

An analysis of occurrence and development and treatment of nasopharyngeal carcinoma related to dietary factors and herbal extracts

Kristine Guran ^{1,#,*}, Roxana Buzatu ^{2,#}, Alina Dolghi ³, Marioara Poenaru ¹

¹ "Victor Babes" University of Medicine and Pharmacy Timisoara, Faculty of Medicine, Eftimie Murgu Sq 2, Timisoara, Romania

² "Victor Babes" University of Medicine and Pharmacy Timisoara, Faculty of Dentistry, Eftimie Murgu Sq 2, Timisoara, Romania

³ "Victor Babes" University of Medicine and Pharmacy Timisoara, Faculty of Pharmacy, 300041, Eftimie Murgu Sq 2, Timisoara, Romania
Equal contribution

Abstract

Nasopharyngeal carcinoma is an epithelial carcinoma derived from the nasopharyngeal mucosa and compared to other cancers, it is relatively unusual. Among the main factors responsible for the development of this disease are genetic predisposition, infection with Epstein Barr virus but also diet and lifestyle. Here, was analysed the incidence of nasopharyngeal carcinoma in western Romania in last six years and the influence of diet and lifestyle. N-nitroso carcinogenic compounds play an important role in the development of the disease, while dietary polyphenols can successfully combat the development of malignant processes.

Keywords: nasopharynx, carcinoma, diet, herbal medicines

1. Introduction

Nasopharyngeal carcinoma (NPC) is a rare type of cancer identified globally, with a total number of new cases of 133,354 during 2020 [1]. The global geographical distribution of nasopharyngeal carcinoma is extremely varied, taking into account that more than 70% of cases are diagnosed in Asia, with a rate of ~3.0 / 100,000 in China and ~0.4 / 100,000 in populations that are mainly Caucasians [2]. In last year, the highest incidence was in Southeast Asia (7.7 / 100,000 for men and 2.5 / 100,000 for women) and the lowest in Central America (0.27 / 100,000 for men and 0.08 / 100,000 for women) [1]. In Europe, the age-standardized incidence rates (ASR) per 100,000, is around 1 for men and less than 1 for women, as follows: 1 (men) and 0.28 (women) in Southern Europe; 0.68 (men) and 0.22 (women) in Central and Eastern Europe; 0.59 (men) and 0.21 (women) in Western Europe and 0.36 (men) and 0.16 (women) in Northern

Europe [1]. Among the risk factors associated with this type of disease are: geographic position, environmental factors, lifestyle and dietary habits [3]. Genetic susceptibility, Epstein-Barr virus infection and a number of environmental factors (carcinogens, dietary factors) underlie the emergence and development of NPC [4]. Dietary factors are incompletely studied due to the different spread of the disease, but it has been established that they can exert a decisive effect on the initiation and promotion of NPC [5,6]. The association between consumption of canned fish with salt, salted vegetables and canned meat and the risk of NPC has been reported frequently [7]. Also, low intake of fresh vegetables and fruits has been associated with the development of the disease [8,9]. Population screening and testing, advances in recent years in diagnosis (by imaging methods) and treatment have contributed to a slight decrease in the incidence of the disease, especially in endemic regions [10].

* Corresponding author: guran.kristine@umft.ro

The main purpose of the present study was to evaluate the role of diet from Western Romania in nasopharyngeal carcinoma development, the importance of medicinal herbs with an anticancer property and their bioactivity in squamous cell carcinoma related to the incidence and curative approach of the disease.

2. Materials and Method

2.1. Study design

Between 2014 and 2020, over ninety patients, aged between 23 and 75 years, were diagnosed with NPC in Western Romania. The data were collected from the clinical records of the patients and the processed information was strictly related to diet, lifestyle and treatment. Other studies have been identified through detailed search using the PubMed platform (<http://www.ncbi.nlm.nih.gov/pubmed>) both for the study of the role of diet and for the frequency of use of medicinal plants and the influence on treatment. The search was limited to studies published in English, using the following keywords: nasopharyngeal carcinoma and diet, or herbal medicines, or plant extract.

