Nutritional Assessment was used to determine the patients' malnutrition statuses. In addition, patients' transthoracic echocardiography results, biochemistry tests, hemograms, medications and habits were evaluated with patients' nutritional scores. One-way ANOVA and Kruskal-Wallis tests were used for comparing the results and Pearson$\chi 2$ test and Fischer's exact test were used to obtain categorical data.

Results: The total number of subjects in the study was 66 (61 patients from Trakya University Hospital; 5 patients from Edirne Sultan 1st Murat State Hospital). The mean age of all subjects was 65.3 ± 11.1. Out of all patients, 17 of them were female, while 49 of them were male. The mean Mini Nutrition Assessment score was 21.9 ± 4.7. In total, 34 patients had an adequate nutritional status, 25 patients were at risk of malnutrition and 7 patients were malnourished.

Conclusion: Although malnourished patients were detected, well – nourished patients were higher. Therefore, it could be said that patients with heart failure are conscious of their health and diet. It is crucial for the health care providers to maintain giving advices on healthy living and nutrition to keep this elderly population well–nourished.

Keywords: Nutrition assessment, systolic heart failure, malnutrition

INTRODUCTION

Heart failure (HF) is a pathophysiological condition defined as the failure of the heart to supply the requirements of the metabolizing tissues under normal cardiac pressures due to an abnormality in cardiac function (1, 2). HF is a major health problem which is a leading and increasing cause of mortality and morbidity around the world (3). Broadly, the prevalence of HF in the western world is at 1–2% and the incidence accounts for 5–10 per 1000 people per year (4). Symptoms of HF include fatigue, dyspnea, limited exercise tolerance and accumulation of concomitant fluid. With the aging population, higher portion of individuals such as people with hypertension, diabetes mellitus and obesity are exposed to the possibility of developing HF (2).

Clinically, HF patients can be divided into two categories; patients with HF with preserved (≥50%) ejection fraction (HFpEF) and patients with HF with reduced left ventricular ejection fraction (HFrEF) (≤40%). Leastwise, half of the HF patients have a reduced ejection fraction (5).

Heart failure with reduced left ventricular ejection fraction is caused by an initial injury or disease state, which can be cardiovascular-originated or originally include other organ systems followed by the evolution of secondary cardiovascular abnormalities that lead to reduced ventricular contraction. With the most important risk factor for HFrEF is being hypertension, other cardiovascular etiologies of HFrEF are myocardial infarction, cardiomyopathies, myocarditis, cardiac infection and valvular diseases. Extra-cardiac causes of HFrEF are systemic diseases, endocrine etiologies, high in take of illicit drugs and alcohol, side effects of chemotherapy treatment. When it comes to stable patients with pre-existing HFrEF, various factors can accelerate

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clinical decompensation, worsen the symptoms thus create the need for hospitalization of patients. These factors consist of ischemia, arrhythmia, infection, failing to comply with medications and dietary restrictions (2).

According to World Health Organization (WHO) malnutrition is defined as excesses, imbalances, inadequacies in a person's intake of energy and/or nourishments (6). WHO also states that the term malnutrition covers two groups of conditions; one being undernutrition and the other one being overweight, obesity and noncommunicable diseases related to diet. Undernutrition includes wasting, stunting, underweight and micronutrient insufficiencies or deficiencies. Examples for diet-related noncommunicable diseases can be diabetes, stroke, cancer and heart disease (6).

Intestinal edema, anorexia, malabsorption, rise of basal metabolism ratio and the increase in energy and nutrition needs of the heart lead to malnutrition among HF patients (7). Moreover, pharmacological therapy can also lead to insufficient energy and nutrition intake by causing loss of appetite that (8). In conclusion, malnutrition induces intensified edema, inflammation, neurohormonal activity and is highly associated with adverse prognosis among HF patients (7).

Malnutrition is often seen among patients with HF and it is associated with the lower quality of life and increased risk of mortality (9, 10). Despite increasing evidence concerning the crucial role of micronutrient deficiencies in chronic HF, the HF guidelines have not yet declared definitive nutritional strategies and the number of clinical research inspecting the effects of micronutrient supplementations on the prevention of malnutrition are still confined. Therefore, to be able to assess generally recognized nutritional strategies to improve the functional capacity and quality of life more researches are needed to be carried out regarding malnutrition of HF patients (9).

The aim of this study is to determine the malnutrition statuses of systolic heart failure patients evaluated between 15 January - 10 February 2019 in Trakya University Medical Research and Practice Hospital and Edirne Sultan 1st Murat State Hospital by using the results of Mini Nutritional Assessment (MNA®), patients' transthoracic echocardiography, biochemistry tests, hemograms, medications and habits (11).

MATERIAL AND METHODS

This study was approved by the Scientific Research Ethics Committee of Trakya University Medical Faculty. Informed consent was obtained from all of the patients. In this cross-sectional and descriptive study, the study population was composed of patients over 18 years old who were diagnosed with systolic heart failure with EF ≤40%, and applied to the cardiology department of Trakya University Hospital, and Edirne Sultan 1st Murat State Hospital between 15th of January – 10th of February, 2019. Seventy patients were asked to fill the MNA® survey to evaluate their anthropometric characteristics, general status, dietary habits, and subjective assessments. After data collection, 66 patients (61 patients from Trakya University Hospital, 5 patients from Edirne Sultan 1st Murat State Hospital) were included in the study. The rest were excluded because of inconsistent and incomplete data. MNA® consists of 18 questions, and four parts to determine patients' nutritional status. If malnutrition indicator score is less than 17 points, the patient is malnourished. 17 to 23.5 points indicate a risk of malnutrition, and 24 to 30 points indicate that the patient is in normal nutritional status (11).

Mini Nutritional Assessment

A. Has food intake declined over the past 3 months due to loss of appetite, digestive problems, chewing or swallowing difficulties?
(0: Severe decrease in food intake. / 1: Moderate decrease in food intake. / 2: No decrease in food intake.)
B. Weight loss during the last 3 months
(0: Weight loss greater than 3kg (6.6lbs). / 1: Does not know. / 2: Weight loss between 1 and 3kg (2.2 and 6.6 lbs). / 3 : No weight loss.)
C. Mobility
(0: Bed or chair bound. / 1: Able to get out of bed / chair but does not go out. / 2: Goes out.)
D. Has suffered psychological stress or acute disease in the past 3 months?
(0: Yes. / 2: No.)
E. Neuropsychological problems
(0: Severe dementia or depression. / 1: Mild dementia. / 2: No psychological problems.)
F. Body Mass Index (BMI) = weight in kg / (height in m)2
(0: BMI less than 19. / 1: BMI 19 to less than 21. / 2: BMI 21 to less than 23. / 3: BMI 23 or greater.)
G. Lives independently (not in nursing home or hospital)
(1: Yes. / 0: No.)

H. Takes more than 3 prescription drugs per day
(0: Yes. / 1: No.)
I. Pressure sores or skin ulcers
(0: Yes. / 1: No.)
J. How many full meals does the patient eat daily?
(0: 1 meal. / 1: 2 meals. / 2: 3 meals.)
K. Selected consumption markers for protein intake
At least one serving of dairy products (milk, cheese, yoghurt) per day (Yes / No)
Two or more serving of legumes or eggs per week (Yes / No)
Meat, fish or poultry every day (Yes / No)
(0: If 0 or 1 yes. / 0.5: If 2 yes. / 1: If 3 yes.)
L. Consumes two or more servings of fruit or vegetables per day?
(0: No. / 1: Yes.)
M. How much fluid (water, juice, coffee, tea, milk...) is consumed per day?
(0: Less than 3 cups. / 0.5: 3 to 5 cups. / 1: More than 5 cups.)
N. Mode of feeding
(0: Unable to eat without assistance. / 1: Self-fed with some difficulty. / 2: Self-fed without any problem.)
O. Self view of nutritional status
(0: Views self as being malnourished. / 1: Is uncertain of nutritional state. / 2: Views self as having no nutritional problem.)
P. In comparison with other people of the same age, how does the patient consider his / her health status?
(0: Not as good. / 0.5: Does not know. / 1: As good. / 2.0: Better.)
Q. Mid-arm circumference (MAC) in cm
(0: MAC less than 21. / 0.5: MAC 21 to 22. / 1: MAC greater than 22.)
R. Calf circumference (CC) in cm
(0: CC less than 31. / 1: CC 31 or greater.)

In addition to the MNA®, patients' medications were recorded according to the following classes: acetylsalicylic acid, angiotensin – converting enzyme inhibitors angiotensin 2 receptor blocker, β-blockers, aldosterone antagonists, statins, diuretics, antiarrhythmics, anticoagulants, sacubitril/valsartan. Findings of echocardiography that included their left ventricular enddiastolic diameter (LVEDD) and ejection fraction (EF) values were collected from the patients' files of Trakya University Medical Faculty and Edirne Sultan 1st Murat State Hospital. Patients' habit of smoking, sex, age, weight (kg), and height (cm) were recorded by their verbal statement. Pre-prandial blood glucose, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride (TG), hemoglobin, hematocrit (HCT), albumin, white blood cell (WBC), neutrophile, lymphocyte, platelet (PLT), c-reactive protein (CRP) levels and history of patients' chronic diseases – if any [hypertension (HT), hyperlipidemia (HL), diabetes mellitus (DM)] were recorded from the archives of Trakya University Hospital and Edirne Sultan 1st Murat State Hospital through patients' medical record numbers to determine patients' nutritional status.

After data collection, statistical analysis was carried out with IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. A p-value of <0.05 was set for statistically significance. As descriptive statistics, mean ± standard deviation or median with 25th-75th percentiles were used for continuous variables. Categorical variables were expressed as numbers and percentages. Compatibility of continuous variables to normal dissemination was evaluated with the Sharipo Wilk test. For comparing the groups, One-way ANOVA and Kruskal-Wallis tests were used. Pearson $\chi 2$ test and Fischer's exact test were used to achieve categorical data.

