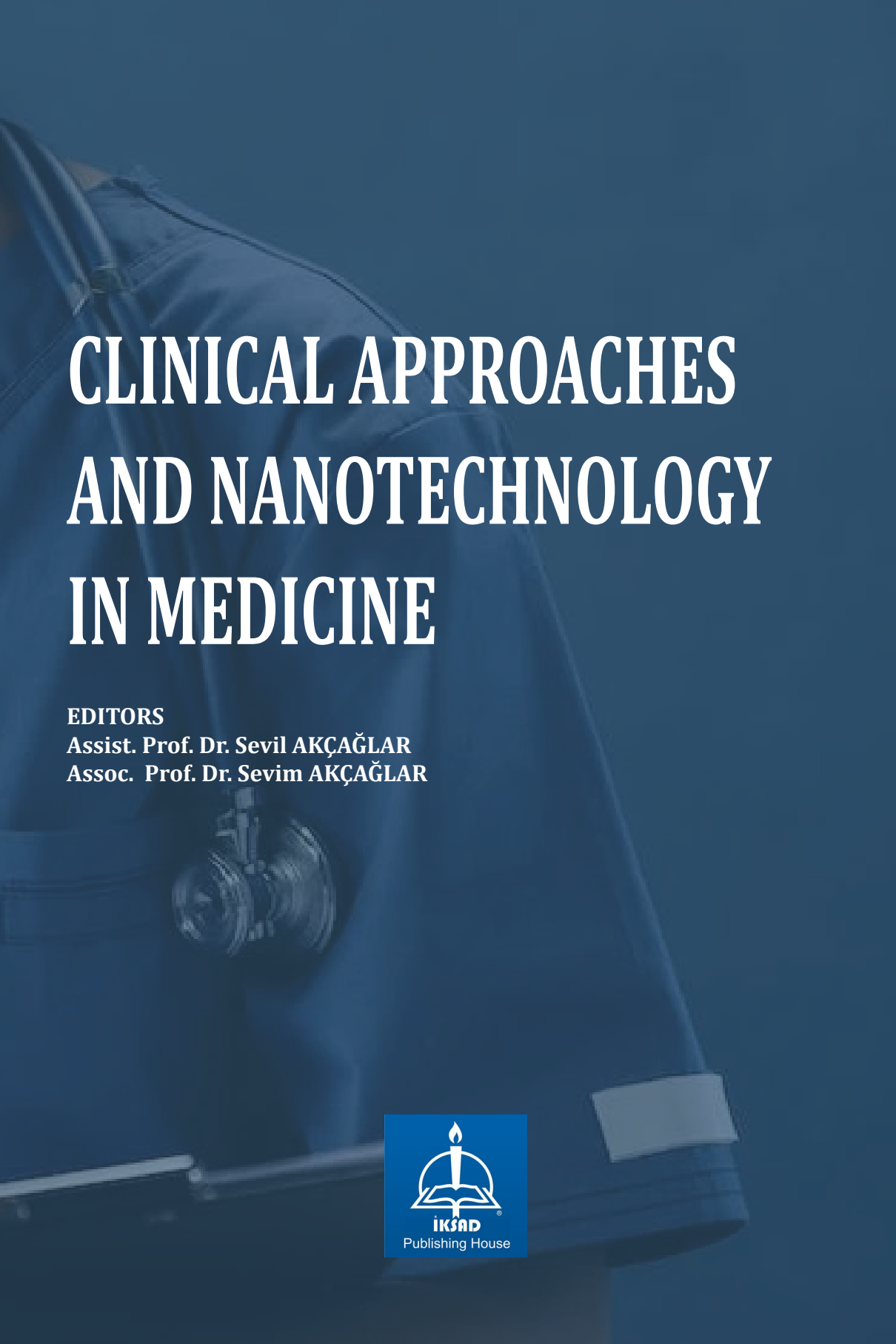


CLINICAL APPROACHES AND NANOTECHNOLOGY IN MEDICINE

EDITORS
Assist. Prof. Dr. Sevil AKÇAĞLAR
Assoc. Prof. Dr. Sevim AKÇAĞLAR

İKSAD
Publishing House

CLINICAL APPROACHES AND NANOTECHNOLOGY IN MEDICINE

EDITORS
Assist. Prof. Dr. Sevil AKÇAĞLAR
Assoc. Prof. Dr. Sevim AKÇAĞLAR

AUTHORS
Prof. Dr. Abdulmecit YILDIZ
Prof. Dr. Alparslan ERSOY
Prof. Dr. Cuma Bulent GUL
Assoc. Prof. Dr Aysegul ORUC
Assoc Prof. Dr Berfu KORUCU
Assist. Prof. Dr. Mustafa Çağatay BÜYÜKUYSAL
Assist. Prof. Dr. Mustafa ASLIER
Asist. Prof. Dr. Sevil AKÇAĞLAR
Dr. Mehmet. F. AYDIN
Dr. Oktay UNSAL
Dr. Suat AKGUR
Lecturer Dr. Utku METE
M.D. İbrahim Ethem PINAR
M.D. Parvin PINAR
Res. Assist. Dr. Kemal YUCE
Dr. Ahmet İsmail ÖZKAN
M.D. Parvin PINAR
Gizem SOLAK

İKSAD
Publishing House

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

ISBN: 978-625-6404-35-9
Cover Design: İbrahim KAYA
December / 2022
Ankara / Türkiye
Size = 16x24 cm

CONTENTS

PREFACE

Nanotechnology; in many fields of science such as physics, materials, biology, environment, chemistry, medicine and pharmacy, technological advances and advanced research have emerged as one of the keys. Thanks to these multidisciplinary studies, developments in every branch of science have gained great momentum.

As nanotechnology develops, the collaboration of scientists has improved, and the value of laboratory and clinical data has increased even more with a multidisciplinary approach.

EDITORS
Assist. Prof. Dr. Sevil AKÇAĞLAR
Assoc. Prof. Dr. Sevim AKÇAĞLAR

CHAPTER I

Excess Fluid Intake Due To Medical Advice In Chronic Kidney Disease Patients: A Common But Nameless Cause Of Hyponatremia

Prof. Dr. Abdulmecit YILDIZ[1]
Dr. Oktay UNSAL[2]
Assoc. Prof. Dr Aysegul ORUC[3]
Dr. Suat AKGUR[4]
Dr. Mehmet. F. AYDIN[5]
Prof. Dr. Cuma Bulent GUL[6]
Assoc Prof. Dr Berfu KORUCU[7]
Prof. Dr. Alparslan ERSOY[8]

[1] Department of Nephrology, Uludag University Faculty of Medicine, Bursa, Turkey
[2] Department of Nephrology, Uludag University Faculty of Medicine, Bursa, Turkey
[3] Department of Nephrology, Uludag University Faculty of Medicine, Bursa, Turkey
[4] Department of Nephrology, Uludag University Faculty of Medicine, Bursa, Turkey
[5] Department of Nephrology, Uludag University Faculty of Medicine, Bursa, Turkey
[6] Department of Nephrology, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa, Turkey
[7] Department of Nephrology, Gazi University Faculty of Medicine, Ankara, Turkey
[8] Department of Nephrology, Uludag University Faculty of Medicine, Bursa, Turkey

INTRODUCTION

Hyponatremia, usually synonymous with hypoosmolality, is the most common electrolyte disorder.[1] Hyponatremia generally results from excess free water intake. Water is taken into the body in two ways, by mouth, which is triggered by thirst and by aquaporin channels in the collecting tubule, which is stimulated by arginine vasopressin (AVP).[1,2] Both thirst and AVP secretion results from the osmosensitive neural activation. If water is taken into the body without any osmotic stimulation and cannot be excreted by the kidneys, hyponatremia may occur. Most of the patients who develop hyponatremia due to inappropriate water intake are patients with a neuropsychiatric disorder or individuals that are massive beer drinkers who consume high amounts of water in the setting of very low solute intake.[3] The former of these relatively rare but famous syndromes is called primary polydipsia and the letter is called beer-potomania syndrome. However, there is another group of patients in whom hyponatremia with a similar mechanism is common but not yet named. In fact, in clinical practice, chronic kidney disease (CKD) patients are the most common group of patients who present with hyponatremia due to high water and low solute intake. CKD patients are very vulnerable to hyponatremia because of both the impaired dilution capacity of the kidneys and peer pressure to drink high amounts of water.[4,5] A common misbelief among physicians and the community is that one should consume large amounts of water to prevent kidney disease progression.[4] In our country, a high amount of water intake is recommended as an important component of a healthy lifestyle via media. Hyponatremia is associated with serious complications such as cognitive changes, falls, and fractures and even mortality.[6] Recent studies also report other complications of hyponatremia such as osteoporosis, cardiomyopathy, sarcopenia, adipose tissue changes, and gonadal disturbances.[7] In this article, we present eight CKD patients who developed hyponatremia after they strictly

followed their physician`s recommendations to keep on high water and low solute diet to keep their kidney function stable.