2.2. Extract preparation and characterization

Salvia officinalis L. was collected in Caras-Severin County in the period of growing season of the year 2018. First step was to crush the vegetal material by using a laboratory mill (analytical type - A11 basic Analytical Mill, IKA). The reagents, standard chemicals and solvents used for the extraction and chromatographic characterization were acquired from Sigma-Aldrich, Germany. In order to obtain sage crude alcoholic extract, maceration method was selected. Briefly, the extraction procedure involved the following steps: (i) plant material (2.5 g aerial parts) were mixed with 25 mL of ethanol 70% and the mixture was stored away from light, with occasional stirring, for seven days, (ii) removal of the solvent by means of a rotary evaporator, after a prior filtration, (iii) lyophilisation and storage away from light at 2-8°C until further evaluations. The total phenols (TP, mg GAE/g d.m.) quantification was made using Folin-Ciocalteu reagent, flavonoids/flavonols (TF/TFv, expressed as rutin equivalents) content was assessed by utilizing Al colorimetric test, and the total condensed tannin (TT, mg (+)-catechin equivalents) was evaluated by the vanillin test. All methods were conducted according to the ones described in the literature [11]. Individual phenolics were quantified by LC-

MS method, using gradient and two mobile phases, water and acetonitrile, both acidified following a method of analysis presented in the literature [11].

2.3. Biological activity assessment

The cell lines employed for the current research were SCC-4 (tongue squamous cell carcinoma, ATCC® CRL-1624™) and FaDu (pharynx squamous cell carcinoma, ATCC® HTB-43™) purchased from ATCC (American Type cell Collection). The media and appropriate materials for cell cultivations were acquired from ATCC and Sigma Aldrich. Cell cultures were prepared as described by the manufacturer's protocol [12]. After meeting the specific culture conditions, once the appropriate confluence was reached, the cells were stimulated with five different concentrations of sage extract (25, 50, 100, 200 and 500 µg/mL) to study cell viability. The Alamar blue test was applied as presented in literature [13].

2.4. Statistical analysis

The statistical programme and software used in this study was GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA) and the results are expressed as mean ± standard deviation.

3. Results and Discussion

Of the cases diagnosed with nasopharyngeal carcinoma, 20% belong to women and the remaining 80% to men. Globally, the incidence among men is significantly higher than that among women, as presented in Figure 1.

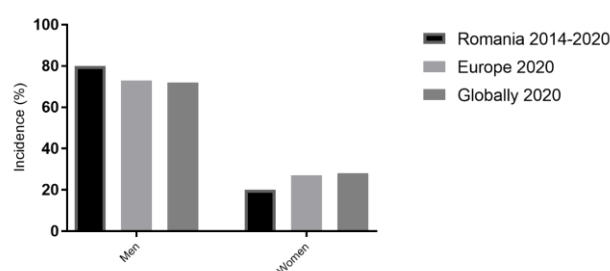


Figure 1. Incidence by sex of people diagnosed with nasopharyngeal carcinoma

The worldwide distribution of NPC, except for China which is considered a pandemic zone (cases exceed 130,000 in the last year), is presented in Figure 2.

The relatively small number of cases encountered in Western Romania during six years represents a joyful proof that European population have an

unfavourable genome for NPC. The European lifestyle and food habits discourages the activation of this type of cancer. A series of case-control studies were performed in order to evaluate the associations between diet and the development of nasopharyngeal carcinoma. A significant dose-response relationship was found in the analysis of the consumption of fresh fish, green tea and coffee [7].