RESULTS

The total number of subjects in the study was 66. The mean age of all subjects was 65.3 ± 11.1. Out of all patients, 17 of them were female, while 49 of them were male. The mean age of female subjects was 67.9 ± 11.2, whereas the mean age of male subjects was 63.9 ± 10.9. Taking into consideration the MNA results, the mean score was 21.9 ± 4.7. Our MNA results also portray that 7 female and 27 male subjects had an adequate nutritional status, 7 female and 18 male subjects were at risk of malnutrition, 3 female and 4 male subjects were malnourished. The mean MNA score for females was 21.5 ± 5.04, while the mean score for males was 23.3 ± 3.9. Evaluation of MNA questions and subjects' answers according to groups are presented at Table 1. The difference of subjects' answers to questions A, B, D, E, O among groups were found to be statistically significant ($p<0.001$). Biochemical, echocardiography parameters and medications of patients can be observed at Table 2. Statistically significant difference was found at platelet counts between groups ($p=0.045$).

Table 1: Evaluation of MNA questions according to groups.

Questions	Adequate nutritional status (n=34)		Risk of malnutrition (n=25)		Malnourished (n=7)		P value
	The option chosen the most	n (%)	The option chosen the most	n (%)	The option chosen the most	n (%)	
A	no decrease in food intake	33(97.1)	no decrease in food intake	17(68)	severe decrease in food intake	5(71)	0.0001
B	no weight loss	26(76.5)	no weight loss	13(52)	weight loss greater than 3kg	6(85.7)	0.0001
C	goes out	31(91.2)	goes out	19(76)	goes out	3(42.9)	0.004
D	no	3(88.2)	no	13(52)	yes	7(100)	0.0001
E	no psychological problems	31(91.2)	no psychological problems	16(64)	mild/severe dementia or depression	6(85.8)	0.0001
G	yes	34(100)	yes	22(88)	yes	7(100)	0.124
H	yes	29(85.3)	yes	23(92)	yes	7(100)	0.614
I	no	34(100)	no	23(92)	no	5(71.4)	0.012
J	3 meals	27(79.4)	3 meals	22(88)	3 meals	4(57.1)	0.106
K	if 2 yes	26(76.5)	if 2 yes	15(60)	if 0 or 1 yes	4(57.1)	0.007
L	no	27(79.4)	no	15(60)	no	5(71.4)	0.302
M	more than 5 cups	26(79.5)	more than 5 cups	16(64)	3 to 5 cups	3(42.9)	0.317
N	self-fed without any problem	33(97.1)	self-fed without any problem	24(96)	self-fed without any problem	5(71.4)	0.058
O	views self as having no nutritional problem	29(85.3)	views self as having no nutritional problem	10(40)	views self as being malnourished	5(71.4)	0.0001
P	As good	12(35.3)	Not as good	12(48)	Not as good	6(85.7)	0.024
Q	Mid-arm circumference greater than 22 cm	31(91.2)	Mid-arm circumference greater than 22 cm	23(92)	Mid-arm circumference greater than 22 cm	5(71.4)	0.193
R	Calf circumference 31 cm or greater	34(100)	Calf circumference 31 cm or greater	24(96)	Calf circumference 31 cm or greater	5(71.4)	0.012

Table 2: Biochemical, echocardiography parameters and medications of patients.

	Adequate nutritional status MNA >24 (n=34)	Risk of malnutrition MNA 17-23.5 (n=25)	Malnourished MNA<17 (n=7)	P value
Age (years)	64.5 (58-67.2)	66 (58-74)	68 (65-69)	0.145
Male n, (%)	27 (79.4)	18 (72)	4 (57.1)	0.447
BMI, kg/m^2	29.1 ± 4.2	27.5 ± 4	25.4 ± 4.1	0.081
Hypertension n, (%)	24 (70.6)	17 (68)	6 (85.7)	0.793
Hyperlipemia n, (%)	11 (32.4)	8 (32)	1 (14.3)	0.746
Diabetes n, (%)	10 (29.4)	12 (48)	3 (42.9)	0.333
Smoking n, (%)	6 (17.6)	8 (32)	2 (28.6)	0.428
Ejection Fraction (%)	31.5 (29-35)	32 (30-35.5)	30 (23-34)	0.393
LVEDD (mm)*[d]	60.5 (56-67.7)	61.5 (56-67.5)	53 (50-64)	0.416
Glucose (mg/dl)[b]	112 (98-130.5)	122.5 (106-206.7)	152 (103-209)	0.154
Urea (mg/dl)[b]	40 (33.5-48.5)	42.5 (33-54.2)	101 (42-116)	0.098
Creatinine (mg/dl)[a]	1 (0.8-1.1)	1 (0.8-1.2)	1.3 (0.8-3.2)	0.254
AST (U/lt)[b]	21.5 (16-25)	24 (18-28)	23 (18-60)	0.239
ALT (U/lt)[b]	15 (10-18.5)	15 (12-23)	23 (13-63)	0.181
LDL (mg/dl)[c]	107.4 ± 26.8	112 ± 29.4	109.8 ± 38.2	0.843
HDL (mg/dl)[c]	35 (32.7-40.2)	35.5 (28.2-42.2)	31 (17-46.8)	0.793
TG (mg/dl)*[c]	148.5 (98.7-185)	116.5 (65.7-149)	96.5 (63.2-165)	0.072
Hemoglobin (mg/dl)[a]	12.9 ± 1.8	12.5 ± 1.9	12.4 ± 1.7	0.659
HCT (%)*[a]	38.5 ± 4.4	37.9 ± 5.7	38.2 ± 5.6	0.901
PLT 10^3/mm^3 *[a]	210 (181-259.7)	222 (171.2-297.7)	155 (126-178)	0.045
WBC 10^3/ml *[a]	8.6 (7-10)	8.2 (7-9.8)	6.9 (5.4-8.4)	0.318
Neutrophil (%)[a]	62 ± 12.3	65.6 ± 7.8	70.6 ± 13.5	0.133
Lymphocytes/µl[a]	2 ± 0.6	2 ± 1.1	1.4 ± 0.6	0.179
CRP (mg/l)[c]	0.6 (0.4-1.6)	0.8 (0.3-1.5)	7.5 (1-7.9)	0.057
Albumin (g/dl)[e]	3.7 (3.3-4.2)	3.5 (3.1-4.1)	3 (2.7-3.4)	0.070
Medications				
ASA n, (%)*[a]	19 (55.9)	13 (54.2)	2 (28.6)	0.458
ACEI n, (%)*[a]	15 (44.1)	11 (45.8)	3 (42.9)	1.000
ARB n, (%)*[a]	1 (2.9)	5 (20.8)	0 (0)	0.085
Beta-blocker n, (%)[a]	33 (97.1)	22 (91.7)	7 (100)	0.692
Ald-antg n, (%)*[a]	18 (52.9)	14 (58.3)	4 (57.1)	0.935
Statin n, (%)[a]	16 (47.1)	13 (54.2)	1 (14.3)	0.202
Diuretic n, (%)[a]	21 (61.8)	18 (75)	5 (71.4)	0.643
Antiarrhythmics n, (%)[a]	5 (14.7)	3 (12.5)	0 (0)	0.870
Anticoagulant n, (%)[a]	15 (44.1)	7 (29.2)	4 (57.1)	0.312
Sacubitril/Valsartan n, (%)[a]	3 (8.8)	3 (12.5)	2 (28.6)	0.339

**LVEDD: left ventricular end-diastolic diameter, TG: Triglycerides, HTC: Hematocrit, PLT: Platelets, WBC: White blood cells, CRP: C-reactive protein, ASA: Acetylsalicylic acid, ACEI: Angiotensin-converting-enzyme inhibitor, ARB: Angiotensin II receptor blocker, Ald-antg: Aldosterone antagonist,*

a: one missing data, b: two missing data, c: four missing data, d: five missing data, e: eight missing data

As descriptive statistics, quantitative data were expressed as mean ± standard deviation or median (25th-75th percentiles) and qualitative data were expressed as numbers (percentages).

DISCUSSION

Heart failure is a worldwide health problem among the elderly population and mainly appears with reduced ejection fraction that causes reduced living standards and morbidity (2). Due to this condition, heart is inoperative to supply the requirements of the tissues to maintain normal cardiac function (1).

In line with previous studies, risk factors such as obesity, hypertension, and diabetes mellitus are seen frequently among HF patients (1). In our study the result of body – mass index was lower in undernourished patients, compared to the other groups, but there was not a significant difference since the malnourished group was already in normal weight range. According to the local studies, body mass index may cause misdirection because it does not reflect weight loss completely (12). In our findings, when body mass index is compared to weight loss in the last 3 months, in the malnourished group, there is a significant weight loss greater than 3 kg (p=0.0001), even though they are in normal weight range. Additionally, the comorbid diesases of our patients' hypertension and diabetes were consistent with the study of Bonilla-Palomas et al. (13) which was conducted on 208 patients.

In our study, although there was no significant difference in smoking rate among all groups (p=0.428), quitting smoking is important to reduce the risk of smoking-related cardiovascular diseases (14).

According to the literature, the majority of the HF patients are classified as at risk of malnutrition (12, 13). Our study revealed that 51.5% of the patients were in adequate nutritional status. As for the reason, it is thought that patients with heart failure are cautious about their health. Nevertheless in our study the percentages of the malnourished patients are in accordance with the literature (12, 13).

Our results demonstrated that the percentage of medication usage was higher in well–nourished group for ASA and antiarrhythmic drugs. Beta–blocker was prescribed for all malnourished patient group (n=7), meanwhile ARB and antiarrhythmic drugs were not in their drug therapy. A similar conclusion was reached by Bonilla–Palomas et al. (13) in beta–blocker usage but they indicate that there was no prognostic benefit specified with this drug.

In this study, the main limitation was the patient profile that we conducted the questionnaire. It could be argued that a higher percentage of the malnutrition and the risk of malnutrition levels could be obtained if this survey would be performed on severely ill patients, but these severe patients have refused were incapable of attending to our survey.