Cases

We summarized hyponatremic patients due to high water intake by their preference or by physicians' recommendations diagnosed in our outpatient nephrology clinic. Hypervolemic hyponatremia due to heart failure or cirrhosis, thiazide induced hyponatremia or SIADH patients were excluded. Out of them, a total of 8 patients with CKD developed symptomatic hyponatremia, one of which required hypertonic saline infusion. All of these patients stated that they were on a strict CKD diet (low salt and protein) while they were consuming at least 3-4 L of water. Two of these patients (Case 1 and 2) were kidney donors. Case 1 donated her son and developed hyponatremia one year later after donation. Case 2 donated his spouse and developed hyponatremia one month later after donation. Characteristics of patients shown in Table 1. Glucocorticoid deficiency, hypothyroidism, advanced heart failure or cirrhosis, and hypovolemia have been ruled out clinically or laboratory. All patients responded well to water restriction that we want them to consume water when they feel thirsty and solute load recommended that added salt their food and roughly 1.2 gr/kg -protein, especially from meat-based food. After water intake is restricted and new dietary recommendations are given, their symptoms entirely resolved and hyponatremia did not recur in the long term follow up. Of those, kidney function compromised in only one case.

Clinical and laboratory characteristic of patients presented in Table 1

	Age	Sex	Major symptoms	GFR (0)	GFR(1)	Urine osmolality (mOsm/kg)	Urine density (1.)
Patient1 (Donor)	61	F	Encephalopathy	82	71	218	1015
Patient2 (Donor)	81	M	Nausea and malaise	25	29	212	1016
Patient3	73	F	Fatigue, malaise	36	43	187	1007
Patient4	86	F	Unstable gait, Nausea	33	17	158	1008
Patient5	64	F	Malaise	46	48	111	1009
Patient6	68	F	Malaise	30	30	223	1017
Patient7	79	E	Unstable gait	54	48	215	1014
Patient8	56	K	Headache, Nausea	13	14	179	1009
Mean (min-Max)	71 (56-86)			39.8 (13-82)	37.5 (14-71)	187 (111-223)	1010 (1007-1016)

GFR: Estimating Glomerular Filtration Rate by CKD-EPI Equation

0: On admission, 1: First week

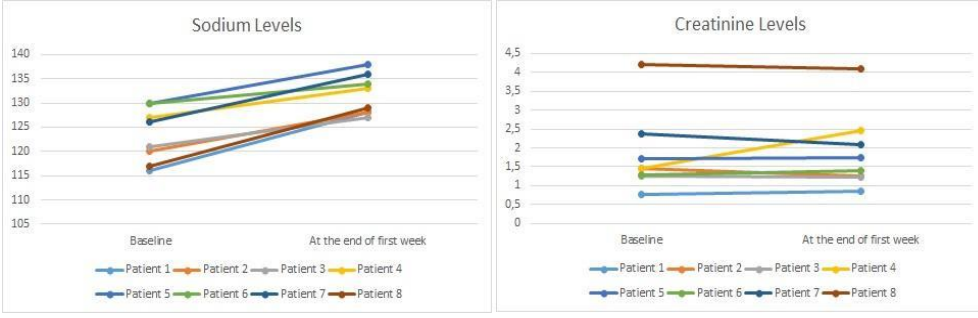


Figure 1: Slope of creatinine and sodium

Discussion:

True hyponatremia is roughly classified in two groups, ADH dependent and non-dependent. ADH dependent hyponatremia causes high urinary osmolality (over 285 mOsm/kg) relative to plasma. ADH is mainly secreted by two stimulating factors in the body. These are high serum osmolarity and low plasma volume. Since high osmolarity will not occur in the presence of hyponatremia, the most important factor for ADH secretion is reduced plasma volume. Decreased plasma may be due to actual losses, or result of diminished effective plasma flow seen in patients with advanced heart failure and cirrhosis.[8,9] Latter called as a hypervolemic and former as hypovolemic hyponatremia. ADH can be secreted without these two factors called The syndrome of inappropriate antidiuretic hormone secretion (SIADH) 1.

ADH independent hyponatremia which is easily understood by urine osmolality below 280 mOsm/kg may result from high volume water or low solute volume with relative high volume water. Decreased dilution capacity of the kidney in the presence of kidney disease facilitates the development of ADH-dependent hyponatremia.[10]

Hyponatremia with inappropriately high ADH levels results from an underlying disease and may require specific treatment options such as V2 receptor antagonists.[11]

Hyponatremia with suppressed AVP results from high oral water intake which overwhelms the excretory capacity of the kidneys. Healthy kidney dilutes the urine to as low as 50 mosm/day and one should exceed 12 L/day water ingestion when consuming an average solute of 600m Osm per day to develop hyponatremia.[5,10] This is an exceedingly rare condition except for severe depression or psychosis. However, if one consumes a low amount of solutes, he/she may develop hyponatremia with a relatively low amount of water intake

like that in beer potomania or tea and toast diet which are rarely encountered in daily practice.[3,5]

We may consider another and relatively more common but nameless condition in addition to these situations: high-water-low-solute intake in CKD patients. All these conditions with low ADH levels can be grouped under a basic heading which may be called excess fluid intake syndrome. Physicians tend to suggest their patients with lower kidney function to consume high amounts of water.[12]. The reason for this traditional and contemporarily abandoned behavior is the idea that plenty of water is kidney-friendly. Except for some conditions such as kidney stone disease, the benefit of high water intake has not yet been established in CKD patients.[13] CKD patients are instructed to consume low solute (low sodium and protein) and a high water diet in order to maintain stable creatinine levels. Without knowing the capacity of the kidneys to excrete free water that can be estimated easily by spot urine sodium and potassium and plasma sodium levels, these patients may end up with severe hyponatremia if suggested to consume a daily water amount of 2 L or more.[5]

Our two patients with solitary kidney secondary to nephrectomy for kidney donation developed severe hyponatremia despite having normal kidney function. The solitary kidney is probably not able to dilute the urine as much as a pair of kidneys. Both of the patients presented in the winter. The patients might have been more susceptible to hyponatremia due to less insensible water loss in winter. A CKD patient with an impaired diluting capacity (e.g. 150 mOsm/kg) who is on a low solute diet (e.g. 300 mOsm/day solute intake) may develop hyponatremia above 2 L of daily water consumption in the absence of insensible fluid loss. Hyponatremia is more common in CKD patients and the prognosis is even worse when both CKD and hyponatremia coexist.[14] It is not surprising that CKD patients are eager to consume more water than their daily needs because of the physician's suggestions or personal preferences.

Hyponatremia is possibly more devastating in these patients than that in subjects with normal kidney functions. [14]

The major limitation of our observation is the lack of long term follow up and limited case number. We have not designed as a study so couldn't provide detailed characteristics of patients. However, we can say that in CKD patients, low solute with disproportional high fluid may make the course worse.

In conclusion, in order to suggest an appropriate level of water intake to patients with CKD, the dilution capacity of kidneys and actual daily solute intake should be taken into account.

References

1. Henry DA. In The Clinic: Hyponatremia. *Ann Intern Med.* 2015;163(3):Itc1-19.
2. Danziger J, Zeidel ML. Osmotic homeostasis. *Clin J Am Soc Nephrol.* 2015;10(5):852-862.
3. Bhattarai N, Kafle P, Panda M. Beer potomania: a case report. *BMJ Case Rep.* 2010;2010.
4. Chang A, Kramer H. Fluid intake for kidney disease prevention: an urban myth? *Clin J Am Soc Nephrol.* Vol 6. United States2011:2558-2560.
5. Berl T. Impact of solute intake on urine flow and water excretion. *J Am Soc Nephrol.* 2008;19(6):1076-1078.
6. Hoorn EJ, Rivadeneira F, van Meurs JB, et al. Mild hyponatremia as a risk factor for fractures: the Rotterdam Study. *J Bone Miner Res.* 2011;26(8):1822-1828.
7. Barsony J, Manigrasso MB, Xu Q, Tam H, Verbalis JG. Chronic hyponatremia exacerbates multiple manifestations of senescence in male rats. *Age (Dordr).* 2013;35(2):271-288.
8. Francoz C, Durand F, Kahn JA, Genyk YS, Nadim MK. Hepatorenal Syndrome. *Clin J Am Soc Nephrol.* 2019;14(5):774-781.
9. Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. *N Engl J Med.* 1999;341(8):577-585.
10. Seay NW, Lehrich RW, Greenberg A. Diagnosis and Management of Disorders of Body Tonicity-Hyponatremia and Hypernatremia: Core Curriculum 2020. *Am J Kidney Dis.* 2020;75(2):272-286.
11. Schrier RW, Gross P, Gheorghiade M, et al. Tolvaptan, a selective oral vasopressin V2-receptor antagonist, for hyponatremia. *N Engl J Med.* 2006;355(20):2099-2112.
12. Wenzel UO, Hebert LA, Stahl RA, Krenz I. My doctor said I should drink a lot! Recommendations for fluid intake in patients with chronic kidney disease. *Clin J Am Soc Nephrol.* 2006;1(2):344-346.
13. Jain N, Reilly RF. Effects of dietary interventions on incidence and progression of CKD. *Nat Rev Nephrol.* 2014;10(12):712-724.
14. Kovesdy CP, Lott EH, Lu JL, et al. Hyponatremia, hypernatremia, and mortality in patients with chronic kidney disease with and without congestive heart failure. *Circulation.* 2012;125(5):677-684.