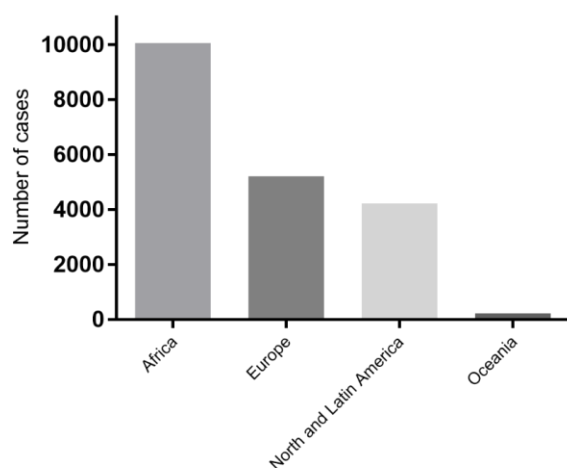


Figure 2. The distribution of the estimated number of cases of nasopharyngeal carcinoma different continents and areas of the world

A meta-analysis explored the dependence between consumption of red meat, the processed meat and the NPC risk and it was established that these foods increase considerable the carcinogenic hazard [14]. Alcohol consumption is also suspected to be another favourable factor in the carcinoma increment [15]. In China, consumption of salted fish is common due to lack of food resources. The process of preparing salted dried fish involves some steps (like sun drying and storage for at least four months before consumption) that can lead to the development of bacteria. Also, in this period N-nitroso compounds are formed. Frequent consumption of foods containing nitrogen-based carcinogens leads to the accumulation of these substances in the cells of the nasopharyngeal area with the accelerated generation of procarcinogenic compounds [16]. N-nitrosodimethylamine can induce tumors in the nasal cavity according to data obtained from analysis in animal models, and other chemical carcinogens could act synergistically, for example, with Epstein Barr virus to increase the risk of developing NPC [17]. In the same time, N-nitroso

compounds can be found in drinking water and cured meat, sign that lifestyle, eating habits and sanitary conditions are fundamental in induction and development of the cancer. In Eastern Europe, diet do not include salted fish, but the majority of people are meat lovers and the alcohol consumption is in continuous ascendancy, imposing a special attention and a desirable screening strategy for NPC to reduce the incidence as much as possible. Certainly, a carefully constructed diet rich in fruits and vegetables helps maintain cellular health. A meta-analysis which involved more than 3,000 cases approached the efficiency of citrus fruit against NPC. Results were dose-dependent and highlighted that in case of an intake four times per week of citrus fruit, the cancer development can decrease with 21% [18]. Carrots, tomatoes, peppers, pome and dark green leafy vegetables combined presented good results in case of men and less in case of women with NPC, distinguishing an interesting approach to this type of cancer, which affect mostly men [19]. In Romania, as citrus fruit are not specific, they are not part of the classic diet, but other food with a strong antioxidant effect are consumed; black grapes, garlic, sea buckthorn, cranberries and blueberries and it will be intriguing to analyse their antitumor properties in the event of NPC cases.

The correlation between lifestyle and the development of nasopharyngeal carcinoma is still deficient and poorly understood. For example, smoking, the use of herbal nasal sprays, or the use of excess tea have been associated with nasopharyngeal carcinoma [20,21]. The treatment of the disease involves first of all irradiation and if necessary the association with chemotherapeutics. These procedures are accompanied by a number of significant side effects. Side effects can be alleviated by adopting a balanced diet and a carefully implemented lifestyle.

The beneficial effects (antioxidant, anti-proliferative, anti-inflammatory) of phenolic compounds in medicinal plants were evaluated, especially in vitro in order to establish preliminary pharmacological and toxicological profiles for use in chemoprevention and treatment. There are still a number of gaps in their biological activity. Each geographical area has its own plants and environment and studies should be conducted in relation to these aspects.

In an advanced search on the PubMed platform for nasopharyngeal carcinoma along with various key terms, the association of the disease with plant extracts was highlighted in the research studies, as can be seen in Figure 3.