High in reliability and validity, MNA® was originally developed for the elderly (mean age 73 ± 10.1) patients , and the mean age of our subjects was 65.3 ± 11.1 which was another limitation for our study (13). In addition, in our study, no significant difference was found in the measure of mid-arm circumference (p=0.193), in contrast with the literature that evaluated Japanese elderly (15). We attribute this to the fact that these measurements can vary in populations, and should be modified for the specific population that was conducted.

As a conclusion, although malnourished patients were detected, the number of well–nourished patients were higher. Therefore, it could be said that patients with heart failure are conscious of their health and diet. It is crucial for the health care providers to give advices on healthy living and nutrition to keep this elderly population well–nourished.

Ethics Committee Approval: This study was approved by the Scientific Researches Committee of Trakya University School of Medicine.
Informed Consent: Written informed consent was obtained from the participants of this study.
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Consept: İİÖ, BK, BS, FMU. Design: İİÖ, BK, BS, FMU. Supervision: İİÖ, BK, BS, FMU Resources: İİÖ, BK, BS, FMU. Materials: İİÖ, BK, BS, FMU. Data collection and/or processing: İİÖ, BK, BS, FMU. Analysis and/or interpretation: İİÖ, BK, BS, FMU. Literature search: İİÖ, BK, BS, FMU. Writing manuscript: İİÖ, BK, BS, FMU. Critical rewievs: İİÖ, BK, BS, FMU.
Financial disclosure: The authors declared that this study received no financial support.
Editor-in-chief's Note: Three authors of this article, Irmak İrem Özyiğit, Beliz Koçyiğit, Begüm Söyleyici are members of the editorial board of Turkish Medical Student Journal. However, they did not take place in any stage on the editorial decision of the manuscript. The editors who evaluated this manuscript are from other institutions.

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TMSJ
Turkish Med. Stud. J. 2018; 5: 64-8 DOI: 10.4274/tmsj.galenos.2019.06.01.04

Received: 05.01.2019 - Accepted: 05.02.2019

THE INFLUENCE OF INTERCURRENT DISEASES ON THE COURSE OF HIV IN ASSOCIATION WITH ACTIVE TUBERCULOSIS

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ABSTRACT

Aims: We aimed to establish the features of the course of HIV in association with active tuberculosis in the presence of other secondary diseases and opportunistic infections.

Methods: In this research, 155 HIV patients with active newly diagnosed tuberculosis and 76 diagnosed only with tuberculosis have been comprehensively immunologically examined. The HIV/Tuberculosis group was divided into 3 subgroups depending on the time of adherence of Tuberculosis to HIV infection. STATISTICA 6.0 was used for data analysis, and when the number of observations is less than 20, the Wilcoxon nonparametric method for independent populations or the dispersive analysis of Kruskal-Wallis was used. In addition, Mann-Whitney U test was used to compare two independent groups.

Results: The number of CD4 + T-lymphocytes was lower compared with patients without secondary ailments. However, the level of serum concentration and spontaneous production of IL-4 and IFN-γ, as well as VL HIV was higher in the presence of other secondary diseases.

Conclusion: In the presence of secondary diseases and opportunistic infections, the number of CD4 + T-lymphocytes was lower, and the level of serum concentration and spontaneous production of IL-4, IFN-γ and viral load was higher compared to patients without these ailments. In the case of secondary diseases and opportunistic infections, the frequency of death of patients was significantly higher than in their absence.

Keywords: HIV, tuberculosis, viral load, mortality, cytokines

INTRODUCTION

The prevalence of Human immunodeficiency virus (HIV) infection has been increasing since the mid-1990s. An increase in HIV prevalence is usually followed by an increase in TB. In Kiev City, the number of TB patients registered for treatment increased from 629 in 1992 to 1274 in 2004. This increase is mainly explained by economic and social changes after independence, but the progressing HIV epidemic may also play a role in the increase in the number of TB patients (1).

Today, TB patients co-infected with HIV are very challenging cases to treat, and the patients suffer from side effects of the combined drugs (2). As it is reported in the literature that the incidence of HIV and TB coinfection has been increasing, the condition could be one of the major concerns in medicine in the future (3).

Tuberculosis in HIV-infected patients is malignant, has a tendency to generalization and progression due to immunodeficiency caused by HIV infection. Generally, TB treatment is the same for people both with HIV and people without HIV. TB medicines are used to prevent latent TB from advancing to TB disease and to treat TB disease. The choice of medicines and the duration of treatment depend on whether a person has latent TB or TB disease. People with HIV/TB coinfection should be treated for both diseases; however, when to start treatment and what medicines to take depends on a person's individual circumstances. Taking certain HIV and TB medicines at the same time can increase the risk of drug-drug interactions and side effects. People being

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treated for HIV/TB coinfection are carefully monitored by their health care providers (4).

In spite of the fact that TB is the most frequent and the earliest opportunistic infection in AIDS patients, many other secondary diseases usually join in these individuals, which certainly impose a reflection on the course of such an associated infection (5, 6).

In this study, we aimed to establish the features of the course of HIV in association with active tuberculosis in the presence of other secondary diseases.

MATERIAL AND METHODS

This study was approved by the ethical committee of Bukovinian State Medical University and carried out between the years 2016-2018 in the Center for the Prevention and Control of AIDS, Chernivtsi. The study design was retrospective and descriptive. All patients with active TB infection diagnosed in the last 5 years and followed up in our center were included in the study. The total number of patients were 231 people under supervision - 184 (79.7%) men and 47 (20.3%) women aged 23 to 60 years. The average age was 38.8 ± 1.2 years.

All patients were subjected to a comprehensive laboratory examination and divided into the following groups:

I. Not HIV-infected (immunocompetent) induviduals with active newly diagnosed TB - 76 patients (TB group).

II. HIV-infected with active newly diagnosed TB - 155 patients (HIV / TB group). Within the HIV / TB group, 3 subgroups were identified depending on the time of adherence of TB to HIV infection.

The first subgroup is HIV-infected, in which TB was detected at different stages of HIV infection (the primary disease) - 72 patients;

The second subgroup includes patients who had been diagnosed with TB but were superinfected by HIV in several years after, being TB the primary disease (according to the epidemiological history) -26 people.

The third subgroup is HIV-infected, in which the diseases were registered approximately at the same time and it was impossible to find out which infection was primary -57 people.

CD4 + T-lymphocytes, VL HIV, a number of cytokines, as well as mortality in the presence of other secondary diseases and opportunistic infections in HIV-infected people with active TB infection were analyzed. The encountered secondary diseases were oropharyngeal candidiasis, mycosis of the hands and feet, clinically manifest of reactivation of CMVI, cerebral toxoplasmosis, frequent recurrences of herpes zoster with the formation of a "herpetic mark", unmotivated weight loss (more than 10% within 6 months) – cachexia. Patients' immune status were investigated within 3-6 months after the diagnosis of the associated infection. The data were recorded from patients' files.

Statistical analysis of the obtained results was performed using the Windows software package, Word and Excel; STATISTICA 6.0 using the method of variation statistics with the criterion: when the number of observations is less than 20, the Wilcoxon nonparametric method for independent populations, or the dispersive analysis of Kruskal-Wallis was used. For the correlation analysis, the Spearman's rank method (7) was used. To compare two independent groups, the Mann-Whitney U test was used. Numbers, percentages, mean +- standard deviation (minimum - maximum) were used as descriptive statistics. A p value <0.05 was set for the statistically significance.

RESULTS

In the group of patients with TB monoinfection, there were no other secondary diseases and opportunistic infections, but in the HIV / TB group there were 58 people (37.4%) with secondary diseases.

In this study it was found that in all three subgroups of patients with HIV / TB, the percentage of secondary diseases did not differ significantly ($p>0.05$). However, significant differences were found between the groups of patients with other secondary diseases and without them ($p<0.05$). Thus, in the presence of secondary diseases and opportunistic infections, the number of CD4 + T-lymphocytes was significantly lower compared with patients without these ailments ($p <0.02$). But the level of serum concentration and spontaneous production of IL-4 and IFN-γ, as well as viral load was higher in the presence of other secondary diseases ($p <0.02$). It is important that there were no significant differences between the considered indicators in all three subgroups of patients ($p>0.05$) (Table 1).

Table 1: The average number of CD4 + T-lymphocytes, the level of IL-4, IFN-γ and viral load in patients with other secondary diseases and opportunistic infections.

Secondary diseases		Indicator		HIV / TB			
				1-st subgroup, n=72	2-nd subgroup, n=26	3-rd subgroup, n=57	total, n=155
Present	I	n		27	10	21	58
		$CD4^+$T-lymphocytes, cl./ mm3		$308,3\pm36,5^{II}$	$343,1\pm40,6^{II}$	$322,5\pm38,2^{II}$	$324,6\pm38,3^{II}$
		IL-4	serum., pg. / ml	$3,5\pm0,7^{II}$	$3,2\pm0,6$	$3,1\pm0,5^{II}$	$3,3\pm0,6^{II}$
			spontaneous., pg. / ml /10^6	$9,6\pm2,1^{II}$	$8,3\pm1,8$	$7,0\pm1,7^{II}$	$8,3\pm1,9^{II}$
		IFN -γ	serum., pg. / ml	$209,6\pm46,3^{II}$	$244,2\pm59,5^{II}$	$247,3\pm52,6^{II}$	$233,7\pm52,8^{II}$
			spontaneous., pg. / ml /10^6	$44,3\pm9,1^{II}$	$51,6\pm12,2$	$47,2\pm12,6^{II}$	$47,4\pm11,3^{II}$
		VL, kop / ml		598389 ± 93583^{II}	632208 ± 113529^{II}	620136 ± 108077^{II}	616911 ± 105063^{II}
Absent	II	n		45	16	36	97
		$CD4^+$T- lymphocytes, cl./ mm3		$443,6\pm41,0^{I}$	$469,4\pm45,7^{I}$	$458,3\pm49,9^{I}$	$457,1\pm48,9^{I}$
		IL-4	serum., pg. / ml	$1,8\pm0,4^{I}$	$1,6\pm0,5$	$1,6\pm0,5^{I}$	$1,7\pm0,5^{I}$
			spontaneous., pg. / ml /10^6	$3,2\pm1,7^{I}$	$3,0\pm1,4^{I}$	$2,4\pm0,9^{I}$	$2,9\pm1,3^{I}$
		IFN -γ	serum., pg. / ml	$99,4\pm28,5^{I}$	$93,6\pm34,7^{I}$	$117,5\pm28,6^{I}$	$103,5\pm30,6^{I}$
			spontaneous., pg. / ml /10^6	$15,7\pm7,9^{I}$	$27,9\pm11,2$	$13,4\pm5,8^{I}$	$19,0\pm8,3^{I}$
		VL, kop / ml		96358 ± 24671^{I}	89952 ± 20462^{I}	94460 ± 22132^{I}	93590 ± 22422^{I}