CHAPTER II

Zinc Oxide Nanoparticles Using Cynara scolymus L (Artichoke) Leaf Aqueous extract Green Synthesis, Characterization, Anti-Bacterial Effects

Asist. Prof. Dr. Sevil AKÇAĞLAR[1]

[1] Department of Mechanical Eng., Dokuz Eylül University, İzmir- Turkey
sevil.akcaglar@deu.edu.tr Orcid ID: 0000-0002-5386-1862

INTRODUCTION

Nanotechnology is a rapidly growing field that has made great progress in human life. An interdisciplinary area, Nanotechnology includes bio nanoscience, technology and materials science [1], and biology, physics and chemistry fields take up a very large amount of space, [2]. Since nanoparticles have biological, optical and physicochemical properties, their application areas are very common and large. [3–7].

Nanoparticles can be produced via biological, chemical, and physical methods [8, 9].

It is necessary to make use of plants, microorganisms, algae and fungi to assemble biological nanoparticles. The green production process has been commonly used lately, because it uses less toxic products [1, 10] that are harmless when put into the nature Cynara scolymus L., it is often popularly called artichoke, is used in the produce of Ag NPs. C. scolymus is usually of Mediterranean origin and comes from the Asteraceae family and Aegean in the area, although now grown all over the world [11].

Artichoke largely appeals to the field of medicine because of its hepato-protective properties. Regarding this feature, Artichoke leaf extract is especially useful in the treatment of hepatitis and hyperlipidemia. It is widely accepted in European medicine. It is well known that C. scolymus leaves and head extracts increase liver functions, thereby increasing hepatoprotective and choleretic activities. Because of these features, it is widely accepted in folk medicine. In addition to all this atherosclerosis, hepatitis, jaundice, diabetes symptoms, chronic liver and gall bladder diseases, enters the treatment area [11]. C. scolymus inhibits the nutritive enzymes like pancreas lipase, α-amylase, α-glucosidase, rises the bile excretion, obstructs of purulence and

ROS, develops liver activity, gut microbiota, decreases lipolysis and lipid metabolism, and decreases blood glucose in preclinical and clinical researches [12]. It has been reported that artichoke extract has a rich content of metabolites such as chlorogenic acid, luteolin, caffeic acid derivatives, and flavonoids [13-17].

There are limited studies in the literature on the synthesis of nanoparticles from metal oxides by green chemistry using Cynara scolymus L(artichoke). Facilitation of nanoparticle synthesis and harmless effects of bioactive components in its content. Studies in this area have increased in recent years due to shows.

In this study, the synthesis of ZnO NPs by green chemistry was carried out using Cynara scolymus L(artichoke) leaf extract.

Synthesized nanoparticles characterization studies Ultraviolet-Visible Region Spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) was using. Anti-bacterial effects of nanoparticles synthesized from Cynara scolymus L(artichoke) on S. aureus E. coli, and C albicans were investigated.

MATERIALS AND METHODS

Preparation of Cynara scolymus L (artichoke)Extract

Artichokes were purchased from the izmir local public market. Artichoke leaves Rao et al. [18]. recommended by extracted according to the method. Artichoke leaf was washed 5 times with deionized water. The leaves were cut into small pieces. 200 g of artichoke leaves and 400 g of artichoke leaves in a liter of erlenmeyer mL of deionized water was added. This mixture is in the magnetic heater. It was heated at 100^{oC} for 2 hours. The resulting mixture through Whatman (Grade GF/B: 1.0 µm)

filter paper. The extract was obtained by filtration and fresh in synthesis prepared and used.

Figure 1. Preparation of Cynara scolymus L (artichoke)

Synthesis of Zinc Oxide Nanoparticles

ZnONPs, Siby and Beena [18-19]. and Haris et al.[20]. modified by the methods suggested by synthesized. For this, 20 mL is poured into a 100 mL beaker zinc sulfate (ZnSO4) solution (10 mM) was added. This
20 mL artichoke extract drop by drop on the mixture added. in an ultrasonic bath for 30 minutes was mixed 360 W in microwave oven. It was left for 5 minutes. The mixture was taken into falcon tubes and kept at 4000 rpm for 10 minutes centrifuged. The resulting pellet was washed 4 times with ethanol to remove organic residues centrifuged again. Taking the pellet part, 100^{oC} dried

in an oven for 12 hours. It was preserved in Eppendorf tubes for use in studies (Figure 1).

Characterization of Zinc Oxide Nanoparticles

The biosynthesis of CsLZnO-NPs was confirmed by the UV-Vis absorption peak obtained in the 300 to 400 nm wavelength range and the FTIR spectra peaks obtained in the 4000 to 400 cm^{-1} range. The surface morphology of the synthesized CsLZnO-NPs was defined by SEM analysis.

Minimal inhibitive Dose

Green produced zinc oxide nanochemicals were identified to perform antimicrobial tasks using a variety of clinically relevant bacterial and fungal strains.

Neomycin and flucanozole was used as a positive control. These strains implicated S. aureus (ATCC 25923), as Gram-positive bacteria (GPB), and E. Coli (ATCC 35218) as Gram-negative bacteria (GNB) and C. albicans (ATCC10231), as fungus. These strains were acquired American Type Culture Collection from 10801 University Boulevard Manassas, USA. Microbroth dilution assays were made to find the MIC of the produced nanochemicals. The bacteria and yeast were used to culture on media Brain heart agar for S. aureus and E. coli, Sabouraud's agar for C. albicans (Merck Millipore, Darmstadt, Germany).The test was used by 96- well plate. One hundred microliters of nutrient medium and Sabraud's dextrose fluid were put into the chambers. Two hundred microliters of nanoparticles were mixed into these chambers and serially diluted. Then inoculum (100µg) was added additionally.

The well was utilization for the positive- negative check inoculum The plate was covered and incubated at 37 C for 24 h. Absorbance's were studied at 490

nm on a microplate reader (ELISA reader) [21, 22]. MIC rates were calculated via the following equations:

İnhibition (%) = 1-OD of test / OD of control x 100

RESULTS AND DISCUSSION

Image Characterization

Image characterization is a proof of ZnO NP production. The color of the particles changes from pale brown to semi-white to light yellow with the addition of leaf extract (Fig. 1a). (Reduction mechanism of artichoke leaf and synthesis of ZnO NPs) (Fig. 1b') Santhoshkumar et al. [one]. Gnanasangeetha and Thambavani [23-24]

Imagining UV Spectrophotometry

UV absorption was identified by studying at wavelengths from 250 nm to 400 nm. When looking at the obtained UV-Vis spectrum, The peaks discovered in the range of 376-380 nm demonstrate the formation of ZnO NPs. As a result of UV absorption, a powerful peak was observed at 370 nm (Fig. 2), which verifies the available of ZnO NPs.

Al-Shabib et al. [25] Vennila and Jesurani [26] additionally found that a peak at 390 nm corresponds to ZnO NPs.