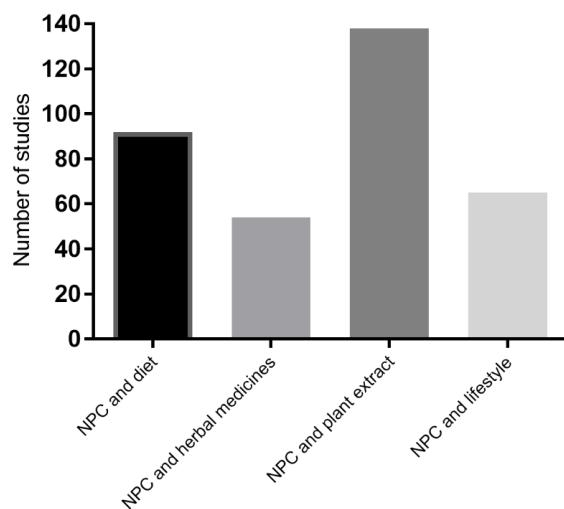


Figure 3. Search results on the PubMed platform for associations between nasopharyngeal carcinoma and different key terms

In the present study were evaluated the total phenolic, flavonoid, flavonols and tannins content, and also individual amounts of phenolic compounds from sage aerial parts extract by using 70% ethanol (maceration). The extraction yield was 22.7% and the other data obtained were as followed: TPC 39.6 mg GAE/g d.m., TFC 27.3 mg RE/g d.m., TFvC 6.7 mg RE/g d.m. and TT 14.6 mg CE/g d.m. Individual quantitative analysis of polyphenols, showed the amounts of bioactive chemical compounds identified in the crude alcoholic extract of sage, rutin (149.9 µg/g) and kaempferol (211.7 µg/g) being the predominant compounds from a quantitative point of view. Furthermore, a number of phenolic acids (in descending order of content: rosmarinic - 74.3 µg/g, caffeic - 35.6 µg/g, ferullic - 9.4 µg/g, gallic - 4.5 µg/g, coumaric - 3.6 µg/g, and protocatechuic - 0.6 µg/g), phytoalexin (resveratrol - 20.4 µg/g), and aglycones flavonoid (quercetin - 47.2 µg/g and epicatechin - 45.8 µg/g) were identified in crude alcoholic extract. The identity of the active molecules was authenticated by LC-MS by comparison with standard compounds.

Cytotoxic potential of sage extract evaluated by Alamar blue assay and presented as percentage of

viable cells compared to untreated cells. Squamous cell carcinoma stimulated with 25, 50, 100, 200 and 500 µg/mL of test samples for 48 h led to a decrease of viable cells, as presented in Table 1.

Table 1. Squamous cell carcinoma viability after stimulation with sage extract

Concentration µg/mL	Viable cells (%)	
	SCC-4	FaDu
25	99.87±1.22	98.97±0.41
50	98.97±1.36	98.00±0.58
100	98.18±0.51	96.48±0.29
200	91.00±0.68	88.38±0.47
500	84.57±0.73	80.07±1.73

The extract obtained from sage, due to its rich content in flavonoids, can have a strong antioxidant capacity, as well as anticancer and anti-inflammatory activities [22,23]. A number of studies have addressed the beneficial effects of rutin and kaempferol, which consumed in the diet (fruit, tea, wine) can prevent inflammatory processes and combat reactive oxygen species. According to literature data, sage extract has anticancer but also anti-metastatic potential against oral squamous cancer cells, through an assigned mechanism for caspase-mediated mitochondrial apoptosis in cancer cells [24,25].

4. Conclusion

This study analyses the cases of nasopharyngeal carcinoma from Western Romania and as expected data showed that men are more suitable to this form of carcinoma. Food containing N-nitroso compounds, which are DNA destroyer, can accelerate the appearance and development of the disease. Fortunately, in the Balkans, foods rich in nitrogen-based carcinogens are significantly less common compared to the pandemic area in China. Natural antioxidants found in fruits, vegetables, certain teas and wine, are healthy and safe solutions to combat pro-inflammatory conditions that lead to the development of malignant processes. Polyphenolic compounds have an activity of reducing the number of cancer cells that must be deepened in order to establish beneficial protocols for chemoprevention of malignant processes.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest.