In the course of the correlation analysis of the obtained data in the HIV / TB group with the presence of other secondary diseases (parametric analysis of Pearson and non-parametric Spearman was used) established the presence of an inverse correlation of the average force between the CD4 + T-lymphocyte count and the serum IL-4 concentration (r = -0, 44, p <0.05) the same relationship between the number of CD4 + T-lymphocytes and the serum concentration of IFN-γ (r = -0.31, p <0.05) is a direct relationship of the average force between the levels of HIV load and serum and spontaneous the concentration of IL-4 (r = 0.38 ... 0.41, p <0.05) is the same correlation between the load of HIV and serum and spontaneous concentrations of IFN-γ (r = 0.33 ... 0.47, p <0.05-0.02). Between the levels of CD4 + T-lymphocytes and viral load, it was possible to establish the presence of an inverse relationship (r = -0.52, p <0.01).

We also estimated the level of HIV load, CD4 + T-lymphocyte IL-4, IFN-γ indices for individual secondary infections and conditions in HIV / TB patients. Taking into account that several diseases certainly affect the studied parameters, from all patients we selected only those people who were diagnosed with only one opportunistic infection (Table 2). Also, the presence or absence of secondary diseases and frequency of death in subgroups are shown in Table 3.

The presence of an inverse correlation of the average strength between the number of CD4 + T-lymphocytes and the serum concentration of IL-4 (r = -0.44, p <0.05) was established. The same relationship between the number of CD4 + T-lymphocytes and the serum concentration of IFN-γ (r = -0.31, p <0.05) a direct relationship of the average force between the levels of HIV loading and the serum and spontaneous concentration of IL-4 (r = 0.38 ... 0.41, p <0.05) is the same correlations between the load of HIV and serum and spontaneous concentrations of IFN-γ (r = 0.33 ... 0.47, p <0.02). The CD4 + T-lymphocyte level and viral load correlate back (r = -0.52, p <0.01).

The concentration of spontaneous production of IL-4 was characterized by a statistically significant increase in the case of registration of cerebral toxoplasmosis, continuous recurrent herpes zoster and cachexia (p <0.01).

The serum concentration of IFN-γ was the lowest in cerebral toxoplasmosis - (7.8 ± 1.6) pg / ml, and the spontaneous production of the indicated cytokine in patients with frequent relapses of herpes zoster - (2.4 ± 0.6) pg / ml / 106 compared with all other secondary diseases and opportunistic infections (p <0.001).

Table 2: The load of HIV, CD4 + T-lymphocytes, IL-4, IFN-γ in patients with other secondary diseases and opportunistic infections (M±m).

Indicator		Secondary diseases					
		Oropharyngeal candidiasis, n=4	Mycosis of the hands and feet, n=8	Clinically manifest reactivation of CMVI, n=3	Cerebral toxoplasmosis, n=3	Frequent recurrences of herpes zoster with the formation of a "herpetic mark", n=7	Unmotivated weight loss (more than 10% within 6 months) - cachexia, n=5
		1	2	3	4	5	6
CD4+T-lymphocytes, cl./ mm3		204,0±63,3[5]	221,0±77,2[5]	165,0±42,4	149,0±37,5	102,0±10,3[1,2]	140,0±117,3
IL-4	serum., pg / ml	4,1±0,7	3,7±0,9	2,2±0,6	2,3±0,4	3,2±0,9	2,6±1,0
	spontaneous., pg / ml /10[6]	3,3±0,7[4,5,6]	2,4±0,6[4,5,6]	3,4±0,8[4,5,6]	15,2±6,6[1,2,3]	14,4±5,2[1,2,3]	15,9±6,8[1,2,3]
IFN -γ	serum., pg / ml	469,3±202,5[4]	154,3±96,3[4]	184,4±106,3[4]	7,8±1,6[1,2,3,5,6]	414,7±193,4[4]	303,5±120,0[4]
	spontaneous., pg / ml /10[6]	21,2±9,1[5]	16,3±6,8[5]	11,4±2,6[5]	6,8±1,2[5]	2,4±0,6[1,2,3,4,6]	9,7±2,0[5]
VL, kop / ml		142614± 29015[3,4,5,6]	281899±50949[4,5,6]	386154±61276[1]	480369±71443[1,2]	616647±99531[1,2]	434350± 88432[1,2]

Table 3: Frequency of death of HIV/TB patients in subgroups depending on the presence or absence of other secondary diseases and opportunistic infections.

Secondary diseases		1-st subgroup, n=72			2-nd subgroup, n=26			3-rd subgroup, n=57			total, n=155		
		n	died		n	died		n	died		n	died	
			abs number	M%± m%		abs number	M%± m%		abs number	M%± m%		abs number	M%± m%
Presence	I	27	16	59,3± 9,5[II]	10	4	40,0± 15,5	21	7	33,3± 10,3	58	27	46,6± 6,6[II]
Absence	II	45	9	20,0± 6,0[I]	16	3	18,8± 9,8	36	4	11,1± 5,2	97	16	16,5± 3,8[I]

DISCUSSION

The association of HIV and TB in 37.4% of cases is accompanied by other secondary diseases and opportunistic infections.

In the presence of secondary diseases and opportunistic infections, the number of CD4 + T-lymphocytes was significantly lower, and the level of serum concentration and spontaneous production of IL-4, IFN-γ and viral load was higher compared to patients without these ailments (p <0, 02).

When comparing mortality in HIV / TB groups, depending on the presence or absence of other secondary diseases and opportunistic infections have established an important feature. So, in the case of secondary diseases, the death rate of patients was significantly higher than in their absence - (46.6 ± 6.6) versus (16.5 ± 3.8)% (p <0.001). This pattern was observed in almost all subgroups of patients, although a statistically significant difference was established only for those who were diagnosed with TB- (59.3 ± 9.5) versus (20.0 ± 6, 0)% (p <0.01). In the 2nd and 3rd subgroups statistically significant difference has not been detected (p<0.05), that may be due to the low number of patients in those

groups. There was no significant difference in the studied index within the subgroups of patients.

Furthermore, the increases in spontaneous production of IL-4, serum concentrations of IFN-γ, viral load and reduction of spontaneous production of IFN-γ are observed with such developed symptoms as cerebral toxoplasmosis, recurrent herpetic infection with the formation of a "herpetic mark", unmotivated weight loss (more than 10% within 6 months.) - cachexia. Also alterations were found in the localized form of mycosis and in the case of clinically manifest reactivation of CMVI. These findings were also in concordence with the literature (8,9). Despite this, the correlations in the study were not taken into account due to the small sample of patients.

As a result of the comparative analysis of CD4 + T-lymphocyte indices, cytokine level, HIV load, and also mortality in the presence or absence of other secondary diseases in HIV-infected people with active TB, it was found that in patients with HIV / TB infection combined with secondary diseases and opportunistic infections, the level of CD4 + T-lymphocytes is reduced and does not significantly differ in all subgroups, regardless of the time, superinfection with the office of TB or HIV ($p<0.05$). At the same time, statistically significant differences were revealed between groups of patients with other secondary diseases and without them. Thus, the level of serum concentration and spontaneous production of IL-4 and IFN-γ, as well as viral load was higher in the presence of other secondary diseases. In addition, in the case of secondary diseases and opportunistic infections, the frequency of death of patients was significantly higher than in their absence, that was also consistent with the findings of Braun et al. (10) who investigated the risk of TB infection in HIV infected women in Zahire.

The limitation factors of this study was the retrospective design and the low number of patients. However, to minimize the limitations all patients that meet our inclusion criterias have been included in our resarch. Multicenter studies with bigger sample groups are needed to be carried out to achieve concreter results.

In conclusion, based on the data obtained, in the case of secondary diseases and opportunistic infections, the content of cytokines in the blood was higher than without secondary diseases, as well as mortality in patients with secondary diseases and opportunistic infections was higher. It can be concluded that comorbidities worsen the course of HIV in association with active tuberculosis.

Ethics Committee Approval: This study was approved by the ethical committee of Bukovinian State Medical University.
Informed Consent: Written informed consent was obtained from the participants of this study.
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: TK, HP, OG. Design: TK, OG. Supervision: TK, HP. Resources: TK, OG. Materials: TK, HP. Data collection and/or processing: TK, OG. Analysis and/or Interpretation: TK, HP, OG. Literature Search: TK, HP. Writing Manuscript: TK, HP, OG. Critical Review: TK, HP.
Financial disclosure: The authors declared that this study received no financial support.

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TMSJ
Turkish Med. Stud. J. 2018; 5: 69-75 DOI:10.4274/tmsj.galenos.2019.06.01.05

Received: 10.10.2018 - Accepted: 12.11.2018

A CASE REPORT: THE ROLE OF PROSTATE-SPECIFIC MEMBRANE ANTIGEN LABELED THERANOSTIC AGENTS IN THE DIAGNOSIS AND TREATMENT OF PROSTATE CANCER

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ABSTRACT

Aims: Prostate cancer is one of the most prevalent cancers worldwide and sometimes it can be deadly which brings out a high quantity of the prostate specific membrane antigen. Gallium-68 prostate specific membrane antigen PET scan is used to detect primary tumors and metastases of prostate cancer. In addition, Lutetium-177 prostate-specific membrane antigen is used for treatment in some special cases. Our case report aims to show the roles of prostate specific membrane antigen labeled theranostic agents in the diagnosis, staging, and treatment of prostate cancer and evaluate the response to the treatment of metastatic castration-resistant prostate cancer.