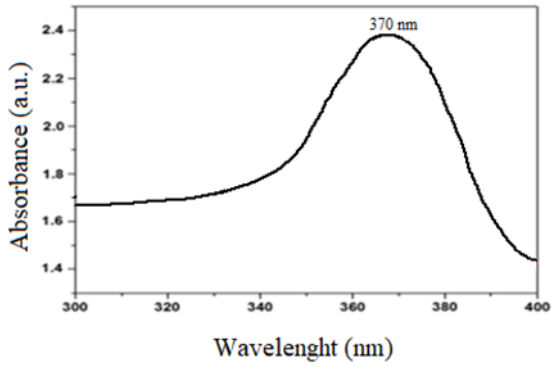
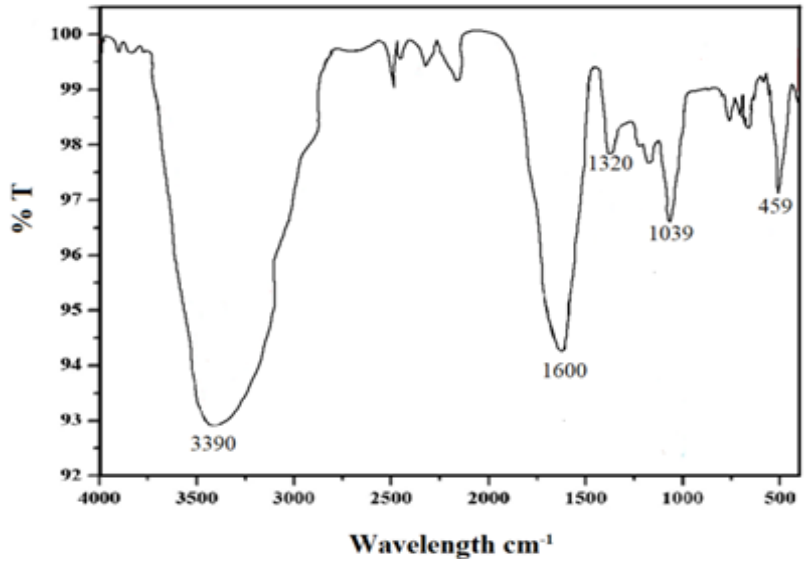


Figure 2. UV spectrum of ZnO NPs

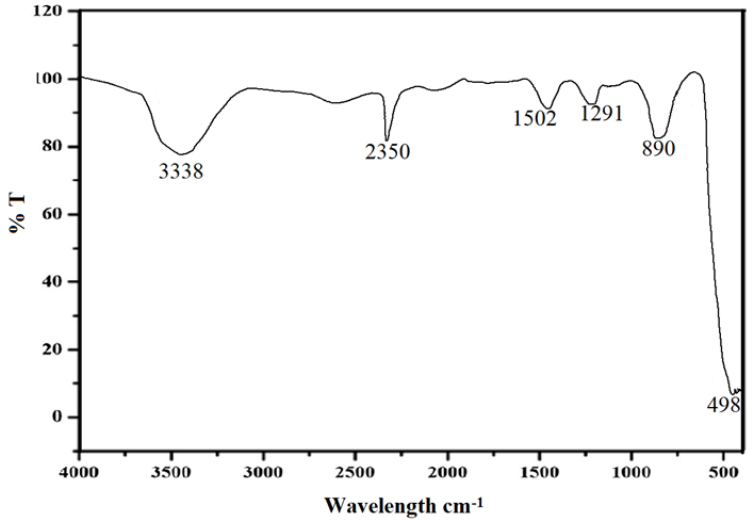
FTIR

FTIR analysis was performed to define the effect of active groups and contribution of biomolecules in the production of ZnO NPs.Peaks were examined from 400 cm-1 to 4000 $^{cm-1.}$ FTIR analysis of artichoke leaf extract indicated a hard broad absorbance at 3405.67 $^{cm-1}$ typical of the (O–H) straining frequency common to alcohol composites. When FTIR analysis was application to the artichoke leaf extract, powerful wide absorbance was followed at 3390.00cm-1 due to the characteristically of (O–H) stretching frequency widespread to alcohol compounds. The peak at 1600.28 $^{cm-1}$ coincides with the (N-H) bending frequency widespread to compounds with an amine group. The peak at 1320.17 $^{cm-1}$ is the sign of the bending density characteristic of compounds with an alkane group (–C–H). The powerful peak at 1039.96 $^{cm-1}$ is determinative of the(C–O) stretching mode characteristic of alcohol compounds. A peak was also determined at 459.912 $^{cm-1}$ (Fig. 3a).

Regarding the spectrum determined via the analysis of ZnO NPs (Fig. 3b), the powerful and broad peak followed at 3338.700 $^{cm-1}$ coincides to an (O–H) bond and indicates the presence of an alcohol group. The very broad, strong peak at 2350 $^{cm-1}$ is determinative of the (O–H) stretching frequency and recommends the existence of an acid.



(a)



(b)

Figure 3 FT-IR spectra of artichoke leaf extract (a) and ZnO NPs (b)

The peak at 1505.02 cm-1 (C=C) agree with to the being of an aromatic group. The low peak at1291.91 cm-1 is determinative of the (C–N) lifting closeness and proposes the presence of an amine group. The peak at 890.636 cm-1 is indicative of the (=C–H) bending frequency and its strong bands suggest the availability of an alkene group. The peaks at 489.995 cm-1 is characteristically of a (Zn–O) bond [8]. This result is promoted by the detections of Vijayakumar et al. [27], in which peaks were discovered between the range of 500–4000 cm-1 and a characteristic (Zn–O) stretching peak was detected at 413 cm-. Santhoshkumar et al. [28] also checked the spectra of nanoparticles and artichoke leaf extract and ensured a parallel commentary.

SEM Analysis

The particle size of the round ZnO NP was found to be 90 nm on average by analysis with Scanning Electron Microscopy (Figure 4). It has also been observed that ZnO NPs combine to form more durable globular and hexagonal aggregates of different sizes.

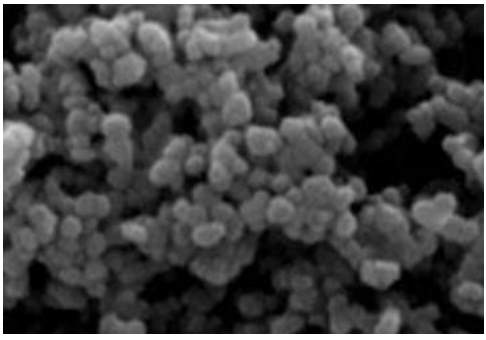


Figure 4. SEM Analysis

Minimum Inhibitory Concentration

The antibacterial effect of the biosynthesized ZnO NPs was evaluated by Micro broth dilution assays against S. aureus (ATCC 25923), as GPB, and E. coli (ATCC 35218) as GNB and C. albicans (ATCC10231), as fungus. Micro broth dilution assays were used to identify the MIC_{50} using the synthesized AT ZnO NPs (Fig. 5) For S. aureus (Gram-positive bacteria), the MIC50 was reckoned as >0.8 μg /ml). In E. coli (Gram-negative bacteria) prevention was reached at a concentration of 24 μg /ml), was make to induce an inhibitory effect. The minimum inhibitory concentration for C. albicans fungal species was found to be >100 μg /ml (Table 1). The antibacterial activity of these nanoparticles could be due to their collection in the bacterial cell walls, which would modify their permeability, resulting in cell death. It has also been proposed that ZnO NPs produce reactive oxygen species, which stimulate cell death.

Table 1. Minimum inhibitory concentration of ZnO NPs

Microorganisms	Species tested	MIC50 Concentration (μg /ml)
Bacteria	S. aureus (ATCC 25923)	>0.8
	E.coli (ATCC 35218)	24
	C. albicans (ATCC10231)	
Fungi		>100

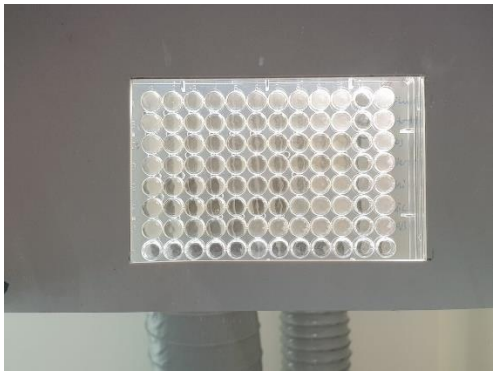


Fıgure 5. Micro broth dilution the antibacterial effect of ZnO NPs

CONCLUSION:

Nanoparticles are an imminent area of research due to their limited size of less than 100nm. The preparation of metallic nanoparticles using a green method is cheap, environmentally friendly and easily scaled up compared to other methods. New developments in nanoparticles can be observed in various fields such as biomedicine, biosensors, bio-nanotechnology due to its different properties compared to the bulk of the same materials due to its small size. Among various metals, Zinc oxide nanoparticles are therefore considered as a potential platform for biomedical research due to their anticancer, anti-inflammatory, antioxidant, antibacterial, antidiabetic, antimicrobial, photocatalytic and wound healing properties. Hence, the enigmas gleaned from nature have contributed to the progress of biomimetic approaches to the growth of advanced nanomaterials. MIC_{50} assay results showed that bacterial strains are more susceptible to ZnO NPs compared to fungi. As a result, ZnONPs synthesized by green chemistry using artichoke extract can be used for new drug delivery systems due to their antibacterial and anticancer effects target molecule.