References

- International Agency for Research on Cancer 2020. <https://gco.iarc.fr/today/data/factsheets/cancers/4-Nasopharynx-fact-sheet.pdf>
- Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J Clin.* **2018**, 68(6), 394-424.
- Lee, H.M.; Okuda, K.S.; González, F.E.; Patel, V.; Current Perspectives on Nasopharyngeal Carcinoma. *Adv Exp Med Biol*, **2019**, 1164, 11-34.
- Chan, A.T.; Felip, E., ESMO Guidelines Working Group. Nasopharyngeal cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up, *Ann Oncol.* **2009**, 20(4), 123-125.
- Dellavalle, C.T.; Xiao, Q.; Yang, G.; Shu, X.O.; Aschebrook-Kilfoy, B.; Zheng, W.; Lan, L.H.; Ji, B.T.; Rothman, N.; Chow, W.H.; Gao, Y.T.; Ward, M.H., Dietary nitrate and nitrite intake and risk of colorectal cancer in the Shanghai Women's Health Study, *International Journal of Cancer* **2014**, 134, 2917-2926.
- Keszei, A.P.; Goldbohm, R.A.; Schouten, L.J.; Jakszyn, P.; van den Brandt, P.A., Dietary N-nitroso compounds, endogenous nitrosation, and the risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study, *The American Journal of Clinical Nutrition* **2013**, 97, 135-146.
- Hsu, W.L.; Pan, W.H.; Chien, Y.C.; Yu, K.J.; Cheng, Y.J.; Chen, J.Y.; Liu, M.Y.; Hsu, M.M.; Lou, P.J.; Chen, I.H.; Yang, C.S.; Hildesheim, A.; Chen, C.J., Lowered risk of nasopharyngeal carcinoma and intake of plant vitamin, fresh fish, green tea and coffee: a case-control study in Taiwan, *PLoS One* **2012**, 7(7), e41779.
- Xie, S.H.; Yu, I.T.; Tse, L.A.; Mang, O.W.; Yue, L., Sex difference in the incidence of nasopharyngeal carcinoma in Hong Kong 1983–2008: Suggestion of a potential protective role of oestrogen, *Eur J Cancer* **2013**, 49, 150-155.
- Xue, W.Q.; Qin, H.D.; Ruan, H.L.; Shugart, Y.Y.; Jia, W.H., Quantitative association of tobacco smoking with the risk of nasopharyngeal carcinoma: A comprehensive meta-analysis of studies conducted between 1979 and 2011, *Am J Epidemiol.* **2013**, 178, 325-338.
- Wu, L.; Li, C.; Pan, L., Nasopharyngeal carcinoma: A review of current updates, *Exp Ther Med.* **2018**, 15(4), 3687-3692.
- Popovici, R.A.; Vaduva, D.; Pinzaru, I.; Dehelean, C.A.; Farcas, C.G.; Coricovac, D.; Danciu, C.; Popescu, I.; Alexa, E.; Lazureanu, V.; Stanca, H.T., A comparative study on the biological activity of essential oil and total hydro-alcoholic extract of *Satureja hortensis* L., *Experimental and therapeutic medicine* **2019**, 18(2), 932-942.
- American Type Culture Collection. Available at <https://www.lgcstandards-atcc.org/>, Accessed: December 8, 2020.
- Pinzaru, I.; Heghes, A.; Marti, D.; Dehelean, C.; Coricovac, D.; Moaca, A.; Moatar, M.; Camen, D., Therapeutically Potential of *Medicago sativa* Extracts. Chemical and in vitro assessments, *Rev. Chim.* **2018**, 69(1), 121-124.
- Li, F.; Duan, F.; Zhao, X.; Song, C.; Cui, S.; Dai, L.; Red Meat and Processed Meat Consumption and Nasopharyngeal Carcinoma Risk: A Dose-response Meta-analysis of Observational Studies. *Nutr Cancer.* **2016**, 68, 1034-1043.
- Okekpa, S.I.; Mydin, R.B.S.M.N; Mangantig, E.; Azmi, N.S.A.; Zahari, S.N.S.; Kaur, G.; et al.; Nasopharyngeal carcinoma (NPC) risk factors: A systematic review and meta-analysis of the association with lifestyle, diets, socioeconomic and sociodemographic in Asian region. *Asian Pacific J Cancer Prev.* **2019**, 20(11), 3505-3514.
- Ward, M.H.; Pan, W.H.; Cheng, Y.J.; Li, F.H.; Brinton, L.A.; Chen, C.J.; Hsu, M.M.; Chen, I.H.; Levine, P.H.; Yang, C.S.; Hildesheim, A., Dietary exposure to nitrite and nitrosamines and risk of nasopharyngeal carcinoma in Taiwan, *Int J Cancer.* **2000**, 86(5), 603-609.
- IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010). IARC monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 94(v-vii), 1-412.
- Feng, X.X.; Wang, M. X.; Li, M.; Tang, X.; Jiang, H.; Wang, R.; et al., Citrus fruit intake and the risk of nasopharyngeal carcinoma. *Asia Pac J Clin Nutr.* **2019**, 28(4), 783-792.
- Liu, Y.T; Dai, J.J; Xu, C.H.; Lu, Y.K.; Fan, Y.Y.; Zhang, X.L.; et al., Greater intake of fruit and vegetables is associated with lower risk of nasopharyngeal carcinoma in Chinese adults: A case-control study. *Cancer Causes Control.* **2012**, 23(4), 589-599.
- Lin, J.H.; Jiang, C.Q.; Ho, S.Y.; Zhang, W.S.; Mai, Z.M.; Xu, L.; Lo, C.M.; Lam, T.H., Smoking and nasopharyngeal carcinoma mortality: A cohort study of 101,823 adults in Guangzhou, China, *BMC Cancer.* **2015**, 15, 906-912.