Case Report: A 78-year-old male patient was admitted to Trakya University Hospital Nuclear Medicine Department with the diagnosis of prostate cancer for cancer staging and treatment. Gallium-68 prostate specific membrane antigen was firstly used to detect any metastases and then to evaluate response to the treatment. Lutetium-177 prostate specific membrane antigen was used for treatment.

Conclusion: After 3 cures of Lutetium-177 therapy, the patient underwent Gallium - 68 prostate specific membrane antigen PET which demonstrated regression of the metastatic tumor. There was a decrease in the uptake of Gallium-68 prostate specific membrane antigen in the primary tumor and lymph nodes metastases. Also, bone metastases have been cleared. Hence, Lutetium-177 seems to be a promising treatment modality to treat metastatic prostate cancer.

Keywords: Prostate cancer, theranostic, lutetium

INTRODUCTION

Cases of prostate cancer are frequently reported and metastatic prostate cancer has a high mortality rate. According to the American Cancer Society's statistics the 5-year survival of metastatic prostate cancer is 30% (1, 2).

Prostate cancer can metastasize to lymph nodes, bones, rarely to lung, skin and liver. It is very important to detect primary tumor before the metastasis. According to studies, the survival rate of prostate cancer with minimal metastases was less than 48 months and the survival rate of metastatic prostate cancer that is resistant to androgen suppression therapy was less than 24 months (3).

Prostate specific membrane antigen (PSMA) is a kind of cell surface receptor which is expressed in various tissues like salivary glands, kidneys, liver, prostate, small intestine and incomparably highly expressed in prostate cancer cells, particularly in its metastatic form. PSMA is non-secretory and membrane-bound. Binding of ligands to PSMA leads to internalization via clathrin-coated pits and these properties make PSMA an ideal target for drug delivery for both diagnostic and therapeutic purposes (4).

PSMA-labeled Gallium(Ga)-68 PET scan is used to detect primary tumors and its metastases with high accuracy for staging. According to studies, Gallium-68 PSMA PET scan can detect very small metastases which are not detected by other scanning techniques like computed tomography (CT) and magnetic resonance imaging (MRI) (5). In addition, it is very successful in showing recurrence in early periods. Afshar et al. (6) found that the positive predictive value of Ga -68 PET scan is 100% and sensitivity is 88.1% for detecting recurrence of prostate cancer. Gallium-68 PSMA PET scan can also help us to evaluate the response of cancer to treatments.

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Newly developed ligands can bind to the extracellular part of PSMA which can help us to scan living prostatic cells. PSMA scan is indicated in patients with a Gleason score of 7 or more, before radiotherapy or in patients that are considered biochemically recurrent cases. PSMA scan should be conducted 2 weeks after chemotherapy and hormone therapy or 6 weeks after radiotherapy (7).

In addition to diagnostic purpose, Lutetium (Lu)-177 PSMA can act as a therapeutic agent in metastatic castration-resistant prostate cancer (mCRPC). According to studies Lu-177 PSMA is very effective and safe in treatment. Emmett et al. (8) found that 28 months following Lu-177 PSMA therapy, the survival rate was 78.6%.

Therefore, PSMA can potentially act as a diagnostic and therapeutic agent. If PMSA is labeled with Ga-68, it is used for diagnosis, whereas if it is labeled with Lu-177, which has more energy than Ga-68, it is used for treatment. Depending on the type of peptide attached to PSMA receptors, PSMA can be a theranostic agent in staging and treating patients with prostate cancer at the same time.

CASE REPORT

A 78-year-old male patient presented to Trakya University Nuclear Medicine Department with the diagnosis of prostate cancer and was referred to scan distant metastasis in February 2017. The patient had prostate adenocarcinoma and the Gleason score was found as 4+4 in January 2017.

After the diagnosis, the patient was scanned for bone metastasis in a different clinic by Technetium 99m MDP SPECT and metastases at the spine, right sacroiliac joint, head of the right femur and right side of rib were detected. Following metastasis scan, both of the patient's testicles were removed at a different medical center to reduce testosterone levels. The patient was followed-up by imaging PET and blood PSA levels.

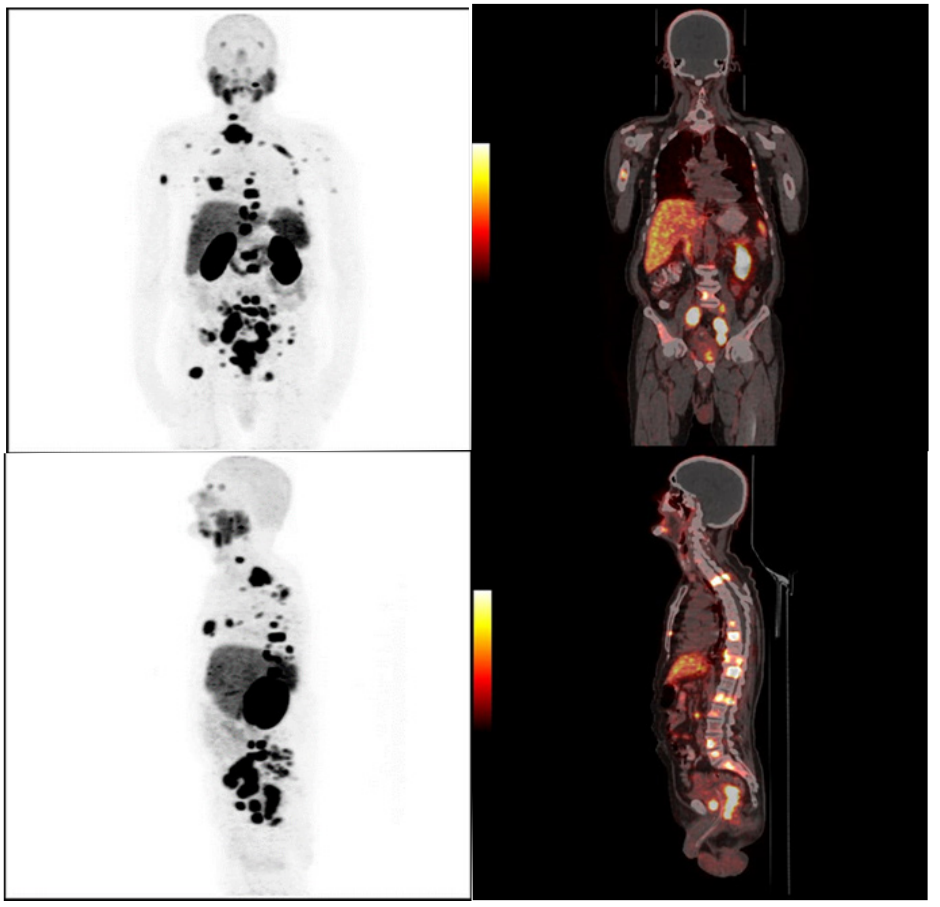

Figure 1: Ga-68 PSMA PET scan in February 2017.

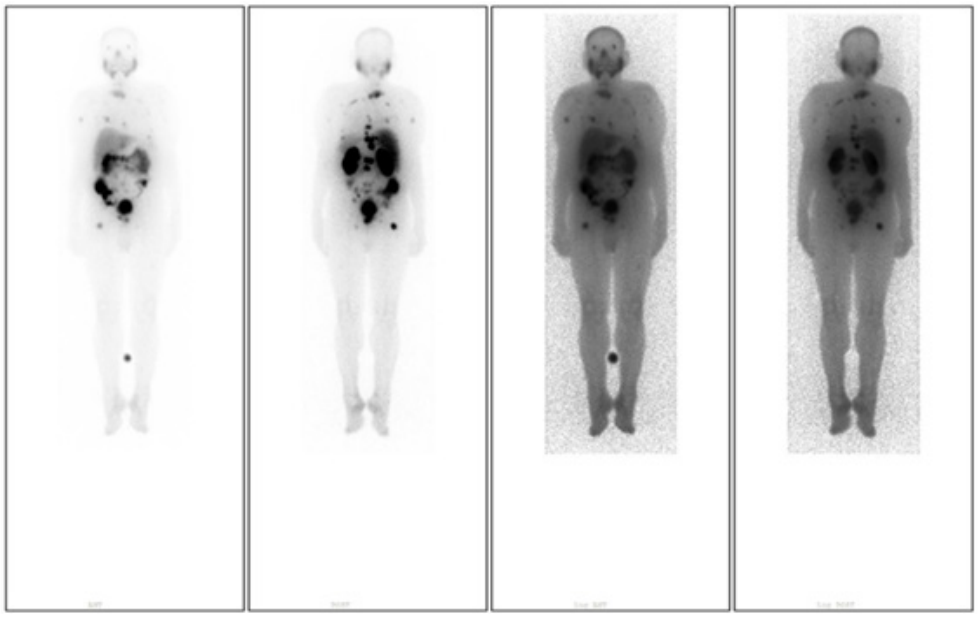

Figure 2: Day 1, whole body scan following Lu 177 therapy.