REFERENCES

1. J. Santhoshkumar, S. V. Kumar, and S. Rajeshkumar (2017).Resour. Eff. Technol. 459 465.
2. M. Divya, B. Vaseeharan, M. Abinaya, S. Vijayakumar, M.Govindarajan, N. S. Alharbi, S.Kadaikunnan, J. M. Khaled, and G. Benelli (2018). J. Photochem. Photobiol. B. 178, 211–218.
3. G. Bhumi and N. Savithramma (2014). Int. J. Drug. Dev. Res. 6, 208–214.
4. P. Ramesh, A. Rajendran, and M. Meenakshisundaram (2014). J.Nanosci. Nanotechnol. 2, 41–45.
5. V. Karthika, A. Arumugam, K. Gopinath, P. Kaleeswarran, M.Govindarajan, N. S. Alharbi, S. Kadaikunnan, J. M. Khaled, and G. Benelli (2017). J. Photochem. Photobiol. B. 167, 189–199.
6. G. Benelli, F. Maggi, R. Pavela, K. Murugan, M. Govindarajan, B. Vaseeharan, R. Petrelli, L. Cappellacci, S. Kumar, A. Hofer, M. R. Youssefi, A. A. Alarfaj, J. S. Hwang, and A. Higuchi (2018). Environ. Sci. Pollut. Res. 25, 10184–10206.
7. P. C. Nagajyothi, T. V. Sreekanth, C. O. Tettey, Y. I. Jun, and S. H. Mook (2014). Bioorg. Med. Chem . Lett. 24, 4298–4303.
8. S. Vijayakumar, B. Vaseeharan, B. Malaikozhundan, and M. Shobiya (2016). Biomed. Pharmacother. 84, 1213–1222.
9. R. Ishwarya, B. Vaseeharan, S. Kalyani, B. Banumathi, M. Govindarajan, N. S. Alharbi, S. Kadaikunnan, M. N. Al-anbr, J. M. Khaled, and G. Benelli (2018). J. Photochem. Photobiol. 178, 249–258.
10. T. Singh, K. Jyoti, A. Patnaik, A. Singh, R. Chauhan, and S. S. Chandel (2017). Genet. Eng. Biotechnol. J. 15, 31–39.
11. O. Erdogan, M. Abbak, G. M. Demirbolat, F. Birtekocak, M.Aksel, S. Pasa, and O. Cevik (2019). PloS one. 14, (6), e0216496.
12. Mahboubi, M. (2018). Bulletin of Faculty of Pharmacy, Cairo University doi:10.1016/j.bfopcu.2018.10.003
13. N. Muthuchamy, R. Atchudan, T. N. Edison, S. Perumal, and Y.R. Lee (2018). J.Electroanal.Chem.816, 195–204.

14. R. Atchudan, T. N. Edison, S. Perumal, M. Shanmugam, and Y.R. Lee (2017). J. Photochem. Photobiol. A Chem. 337, 100–111.
15. O. Erdogan, M. Abbak, G. M. Demirbolat, F. Birtekocak, M.Aksel, S. Pasa, and O. Cevik (2019). PloS one. 14, (6), e0216496.
16. X. Zhu, H. Zhang, and R. Lo (2004). J. Agric. Food Chem. 52,(24), 7272–7278.
17. M. A. Farag, S. H. El-Ahmady, F. S. Elian, and L. A. Wessjohann(2013). Phytochemistry. 95, 177–187.
18. Rao KG, Ashok CH, Rao KV, Chakra CHS,Akshaykranth A. Int J Appl Phys Sci. 2015;2(3):1-6.
19. Joseph S, Mathew B. J Mol Liq. 2015;204:184-91.
20. Haris M, Kumar A, Ahmad A, Abuzinadah F, Basheikh M, Khan SA, et al. Trop J Pharm Res. 2017;16(12):2967-76
21. Al-Bayati FA, Sulaiman KD. In vitro antimicrobial activity of salvadora persica l. Turk J Biol. 2008;32(1):57-62.
22. Jahangirian H, Haron MJ, Ismail MHS, Rafiee- Moghaddam R, Afsah-Hejri L, Abdollahi Y, et al. Dig JNanomater Bios. 2013;8(3):1263-70.
23. Santhoskumar J, Venkat Kumar S, Rajeshkumar S Resource-Efficient Technologies. 2017;3(4):459- 65.
24. Gnanasangeetha D, Sarala Thambavani D. One potsynthesis of zinc oxide nanoparticles via chemical and green method. Res J Material Sci. 2013;1(7):1-8.
25. N. A. Al-Shabib, F. M. Husain, I. Hassan, M. S. Khan, F. Ahmed, F. A. Qais, M. Oves, M. Rahman, R. A. Khan, A. Khan, and A. Hussain (2018). J Nanomater. 2018, 14.
26. S. Vennila, S.S. Jesurani (2017). Int. J. Chemtech. Res. 10, 271–275
27. S. Vijayakumar, B. Vaseeharan, B. Malaikozhundan, and M.Shobiya (2016). Biomed. Pharmacother. 84, 1213–1222.
28. J. Santhoshkumar, S. V. Kumar, and S. Rajeshkumar (2017). Resour. Eff. Tech

CHAPTER III

Management Of Early Stage Laryngeal Cancers

Lecturer Dr. Utku METE[1]

Asst. Prof. Dr. Mustafa ASLIER[2]

[1] Bursa Uludag University, Faculty of Medicine, Department of Otorhinolaryngology, Bursa, Turkey. utkumete@uludag.edu.tr, Orcid ID: 0000-0003-0902-8061
[2] Bursa Uludag University, Faculty of Medicine, Department of Otorhinolaryngology, Bursa, Turkey. mustafaaslier@uludag.edu.tr, Orcid ID: 0000-0001-8257-0979

INTRODUCTION

Laryngeal cancers are the most common group of head and neck cancers. The true vocal cords are affected in approximately half of the patients. [1]. Patients affected by true vocal cords usually present with prolonged hoarseness and can be diagnosed at early stages such as T1 and T2, whereas supraglottic and subglottic cancers do not give early symptoms and signs and present at more advanced disease stages. Since there is anatomically less lymphatic circulation in the glottic region compared to other parts of the larynx, metastasis development is rarely seen in the early stages of glottic cancers compared to supraglottic and subglottic areas.

Therefore, the treatment of early-stage glottic cancer is of critical interest and importance. Although there are many studies suggesting that surgery or primary radiotherapy are successful treatment options, there are no complete, prospective and randomised controlled trials comparing the two treatment modalities. [2]. Early stage glottic cancer treatments are discussed in terms of local control of the disease, laryngeal preservation rate, survival, functional outcome and the necessity of salvage surgery due to treatment failure. Considering the indispensable role of the larynx in speech and communication, it is important to maintain the presence of the voice in the treatment of early stage glottic cancer. In this respect, the American Society of Clinical Oncology recommends that larynx-sparing methods should be initiated in patients with T1 and T2 stage laryngeal cancer. [3]. This book chapter will provide information about the management of early-stage glottic cancers.

EVOLUTION

In the staging of glottic cancer, vocal cord mobility and which region of the larynx is involved by the disease are the most important determinants.

Squamous cell carcinoma of the larynx is classified by the American Joint Committee on Cancer (AJCC) as follows [4].

Tis: Carcinoma in situ

T1a: Tumour limited to single vocal cord level, normal cord mobility (anterior and/or posterior commissure may be involved)

T1b: Tumour involving both vocal cords, normal cord mobility (anterior and/or posterior commissure may be involved)

T2: Tumour with supraglottic and/or subglottic extension and/or restriction of vocal cord movements

T3: Tumour limited to the larynx but causing vocal cord fixation, invading the paraglottic area and/or showing minor thyroid cartilage invasion

T4: Tumour invading the prevertebral space, invading the carotid artery, extending to mediastinal structures

Tis, T1 and T2 tumours are classified as 0, I and II respectively in AJCC staging. Although T1 and T2 tumours are usually mentioned, the definition of early stage glottic cancer includes stages 0, I and II. Based on the definition of early stage, it is known that these tumours do not cause regional lymph node spread and distant metastasis.

In addition to the existing classifications, early-stage glottic cancers can present clinically in a variety of forms, ranging from small, superficial, isolated tumours to large, diffuse, infiltrative tumours. The European Laryngological Society (ELS) has classified and standardised the surgical management of the disease as follows.

Type 1: Subepithelial cordectomy - resection of the epithelium

Type 2: Subligamental cordectomy - resection of epithelium, reinke's distance and vocal ligament

Type 3: Transmusculer cordectomy - resection including the vocalis muscle

Type 4: Total cordectomy - complete resection of the vocal cord up to the anterior commissure

Type 5a: Extended chordectomy - resection including the anterior commissure and the opposite vocal cord

Type 5b: Extended cordectomy - resection including arytenoids

Type 5c: Extended chordectomy - resection including subglottis

Type 5d: Extended chordectomy - resection including the ventricle

Type 6: Anterior commissurectomy - with anterior portion of both vocal cords

In this way, the appropriate type of resection to treat the existing disease can be accurately defined by the ELS classification. This categorisation is important for staging and comparison of treatment outcomes, especially for use in publications.