21. Chellang, P.K.; Narain, K.; Das, H.K.; Chetia, M.; Mahanta, J., Risk factor for cancer of nasopharynx: A case control study from Nagaland, *India. Natl Med J India.* **2000**, *13*, 6-8.
22. Hamidpour, M.; Hamidpour, R.; Hamidpour, S.; Shahlari, M., Chemistry, pharmacology, and medicinal property of sage (*Salvia*) to prevent and cure illnesses such as obesity, diabetes, depression, dementia, lupus, autism, heart disease, and cancer, *J Tradit Complement Med.* **2014**, *4*, 82-88.
23. Martins, N.; Barros, L.; Santos-Buelga, C.; Henriques, M.; Silva, S.; Ferreira, I.C., Evaluation of bioactive properties and phenolic compounds in different extracts prepared from *Salvia officinalis* L., *Food Chem.* **2015**, *170*, 378–385.
24. Rajagopalan, P.; Wahab, S.; Dera, A.A.; Chandramoorthy, H.C.; Irfan, S.; Patel, A.A.; Abullias, S.S; Zaman, G.S.; Ahmad, I., Anti-cancer activity of ethanolic leaf extract of *Salvia officinalis* against oral squamous carcinoma cells in vitro via caspase mediated mitochondrial apoptosis, *Pharmacognosy Magazine*, **2020**, *16*(5), 546-552.
25. Dziedzic, A.; Kubina, R.; Wojtyczka, R.D.; Tanasiewicz, M.; Varoni, E.M.; Iriti, M., Flavonoids Induce Migration Arrest and Apoptosis in Detroit 562 Oropharynx Squamous Cell Carcinoma Cells, *Processes* **2021**, *9*, 426.