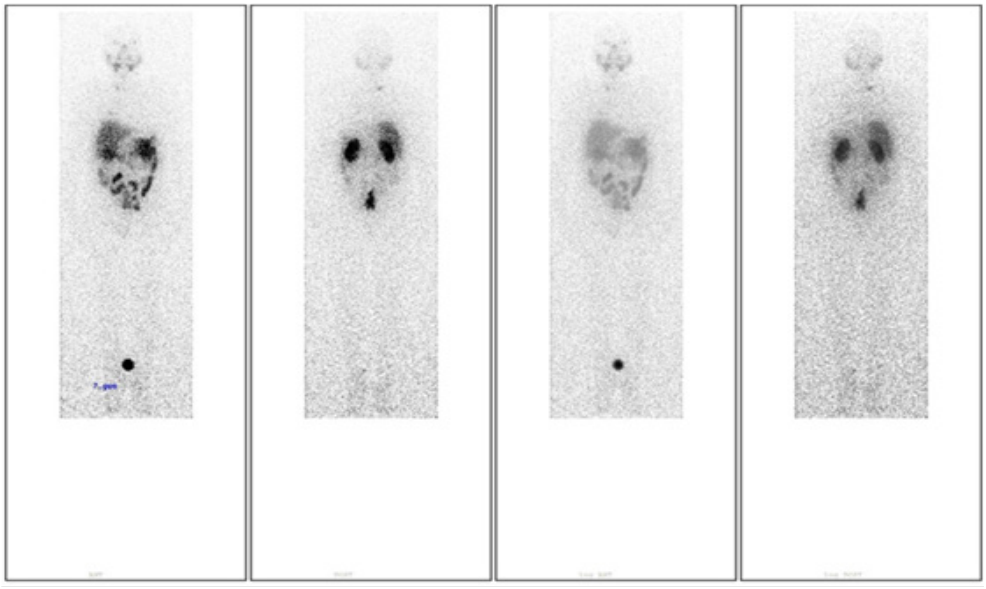

Figure 3: Whole body scan following 3 Lu-177 therapies.

In February 2017, the patient underwent Ga-68 PSMA PET in Trakya University Hospital. The primary prostate tumor metastasized to various lymph nodes, spines, hipbone, sacroiliac joint, right femur, right side of ribs, both scapulas and right humerus (Figure 1). The patient was diagnosed with mCRPC and clinicians decided to start Lu-177 PSMA therapy. The whole body scan on the first day of Lu-177 PSMA therapy and after the treatment regime are demonstrated in Figure 3 and Figure 4 respectively.

After 3 cycles of Lu-PSMA treatment, the patient was assessed for the therapeutic efficacy of Lu-PSMA treatment by Ga-68 PSMA PET (Figure 4). The uptake of Ga-68 PSMA reduced the size of the tumor. There were a lot of pelvic lymph node metastases and all of them were smaller than 1cm. No bone metastasis was detected.

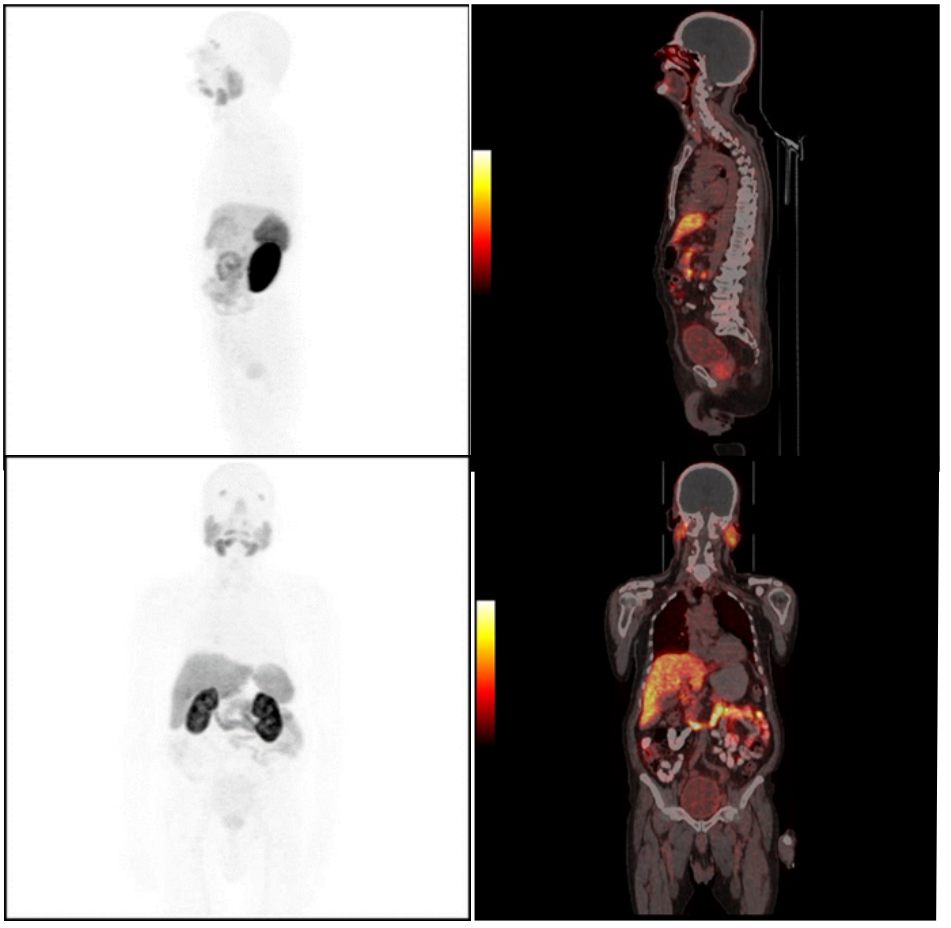

Figure 4: Ga-68 PSMA PET scan following 3 cycles of Lu-PSMA treatment to assess treatment efficacy.

Ga-68 PSMA PET scan was found very effective in detecting metastasis (9). In the present case study, it detected a 6mm large lymph node metastasis, which could not be detected by CT or MRI.

PSA was used as a marker of treatment response (10). Blood total PSA level was 83.59 ng/ml in March 2017 and the total PSA was 156 ng/ml in May 2017. After 3 cycles of Lu-PSMA therapies, patient's blood total PSA level was 0.7 ng/ml and free PSA level was 0.0037 ng/ml in December 2017.

Before the therapy, there was very active primary tumor in prostate (SUVmax= 16.2) and the tumor metastasized to a number of lymph nodes and pelvis. Some of the lymph node metastases were multiple in colonies and bigger than 1 cm such as in presacral, right pararectal and internal iliac lymph nodes. Moreover, some of the lymph node metastases were bigger than 4 cm like in internal, external and common iliac lymph nodes. Additionally, there were a lot of bone metastases in C1 and C2.

After this therapy, primary tumor activity regressed (SUV max = 5.5). The bone metastasis could not be detected on the PET scan. In addition, the lymph node involvement was regressed. All of the lymph node metastases observed were smaller than 1 cm.

DISCUSSION

Prostate cancer is the most common type of cancer in men (1). It can metastasize to various tissues. Prostate cancer should be detected before it metastasises, otherwise it can be lethal (2). It is very important to detect the primary tumor and their metastases and treat properly if there are metastases. Androgen deprivation therapy is applied to reduce testosterone levels since testosterone is the cause of prostate cancer cell growth and proliferation. But in some cases the tumor cells are resistant to androgen deprivation therapy, thus are called mCRPC (1). The survival of these cases is less than 24 months (3). Also it is very important to monitor the treatment properly. Prostate cancer may reoccur and this recurrence may not be detected because there may be some recurrences which are too small to detect by any radiological imaging or doctors may not be suspected of recurrence because the PSA level is low.

The treatment and monitoring methods may be inadequate in some cases. Therefore, new methods are required to treat patients who do not respond to conventional therapy. If only PSA based screening or imaging is used, some recurrent cases may be missed. According to various studies, theranostic agents are more sensitive for screening and more effective for treatment in mCRPC cases (5-8).

In this case, it is observed that theranostic agents can reduce the activity of prostate cancer in mCRPC and it is found that Ga-68 PSMA is reliable to detect metastases and monitoring treatment. In our case a 6 mm large lesion was observed with Ga-68 PSMA PET, which could not be observed in CT. Ga-68 PSMA PET can show recurrences, although PSA levels are low (0.2 ng/ml to 10 ng/ml) (11). But the data and experience is insufficient to state Lu-177 PSMA has a positive effect on survival of mCRPC (12).

In this case, results showed that Lu-177 PSMA therapy is effective in the treatment of metastatic prostate cancer. The patient demonstrated regression of the primary and metastatic tumor. Theranostic agents were also effective in cancer diagnosis and monitoring treatment efficacy. Our results herald prospective studies with larger sample size and longer follow-up period to further discern the treatment efficacy and detection rate of PSMA in prostate cancer.

As a conclusion it should be noted that Lu-177 PSMA is showing exciting treatment responses in men with mCRPC and has an important future role in the treatment of prostate cancer (12).

Ethics Committee Approval: N/A
Informed Consent: Written informed consent was obtained from the participant of this study.
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: ARA, BÖ, GDA. Design: ŞYS. Supervision: ARA, BÖ, GDA. Resources: ARA, BÖ, GDA. Materials: ARA, BÖ, GDA. Data collection and/or processing: ARA, BÖ, GDA. Analysis and/or Interpretation: ARA, BÖ, GDA. Literature Search: ARA, BÖ, GDA. Writing Manuscript: ARA, BÖ, GDA. Critical Review: ARA, BÖ, GDA.
Financial disclosure: The authors declared that this study received no financial support.

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Received: 31.01.2019 - Accepted: 05.02.2019

CAENORHABDITIS ELEGANS AND ANGIOGENESIS

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Abstract: Angiogenesis is sprouting of new capillaries from already existing ones. It is a dynamic process that can be seen in every phase of human life. It is among the dynamic mechanisms of both physiological and pathological processes. Vascular endothelial growth factor is one of the many molecules that play a role in angiogenesis. Vascular endothelial growth factor is released specifically to the endothelium. It regulates mitogenesis, vascular tone, vascular permeability and vasodilatation in the vascular endothelium. Caenorhabditis elegans is a nematode used to detect and screen the developmental processes and genetic mutations. It is appropriate to study at the organism level to isolate cells and to demonstrate intercellular interactions in vivo. Polyvinyl fluoride-1 is a molecule that plays a role in the neural development of Caenorhabditis elegans. In addition, the polyvinyl fluoride-1 molecule is told to be effective in angiogenesis. Studies have shown that polyvinyl fluoride-1 binds to vascular endothelial growth factor receptor-1 and vascular endothelial growth factor receptor-2, but not to vascular endothelial growth factor receptor-3 and platelete derivated growth factor receptor β. In the research of human umbilical vein endothelial lines, it was observed that polyvinyl fluoride-1 induced angiogenesis and vascular tube formation. These results suggest that Caenorhabditis elegans may have a very important role in vascular endothelial growth factor studies. Caenorhabditis elegans model is used in many scientific areas such as aging, nervous system and genetic changes. However, only a few laboratories around the world studied the Caenorhabditis elegans angiogenesis model. Besides, this model is not currently used in Turkey. This provides a great advantage in terms of the utilization of this model in angiogenesis.