MANAGEMENT OF THE PRE-OPERATION PERIOD

Regardless of the course of treatment, it is critical to diagnose carcinoma when a vocal cord lesion is present. The differential diagnosis of vocal cord lesions varies widely, including benign and malignant lesions. Brush swab cytology using a flexible endoscope under office conditions [5] or punch biopsy methods with the help of rigid endoscopy under general anaesthesia can be used for

diagnostic purposes. Approximately 15-20% of biopsies are both diagnostic and therapeutic [6], [7]. Careful examination of the lesion and documentation of the findings are important. In particular, the condition of the anterior commissure region, which presents difficulties in terms of treatment with surgical and radiotherapy methods, should be noted.

Routine use of computed tomography (CT) is usually not necessary in T1 lesions, but may be considered in T2 lesions. CT is recommended in patients with anterior commissure involvement. There are studies reporting that CT does not change the classification of T1 and T2 lesions and that imaging is necessary in advanced laryngeal cancers or tumours involving the anterior commissure. [8]There are also studies indicating that CT imaging changes the T classification in 21% of glottic cancer patients, mostly to a higher stage. [9]. The transition to this higher stage is mostly due to subclinical thyroid cartilage involvement, supraglottic and/or subglottic extension, and soft tissue invasion. Normal-negative CT findings are present in 54% of patients with T1 tumours and 20% of patients with T2 tumours. The use and usefulness of CT in patients with T1 lesions is controversial unless there are other clinical indications.

If surgery is considered after obtaining the tissue diagnosis, excision of the lesion should be performed by paying attention to the surgical margins and depth of invasion. Alternatively, if curative radiotherapy is considered, surgery can be saved as salvage treatment.

SURGICAL

Surgical methods for early glottic cancers can be categorised under partial laryngectomies, including external approaches and transoral endoscopic laser resections. While open approaches are based on en bloc mass resection, laser excision methods have significantly changed the treatment approaches for early

stage glottic cancer. The surgical approach and the content of the resection material are determined by the location, size, stage and patient-physician factors. The amount of functional abilities such as phonation difficulty and dysphagia that develop after surgical treatment is related to the volume of excised tissue and the final defect size.

TRANSORAL LASER MICROSURGERY

Steiner's publication in 1993 made transoral laser microsurgery widely used in laryngeal carcinomas. [10]. Since then, transoral laser microsurgery has established itself as a time-saving treatment option in terms of operative time. Instead of the standard en bloc resection, the laser uses a piecemeal resection technique. This is generally thought to be due to the method of working under microscope magnification and illumination and the unique characteristics of the various tissues during dissection with the carbon dioxide (CO2) laser.

This treatment is based on close follow-up of persistent or recurrent disease. Re-excisions or further treatments may be considered according to the pathological results of the surgical material. In the study by Juckel et al. approximately 30% of patients with T1-T4 laryngeal cancer who underwent transoral laser surgery required revision laser surgery. Although almost one third of the patients required additional procedures, the ease of revision surgery characterised the process as "unproblematic". [11]. Moreover, residual cancer is not found in the repeat resection material in most of the patients who were reported as inadequate surgical margins in the first procedure. Although locoregional control and laryngeal preservation rates of patients with residual disease in revision laser surgery are significantly worsened, survival is not affected. Despite all these, most patients become completely healthy and only 0.7% of patients have persistent cancer after revision laser surgery.

Transoral laser excision in the treatment of early stage glottic cancer is comparable to other surgical methods and radiotherapy in terms of local control of the disease and laryngeal preservation rates. In various studies, local control rates have been reported as 77%-92% in T1 tumours and 66%-88% in T2 tumours. After salvage treatments, laryngeal protection is 90-99% and local control is approximately 97-98%. 5-year disease-specific survival rates have been reported as 90-98%. [12]-[16].

In the management of persistent or recurrent disease after laser resection, there are many salvage treatment options such as revision laser surgery, radiotherapy, conservative laryngeal surgery or total laryngectomy. After laser resections for early glottic tumours, tracheostomy is usually not required, oral feeding can be quickly resumed in the early period and the pain associated with the procedure is very mild. Shortened treatment time and reduced hospitalisation time are additional advantages. [6].

Although the laser method has many advantages, the safe use of the CO2 laser requires special awareness, training and high skills of the surgeon and the operating team. The most serious potential complication to be avoided during laser surgery is airway burns. Other complications include infection, granulomas, emphysema, skin fistula, bleeding, dyspnoea and respiratory distress requiring temporary or permanent tracheostomy, dysphagia and aspiration pneumonia. Although the risk of complications is relatively rare, it has been reported as 4%. [17].

OPEN SURGICAL METHODS

Open surgical methods for early glottic cancers are considered organ-sparing techniques because they aim to preserve speech and swallowing without a permanent stoma. In the past, a tracheostoma was required to perform open

techniques. Today, these techniques have been developed to eliminate the need for a permanent tracheostoma, but a temporary tracheostoma can still be used in some patients after open partial surgeries. Swallowing function may be affected for a period of time and may lead to different degrees of aspiration. Therefore, patients should have adequate lung reserve for the application of protective surgical methods.

There are many open surgical laryngeal methods for the treatment of early glottic cancer [18]. Laryngofissure cordectomy refers to the resection of the vocal cord by cutting the thyroid cartilage (thyrotomy). This method is preferred for masses located in the middle third of the vocal cord and in cases of mobile vocal cord. Vertical partial haemilaryngectomy, which involves removal of half of the larynx, can be used successfully in lesions without anterior commissure involvement. Other selected T1 and T2 lesions involving the anterior commissure can be treated with frontolateral haemilaryngectomy or supracricoid laryngectomy. Frontolateral partial laryngectomy involves resection of the entire vocal cord, anterior commissure, anterior third of the opposite vocal cord and the thyroid cartilage surrounding these structures. Supracricoid laryngeotomy preserves at least one cricoarytenoid unit and the cricoroid cartilage. Tumours located in the anterior commissure can be treated with anterior commissure surgery, which involves removal of the anterior portions of both vocal cords and the surrounding cartilage.

In general, local control rates of open surgical methods are 86-98%. [16], [18]-[22]. After salvage surgeries, local control is 99-100% and laryngeal preservation is 88-100%. 5-year disease-specific survival rates have been reported to be 92-97%.

Complications related to open surgical methods vary depending on the procedure performed. These complications include adhesion, granulation tissue,

stenosis, infection, skin fistula, haemorrhage, aspiration pneumonia, inability to wean from gastrostomy tube, inability to wean from tracheotomy and death.

RADIOTHERAPY

It has become a widely used treatment modality due to the fact that it does not require surgery, local control and survival rates. Cobalt 60 and 4-6 MV photons are the most suitable energy sources used for radiotherapy. The area to be irradiated in early stage glottic cancers covers the area extending from the upper edge of the thyroid notch to the lower edge of the cricoid cartilage and from 1 cm deep to the prevertebral fascia. [23].

Local control rate in T1 and T2 lesions is between 82-87%. 5-year disease-specific survival rate has been reported as 96 [24]. Although various rates (82-94%) are reported in different sources in studies on T1 cancers, local control rates are generally reported as 90%. [24]-[30]. When salvage treatments are performed with surgical methods in radiotherapy failures, local control is 90-96% and laryngeal preservation is 83-95%. 5-year disease-specific survival rate is 95-98%.

There is a direct correlation between the total dose administered and local control, indicating a decrease in local control, especially in patients receiving doses of 65 Gy or less [16], [22], [26], [27], [31]. In studies comparing the daily fraction amount with 5-year local control success, rates of 84% at 2.25 Gy and above, 77% at 2 Gy and 44% below 1.8 Gy were obtained. [26], [29], [32]. In a prospective randomised study investigating the difference between daily 2.5 Gy and 2 Gy applications, it was found that better local control was achieved with higher fractions. [33]. The duration of radiotherapy application is equally important; while the local control rate is 95-100% in treatments lasting less than 40 days, it decreases to 79-84% in treatments exceeding 40 days. [34].

Poor prognostic factors in radiation therapy, bulky tumour [28], [35] , anterior commissure involvement [25], [26]restriction of vocal cord mobility [27], [36] and involvement of more than one laryngeal subregion [26]-[28] as the most important factor in the development of the country's economy.

Salvage surgery is preferred for tumours that persist or recur after radiotherapy. Steiner et al. successfully applied transoral laser microsurgery as salvage surgery in radiotherapy failures and published their case series. In patients with early stage recurrence, 5-year disease-specific survival after laser surgery was 86%. [37]. In some patients, conventional laryngeal surgery or laryngectomy may be performed as salvage surgery [27], [38]-[41]. Pharyngocutaneous fistula and other complications that may develop after salvage surgeries are related to the radiotherapy dose and are usually high.

Side effects related to radiation applied to the larynx include early oedema and mucositis, late fibrosis, xerostomia and stenosis. Hoarseness may develop commonly in the early period and resolves in most patients in the later stages. Although swallowing function may be affected by the development of fibrosis, it usually improves. Serious complications requiring tracheotomy or total laryngectomy are seen in 1-2% frequency. [24], [26].

KARSİNOMA IN SİTU

Treatment options for carcinoma in situ include vocal cord stripping, laser excision and radiotherapy. Surgery should generally be considered as the first line of treatment, while radiotherapy should be reserved for persistent recurrent disease or lesions with widespread involvement of the larynx. Nevertheless, studies have reported 94% local control with radiotherapy, 81% with CO2 laser and 77% with vocal cord stripping. [42] However, this information is difficult to interpret due to differences in the pathological staging, patient population and

the nature of the surgical procedure used in the studies.

ANTERIOR COMMISSURE

Approximately 20% of glottic tumours involve the anterior commissure. Although anterior commissure involvement is said to be a poor prognostic marker, it is not included in the staging system.

Opinions on the importance of anterior commissure involvement are contradictory. There are studies indicating that anterior commissure involvement has a negative effect on local control, organ preservation and survival. [25], [27], [43], the other found no significant relationship [44]. The role of the anterior commissure in tumour invasion is also contradictory.

Although Steiner et al. stated that laser surgery is an effective method in the treatment of anterior commissure involvement, Steiner et al. [45] Eckel stated that the anterior commissure region is a risky area in terms of local recurrences and will limit the use of laser. [46]. It is thought that these differences in results may be due to limitations in the possibilities of wide surgical view and appropriate intervention in the anterior commissure region of the larynx.

Open partial laryngectomy methods provide good local control in tumours with anterior commissure involvement in contrast to poor voice performance. In patients with early-stage glottic tumours with anterior commissure involvement, supracricoid laryngectomy and hyoideopiglottopexy have been reported to provide greater local control and higher laryngeal preservation rates than vertical partial laryngectomy and radiation therapy. [21].

There are different data on local control after radiation therapy in early glottic cancers with anterior commissure involvement. Mendenhall et al. reported that there was no difference in local control at 5 years in patients with and without

anterior commissure involvement. [24]. In other studies, 5-year local control ranged between 82-90% in tumours without anterior commissure involvement and between 56-80% in tumours with anterior commissure involvement. [25], [26], [47], [48].

When all these results are evaluated, it can be said that anterior commissure involvement is associated with worse treatment results. The degree of anterior commissure involvement should be determined and should be taken into consideration when determining the treatment method. It is thought that the safest oncological results can be obtained with open surgical methods. [49].

SOUND QUALITY

Opinions on voice quality after surgery or radiotherapy are conflicting and different results can be found in the literature. Randomised studies comparing quality of life and voice quality between patients treated with radiotherapy, open surgery and laser excision are lacking.

It is widely believed that sound quality is better after radiotherapy and poorer after open surgical methods. The contradictory point is the difference in voice quality between radiotherapy and laser surgery. In a study in which vocal outcomes were analysed with videolaryngoscope, objective and perceptual measurement parameters, electroacoustic analyses of patients with type 1 and type 2 endolaryngeal cordectomy were compared with a control group known to be euphonic, no statistically significant difference was found between them, but a statistical difference was found in more advanced cordectomies. [50]. Videostroboscopic examination after laser surgery revealed incomplete glottic closure in all patients, with reduced or complete disappearance of amplitude and mucosal wave patterns, and incomplete glottic closure consistent with the volume of tissue resected, resulting in breathless speech. [51]. In another study,

no difference was found between laser surgery and radiotherapy in terms of voice quality [52]. In a meta-analysis study investigating laser excision and radiotherapy, similar voice handicap index results were obtained in T1a laryngeal cancer patients [53].

In summary, some changes in voice quality may occur regardless of the treatment option chosen. Therefore, voice therapy should be kept in mind during or after treatment, especially in patients who perform voice-based jobs in terms of career and social relations. [54]. In a study comparing radiotherapy with a control group, it was found that some irregularities in the vocal cords and poor voice quality were observed after radiotherapy and voice therapy was recommended. [55]. Finally, patients should be strongly advised to give up smoking [49].

APPROACH TO NECK TREATMENT

In retrospective analyses of T1 and T2 tumours, the incidence of occult lymph node metastasis in the neck region was reported to be 0%. [56]. In a study specific to T2 glottic squamous cell carcinoma, cervical lymph node involvement was found to be rare and elective neck dissection was not recommended in clinically negative neck, but neck dissection should be added to the treatment if endolaryngeal surgery is to be performed due to local recurrence after radiotherapy. [57].

In this context, elective neck dissections are generally not performed in patients who will undergo surgery, and the inclusion of the neck region is not considered in patients who will receive primary radiotherapy. Close follow-up surveillance and wait-and-see approaches are preferred in the neck management of early-stage glottic cancers.

SUMMARY

The aim of treatment of early glottic cancers is tumour eradication and preservation of function. Surgery and radiotherapy offer similar results in terms of local control, laryngeal preservation and survival. Tumoural factors such as anterior commissure involvement, location, volume and amount of invasion, patient-related factors such as age, medical comorbidities and indications, risks, benefits and alternative treatments should be discussed between the patient and the physician.

SOURCE

[1] H. T. Hoffman *et al.* , "Laryngeal Cancer in the United States: Changes in Demographics, Patterns of Care, and Survival," 2006, doi: 10.1097/01.mlg.0000236095.97947.26.

[2] L. Warner *et al.* , "Radiotherapy versus open surgery versus endolaryngeal surgery (with or without laser) for early laryngeal squamous cell cancer," *Cochrane Database Syst. Rev.* , vol. 2014, no. 12, p. 12, Dec. 2014, doi: 10.1002/14651858.CD002027.PUB2.

[3] D. G. Pfister *et al.* , "American Society of Clinical Oncology clinical practice guideline for the use of larynx-preservation strategies in the treatment of laryngeal cancer," *J. Clin. Oncol.* , vol. 24, no. 22, pp. 3693-3704, Aug. 2006, doi: 10.1200/JCO.2006.07.4559.

[4] "AJCC Cancer Staging Manual," *AJCC Cancer Staging Man.* , 2002, doi: 10.1007/978-1-4757-3656-4.

[5] V. D. Malamou-Mitsi, D. A. Assimakopoulos, A. Goussia, L. Pappa, A. T. Skevas, and N. J. Agnantis, "Contribution of Exfoliative Cytology to the Diagnosis of Laryngeal Lesions," *Acta Cytol.* , vol. 44, no. 6, pp. 993-999, 2000, doi: 10.1159/000328622.

[6] P. R. Moreau, "Treatment of laryngeal carcinomas by laser endoscopic microsurgery," *Laryngoscope*, vol. 110, no. 6, pp. 1000-1006, 2000, doi: 10.1097/00005537-200006000-00022.

[7] M. Ansarin *et al.* , "Endoscopic CO2 laser surgery for early glottic cancer in patients who are candidates for radiotherapy: Results of a prospective nonrandomised study," *Head Neck*, vol. 28, no. 2, pp. 121-125, Feb. 2006, doi: 10.1002/HED.20301.

[8] R. Dullerud, J. G. Johansen, T. Dahl, and H. Faye-Lund, "Influence of CT on tumour classification of laryngeal carcinomas," *Acta radiol.* , vol. 33, no. 4, pp. 314-318, 1992, doi: 10.1080/02841859209173185.

[9] L. Barbera *et al.* , "The role of computed tomography in the T classification of laryngeal carcinoma," *Cancer*, vol. 91, no. 2, pp. 394-407, 2001, doi: 10.1002/1097-0142(20010115)91:2<394::AID-CNCR1014>3.0.CO;2-J.

[10] W. Steiner, "Results of curative laser microsurgery of laryngeal carcinomas," *Am. J. Otolaryngol.* , vol. 14, no. 2, pp. 116-121, 1993, doi: 10.1016/0196-0709(93)90050-H.

[11] M. C. Jäckel, P. Ambrosch, A. Martin, and W. Steiner, "Impact of re-resection for inadequate margins on the prognosis of upper aerodigestive

tract cancer treated by laser microsurgery," *Laryngoscope*, vol. 117, no. 2, pp. 350-356, Feb. 2007, doi: 10.1097/01.MLG.0000251165.48830.89.

[12] H. E. Eckel, W. F. Thumfart, M. Jungehülsing, C. Sittel, and E. Stennert, "Transoral laser surgery for early glottic carcinoma," *Eur. Arch. Otorhinolaryngol.* , vol. 257, no. 4, pp. 221-226, 2000, doi: 10.1007/S004050050227.