Keywords: Caenorhabditis elegans, vascular endothelial growth factors, VEGFR-1, VEGFR-2

INTRODUCTION

Angiogenesis

Angiogenesis is sprouting of new vessels from mature endothelial cells. This process is a condition that may occur in every phase of human life in both physiological-pathological processes in the body. Physiologically, all tissues need capillaries for diffusion of nutrients and metabolites. To maintain this, the capillaries should be formed from the main vessels. Changes in angiogenesis and capillaries are determined by metabolic activity. Oxygen plays a vital role in these processes (1).

Studies on angiogenesis began in 1971 with the hypothesis that angiogenesis might be the reason for tumor growth (Figure 1) (2). After this hypothesis, ideas such as stimulation of angiogenesis may be used therapeutically in ischemic heart diseases, peripheral artery diseases, and wound recoveries have been thought. In addition to this, there has been a supposal that inhibition or reduction of angiogenesis may be used therapeutically in cancer, ophthalmic diseases, rheumatoid diseases, and other diseases (1). Apart from these pathological conditions, capillaries develop or regress in healthy tissues according to functional needs. In other words, physiologically, stimulation and inhibition of angiogenesis occur in the body.

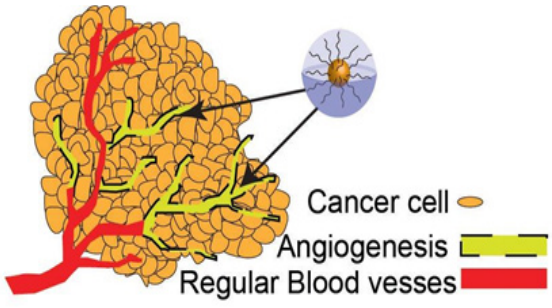

Figure 1: An illustration of angiogenesis on a tumor cell (3).

Various molecules play a role in the regulation of angiogenesis in the body: Fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), angiostatin, thrombostatin etc. (4). Angiogenesis is stimulated and inhibited by such factors.

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Vascular Endothelial Growth Factor

Vascular endothelial growth factor is a molecule that regulates mitogenesis, vascular tone, vascular permeability and vasodilation in the vascular endothelium (5). Members of the VEGF family and VEGF receptors play a critical role in physiological and pathological angiogenesis (6). VEGF is a strong angiogenic factor and it is upregulated in many tumor tissues (7). VEGF and VEGF receptors are expressed from many cells, especially endothelial cells and tumor cells (8).

Glycoproteins which form the VEGF family, show their effects on cell membrane receptors (6). These receptors that VEGF family affects are called vascular endothelial growth factor receptors (VEGFRs). VEGFRs are structurally tyrosine kinase receptors (8). VEGF receptors are similar to platelet-derived growth factor receptor (PDGFR). All subtypes of the VEGFR family have seven immunoglobulin-like domains in the extracellular medium and a tyrosine kinase domain in the intracellular medium (6).

The VEGF family consists of five members that have a homodimeric structure: VEGF-A, VEGF-B, VEGF-C, and VEGF-D and Placental growth factor (PIGF). VEGF-A plays a regulatory role in angiogenesis, progressional vasculogenesis and differentiation of progenitor endothelial cells. VEGF-B and PlGF induce angiogenesis in ordinary tissues. However, increased PlGF production promotes the development of pathological angiogenesis in tumor and inflammatory lesions. The effects of VEGF-B and PlGF on angiogenesis are weaker than VEGF-A. VEGF-C plays a role in embryonic lymphangiogenesis (6). VEGF-D has mitogenic effects for endothelial cells. VEGF-D is a glycoprotein that stimulates remodeling of the blood vessels and lymphatic vessels (9).

VEGFR family consists of three members: VEGFR-1, VEGFR-2, and VEGFR-3. Vascular endothelial cells, hematopoietic stem cells, some tumor cells, monocytes and macrophages express VEGFR-1 (Figure 2). VEGFR-2 is expressed from vascular endothelial cells, hematopoietic stem cells and some tumor cells. VEGFR-3 is specifically expressed from lymphatic endothelial cells in the human body (6). VEGF types show their effects by binding to VEGFRs. The level of binding varies according to the types of VEGF and VEGFRs. VEGF-A binds to VEGFR-1 and VEGFR-2 among VEGFRs. VEGF-A shows higher affinity to VEGFR-1 than to VEGFR-2. In addition, the tyrosine kinase activity of VRGFR-2 is higher than VEGFR-1 (8). The pro-angiogenic signal occurs mainly via VEGFR-2 (8). VEGF-B and PIGF only bind to VEGFR-1 among VEGFRs (6). VEGF-C and VEGF-D bind to VEGFR-2 and VEGFR-3, but not to VEGFR-1 (10).

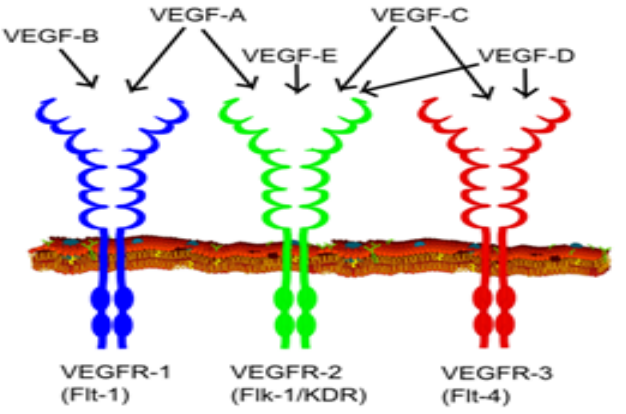

Figure 2: VEGF family members and their receptors (11).

Caenorhabditis Elegans

Caenorhabditis elegans (C. elegans) is a small, non-pathogenic nematode found in nature in its free form. Larvae are 0.25 millimeters long, adults are 1 millimeter long (12). C. elegans are transparent and have individual cells. Their subcellular details can easily be easily observed. C. elegans' proteins can be labeled using fluorescent proteins (12). Thus, advanced details can be obtained. Fluorescent proteins can also be used to characterize developmental processes and mutations that effect cell function, to isolate cells, and to characterize intracellular and extracellular protein interactions in vivo.

Caenorhabditis elegans' life cycle is really fast. The time from the egg to the non-egg-producing adult is 3 days at 25 °C. C. elegans consists male or hermaphrodite sex characteristics. Male C. elegans occur in less than 0.2%. Men are initially observed as a self-fertilizing hermaphrodite. Because of all these features, C. elegans is an important model for eukaryotic genetic studies. At the same time, C. elegans is the first multicellular organism known to hold a full genome sequence; thus allowing the molecular identification of many primary genes in genetic, developmental and biological processes of the cell and the discovery of new key molecules (12).

C. elegans' genome and 60-80% of human genes are found to be orthologue (12). In addition, in the C. elegans' genome, 40% of genes that are associated with human diseases have orthologs. Therefore, the discoveries in C. elegans are valuable for the study of human health and diseases.

Growth and Maintenance

Caenorhabditis elegans can easily be isolated from rotten vegetables and fruits that are rich in bacterial food sources. In the laboratory, C. elegans are grown in agar plates containing Escherichia coli (E. coli). After C. elegans consume the bacteria in the agar, they pass into the dauer form and survive for at least one month. This period can be up to 6 months at 15°C. The C. elegans in the dauer form do not need continuous feeding. When healthy, developing animals are needed in the laboratory a piece of old plaque agar is transferred to a new plate containing bacteria, then C. elegans return to L4 larvae form from the dauer form and maintain their normal developmental processes (12).

Caenorhabditis elegans is suitable for experimental usage because it can be reused after freezing and to produce new C. elegans from a single hermaphrodite. C. elegans can be grown at temperatures between 12 C° to 25 C°. A 10 C° increase accelerates the growth twice (12). Development at different temperatures makes it possible to follow the growth rate in C. elegans. It also helps in the isolation and utilization of temperature-sensitive mutants (12). C. elegans is an easy and cost-effective experimental animal.

Sexual Forms

Caenorhabditis elegans has two forms of wild type: hermaphrodite and male (Figure 3). Hermaphrodite C. elegans' gonads produce amoeboid sperms which first stand in L4 staged spermatheca (12). Then, in the period close to adulthood, the ovary changes the fate of the egg cell to produce larger oocytes (12). Normally, hermaphrodites produce sperm a certain time before producing oocytes. The hermaphrodites store the sperm they produce before producing oocytes and normally fertilize these sperms with their newly produced oocytes. About 300 new eggs occur here. If hermaphrodites mate with men, they can produce 1000 eggs. This shows that sperm produced by hermaphrodite is a limited factor in self-fertilization (12). The chromosomes of the hermaphrodite and male C. elegans are diploid and contain five autosomal chromosomes (12). The sexes differ in terms of sex chromosome. Hermaphrodites have two X chromosomes, while males have one X chromosome (Hermaphrodite: XX, Male: XO) (12). Most of the eggs formed by hermaphrodites are hermaphrodites. Only 0.1-0.2% of the eggs form male C. elegans.

Male C. elegans is fundamental because it allows the exchange of materials necessary to achieve genetic diversity and gene mapping (12).

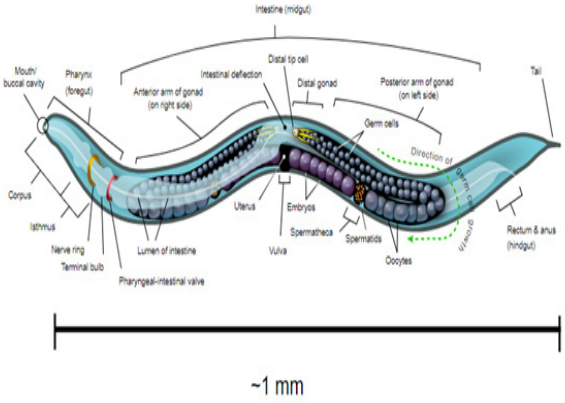

Figure 3: Caenorhabditis elegans hermaphrodite adult (13).

Role of Caenorhabditis elegans in Genetic Studies

Caenorhabditis elegans is one of the most important and useful experimental animals for genetic studies. One of the major reasons for the use of C. elegans in genetic studies is that it is a model suitable for genetic manipulation. In addition, C. elegans has many advantages in terms of genetic use. One of the advantages of the usage of C. elegans is that they show genetically rapid development. Mutant homozygotes can be detected in two weeks after mutagenesis.

Polyvinyl Fluoride-1

Polyvinyl fluoride -1 (PVF-1) is a polymer expressed from the region of the gene in Drosophila melanogaster, Caenorhabditis remanei, Caenorhabditis briggsae and Caenorhabditis elegans. The protein it encodes is called PVF-1. PVF-1 protein is similar to VEGF-A, VEGF-B and PIGF molecules in the molecular structure (14).

Polyvinyl fluoride-1 molecule acts on VEGFR 1-4 receptors in C. elegans (15). Further studies have shown that PVF-1 binds to VEGFR-1 and VEGFR-2 but not to VEGFR-3 and PDGF receptor β (14). This results in PVF-1's VEGF A and PIGF is closer to the suggestion.

These molecules interact with PVF-1, thus play a role in both angiogenesis and neural development (14).

The relation between Polyvinyl Fluoride-1 and Angiogenesis

Polyvinyl fluoride-1, which is a molecule encoded from PVF-1 gene region in C. elegans has an important role for ray formation. Molecular studies show that; PVF-1 molecule binds to VEGFR-1 and VEGFR-2.

Polyvinyl fluoride-1 induces angiogenesis and capillary tube formation in human umbilical vein endothelial cells (HUVECs) (15). These results suggest that C. elegans may have a very important place in VEGF studies.

DISCUSSION

Angiogenesis is in the pathological and physiological processes in our body. This situation shows the importance of angiogenesis in our life cycle. Angiogenesis is the necessary mechanism for the human body. If the production of molecules that lead to angiogenesis is inhibited or the receptors to which the molecules bind are blocked, the formation of angiogenesis is prevented and tumor angiogenesis is inhibited. In this way, the tumor tissue cannot be fed, the vessels required for metastasis cannot be obtained and the body is minimized for damage. Also, tumor angiogenesis is induced to increase the likelihood of tumor-specific drugs. In this way, the drug dose to be given to the patient is reduced, the cost of the drug is decreased and the patient takes a minimum level of medication. These two opposite situations are important for understanding the importance of the balance in angiogenesis.

Many molecules play an important role in angiogenesis that is so critical for our body. VEGF comes at the beginning of these molecules. VEGF is a molecule that regulates mitogenesis, vascular tone, vascular permeability and vasodilation in the vascular endothelium, which is specific to the endothelium (5). VEGF has five different subtypes; VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PIGF) (6). VEGF-A plays a regulatory role in the progression of vasculogenesis, angiogenesis, and differentiation of progenitor endothelial cells (6). VEGF-B and PIGF induce angiogenesis in ordinary tissues (6). However, increased PIGF production promotes the development of pathological angiogenesis in tumor and inflammatory lesions (6). The effect of VEGF-B and PIGF on angiogenesis are weaker than VEGF-A. VEGF-C plays a role in embryonic lymphangiogenesis (6). VEGF-D is a mitogen for endothelial cells VEGF-D is a glycoprotein that stimulates the remodeling of blood vessels and lymphatic vessels. (9) VEGF shows the effect on the VEGF receptor, which is a tyrosine kinase receptor on the cell surface. There are three subtypes of VEGFR: VEGFR-1, VEGFR-2, and VEGFR-3. Vascular endothelial cells and hematopoietic stem cells express VEGFR-1 and VEGFR-2 in physiological conditions (6). VEGFR-1 and VEGFR-2 are also expressed in some tumor cells pathologically (6). Furthermore, monocytes, and macrophages express VEGFR-1 (6). The kinase activity of VEGFR-1 is very low compared to VEGFR-2 (8). VEGFR-3 is usually expressed from lymphatic endothelial cells in the body (5).

The future of angiogenesis can be changed using VEGF agonists and antagonists. This situation provides the ability to stop the development of many cancers and diseases, regression of diseases and full cure.

CONCLUSION

Caenorhabditis elegans is a good and useful model for the detection of VEGF agonists and antagonists. The PVF-1 molecule, which is responsible for the formation of the first of the rays at the tail of male C. elegans, resembles VEGF-A, VEGF-B and PIGF in the molecular structure (14). Studies have shown that PVF-1 binds to VEGFR-1 and VEGFR-2 (14). This suggests that C. elegans is a good, useful, inexpensive, simple model for the detection of VEGF agonists and antagonists. Inhibition of angiogenesis, which plays an important role in the pathogenesis of important diseases, with the molecules to be discovered will prevent the progression of many diseases and prevent further discomfort. It will also increase the diversity of treatment options in tumors and many other diseases.

Ethics Committee Approval: N/A
Informed Consent: N/A
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: AE, İK. Design: AE, İK. Supervision: EŞ, AE, İK. Resources: EŞ, AE, İK. Materials: AE, İK. Data collection and/or processing: AE,

İK. Analysis and/or Interpretation: AE, İK. Literature Search: EŞ, AE, İK. Writing Manuscript: AE, İK. Critical Review: EŞ, AE, İK.

Financial disclosure: The authors declared that this study received no financial support.

Acknowledgements: We thank Prof. Abdullah Olgun from Istinye University Faculty of Pharmacy for his support.

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Turkish Med. Stud. J. 2018; 5: 69-75 DOI: 10.4274/tmsj.galenos.2019.06.01.07
Received: 15.02.2019 - Accepted: 17.02.2019

LETTER TO THE EDITOR

Christos Tsagkaris

University of Crete School of Medicine, Heraklion, GREECE

Dear editor,

I read "Research of urgent biochemistry test ordering habit" with great interest. The authors have applied statistics in order to transform a "common secret" into quantitative evidence illustrating its impact on the medical community of the University Hospital of Edirne. I paid special attention to the causes of ordering superficial laboratory investigations and I would like to underline the potential impact of the so-called defensive medicine (DM) on this kind of medical practice.

DM consists of a contemporary tendency in clinical practice. The two main types of DM are positive and negative DM. In the first case, doctors tend to overuse diagnostic procedures including laboratory testing and imaging in order to avoid missing any potential diagnosis. In the second case, doctors avoid performing risky medical procedures (i.e. surgical operations, invasive techniques etc) so as not to commit a medical error. The elevated biochemistry test ordering could be linked to positive DM (1).

Several synchronic studies as well as personal narratives – letters have indicated the impact of DM so far. Studdert et al. (2) investigated 800 physicians' responses indicating that 92% of them were prone to positive DM and 42% practiced negative DM in order to minimize the risk of complications. In a survey of Gallup and Jackson Healthcare in 2010, it was found that physicians in private healthcare tend to practice DM more than physicians in state institutions (3). Rodriguez et al. (4) study in 2007 suggested a high prevalence of 50% of DM among emergency physicians in California from 2001 to 2005.

All in all, defensive medicine is considered as a negative aspect of contemporary medical practice. Although safety is highlighted, the rationale of DM is structured on personal feeling or anxiety rather than evidence-based medicine (5).

However, most of the available studies have been conducted in the US, where the attitude of physicians is influenced by the high frequency of malpractice suing. To to the best of our knowledge, there is not adequate evidence about this phenomenon in the Balkans region and it would be very interesting if this parameter could be evaluated in a future version of Elmacı et al.'s study.

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CONCEPT	The idea for research or article/hypothesis generation	
DESIGN	Planning the methods to generate hypothesis	
SUPERVISION	Supervision and responsibility for the organization and course of the project and the manuscript preparation	
RESOURCES	Supplying financial resources, equipment, space, and personnel vital to the project	
MATERIALS	Biological materials, reagents, referred patients	
DATA COLLECTION AND/OR PROCESSING	Responsibility for conducting experiments, management of patients, organizing and reporting data	
ANALYSIS AND/OR INTERPRETATION	Responsibility for presentation and logical explanation of results	
LITERATURE SEARCH	Responsibility for conducting literature search	
WRITING MANUSCRIPT	Responsibility for creation of an entire or the substantial part of the manuscript	
CRITICAL REVIEW	Reworking the final, before submission version of the manuscript for intellectual content, not just spelling and grammar check	
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9. Royalties					
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CONSENT FORM for CASE REPORT

Title of Project:_____

1. I have read, and understood the Participant Information Sheet dated _____
2. I freely agree to the use of my medical records for the purpose of this study.
3. I understand that the case report will be published without my name attached and researchers will make every attempt to ensure my anonymity. I understand, however, that complete anonymity cannot be guaranteed.
4. I have been given a copy of the Participant Information Sheet and Consent Form to keep.

Name of Participant_____

Signature of Participant _____ Date _____

The participant was informed through phone call and a verbal consent was obtained.

The following section regarding the witness is not essential but may be appropriate for patients where the research teams feel that the participant should have a witness to the consent procedure.

Name of witness (if appropriate)_____

Signature of witness_____ Date _____

Name of Researcher_____

Signature of Researcher_____ Date_____

Name of Researcher

Signature of Researcher_____ Date_____