[13] S. A. Pradhan, P. S. Pai, S. I. Neeli, and A. K. D'Cruz, "Transoral laser surgery for early glottic cancers," *Arch. Otolaryngol. Head. Neck Surg.* , vol. 129, no. 6, pp. 623-625, Jun. 2003, doi: 10.1001/ARCHOTOL.129.6.623.

[14] G. Motta, E. Esposito, S. Motta, G. Tartaro, and D. Testa, "CO(2) laser surgery in the treatment of glottic cancer," *Head Neck*, vol. 27, no. 7, pp. 566-574, 2005, doi: 10.1002/HED.20135.

[15] H. H. Rudert and J. A. Werner, "Endoscopic resections of glottic and supraglottic carcinomas with the CO2 laser," *Eur. Arch. Otorhinolaryngol.* , vol. 252, no. 3, pp. 146-148, May 1995, doi: 10.1007/BF00178101.

[16] J. G. Spector *et al.* , "Stage I (T1 NO MO) squamous cell carcinoma of the laryngeal glottis: Therapeutic results and voice preservation," *Head Neck*, vol. 21, no. 8, pp. 707-717, 1999, doi: 10.1002/(SICI)1097-0347(199912)21:8<707::AID-HED5>3.3.CO;2-U.

[17] I. Vilaseca-González, M. Bernal-Sprekelsen, J. L. Blanch-Alejandro, and M. Moragas-Lluis, "Complications in transoral CO2 laser surgery for carcinoma of the larynx and hypopharynx," *Head Neck*, vol. 25, no. 5, pp. 382-388, May 2003, doi: 10.1002/HED.10207.

[18] J. V. Thomas, K. D. Olsen, H. B. Neel, L. W. Desanto, and V. J. Suman, "Early glottic carcinoma treated with open laryngeal procedures," *Arch. Otolaryngol. Head. Neck Surg.* , vol. 120, no. 3, pp. 264-268, 1994, doi: 10.1001/ARCHOTOL.1994.01880270012003.

[19] T. S. Sheen, J. Y. Ko, and Y. L. Chang, "Partial vertical laryngectomy in the treatment of early glottic cancer," *Ann. Otol. Rhinol. Laryngol.* , vol. 107, no. 7, pp. 593-597, 1998, doi: 10.1177/000348949810700709.

[20] A. Giovanni, B. Guelfucci, R. Gras, P. Yu, and M. Zanaret, "Partial frontolateral laryngectomy with epiglottic reconstruction for management of early-stage glottic carcinoma," *Laryngoscope*, vol. 111, no. 4 Pt 1, pp. 663-668, 2001, doi: 10.1097/00005537-200104000-00020.

[21] O. Laccourreye, L. Muscatello, L. Laccourreye, P. Naudo, D. Brasnu, and G. Weinstein, "Supracricoid partial laryngectomy with cricohyoidoepiglottopexy for 'early' glottic carcinoma classified as T1-

T2N0 invading the anterior commissure," *Am. J. Otolaryngol.* , vol. 18, no. 6, pp. 385-390, 1997, doi: 10.1016/S0196-0709(97)90058-2.

[22] J. G. Spector, D. G. Sessions, K. S. C. Chao, J. M. Hanson, J. R. Simpson, and C. A. Perez, "Management of stage II (T2N0M0) glottic carcinoma by radiotherapy and conservation surgery," *Head Neck*, vol. 21, no. 2, pp. 116-123, 1999, doi: 10.1002/(SICI)1097-0347(199903)21:2<116::AID-HED4>3.0.CO;2-8.

[23] D. J. Lee, "Definitive radiotherapy for squamous carcinoma of the larynx," *Otolaryngol. Clin. North Am.* , vol. 35, no. 5, pp. 1013-1033, Oct. 2002, doi: 10.1016/S0030-6665(02)00039-7.

[24] W. M. Mendenhall, R. J. Amdur, C. G. Morris, and R. W. Hinerman, "T1-T2N0 squamous cell carcinoma of the glottic larynx treated with radiation therapy," *J. Clin. Oncol.* , vol. 19, no. 20, pp. 4029-4036, Oct. 2001, doi: 10.1200/JCO.2001.19.20.4029.

[25] G. Marshak *et al.* , "Prognostic factors for local control of early glottic cancer: the Rabin Medical Centre retrospective study on 207 patients," *Int. J. Radiat. Oncol. Biol. Phys.* , vol. 43, no. 5, pp. 1009-1013, Mar. 1999, doi: 10.1016/S0360-3016(98)00547-1.

[26] Q. T. X. Le *et al.* , "Influence of fraction size, total dose, and overall time on local control of T1-T2 glottic carcinoma," *Int. J. Radiat. Oncol. Biol. Phys.* , vol. 39, no. 1, pp. 115-126, Aug. 1997, doi: 10.1016/S0360-3016(97)00284-8.

[27] E. Cellai *et al.* , "Radical radiotherapy for early glottic cancer: Results in a series of 1087 patients from two Italian radiation oncology centres. I. The case of T1N0 disease," *Int. J. Radiat. Oncol. Biol. Phys.* , vol. 63, no. 5, pp. 1378-1386, Dec. 2005, doi: 10.1016/J.IJROBP.2005.05.018.

[28] P. Warde *et al.* , "T1/T2 glottic cancer managed by external beam radiotherapy: the influence of pretreatment haemoglobin on local control," *Int. J. Radiat. Oncol. Biol. Phys.* , vol. 41, no. 2, pp. 347-353, May 1998, doi: 10.1016/S0360-3016(98)00062-5.

[29] W. M. Mendenhall, J. T. Parsons, R. R. Million, and G. H. Fletcher, "T1-T2 squamous cell carcinoma of the glottic larynx treated with radiation therapy: relationship of dose-fractionation factors to local control and complications," *Int. J. Radiat. Oncol. Biol. Phys.* , vol. 15, no. 6, pp. 1267-1273, 1988, doi: 10.1016/0360-3016(88)90220-9.

[30] L. V. Johansen, C. Grau, and J. Overgaard, "Glottic carcinoma - Patterns of failure and salvage treatment after curative radiotherapy in 861 consecutive patients," *Radiother. Oncol.* , vol. 63, no. 3, pp. 257-267, 2002, doi: 10.1016/S0167-8140(02)00118-4.

[31] K. Skladowski, R. Tarnawski, B. Maciejewski, A. Wygoda, and K. Slosarek, "Clinical radiobiology of glottic T1 squamous cell carcinoma," *Int. J. Radiat. Oncol. Biol. Phys.* , vol. 43, no. 1, pp. 101-106, Jan. 1999, doi: 10.1016/S0360-3016(98)00375-7.

[32] F. Schwaibold *et al.* , "The effect of fraction size on control of early glottic cancer," *Int. J. Radiat. Oncol. Biol. Phys.* , vol. 14, no. 3, pp. 451-454, 1988, doi: 10.1016/0360-3016(88)90259-3.

[33] H. Yamazaki, K. Nishiyama, E. Tanaka, M. Koizumi, and M. Chatani, "Radiotherapy for early glottic carcinoma (T1N0M0): results of prospective randomised study of radiation fraction size and overall treatment time," *Int. J. Radiat. Oncol. Biol. Phys.* , vol. 64, no. 1, pp. 77-82, Jan. 2006, doi: 10.1016/J.IJROBP.2005.06.014.

[34] J. C. M. Van Der Voet, R. B. Keus, A. A. M. Hart, F. J. M. Hilgers, and H. Bartelink, "The impact of treatment time and smoking on local control and complications in T1 glottic cancer," *Int. J. Radiat. Oncol. Biol. Phys.* , vol. 42, no. 2, pp. 247-255, Sep. 1998, doi: 10.1016/S0360-3016(98)00226-0.

[35] S. P. Reddy, N. Mohideen, S. Marra, and J. E. Marks, "Effect of tumour bulk on local control and survival of patients with T1 glottic cancer," *Radiother. Oncol.* , vol. 47, no. 2, pp. 161-166, May 1998, doi: 10.1016/S0167-8140(97)00196-5.

[36] A. R. Harwood *et al.* , "Radiotherapy of early glottic cancer," *Laryngoscope*, vol. 90, no. 3, pp. 465-470, 1980, doi: 10.1002/LARY.5540900313.

[37] W. Steiner, P. Vogt, P. Ambrosch, and M. Kron, "Transoral carbon dioxide laser microsurgery for recurrent glottic carcinoma after radiotherapy," *Head Neck*, vol. 26, no. 6, pp. 477-484, Jun. 2004, doi: 10.1002/HED.20009.

[38] G. Schwaab, G. Mamelle, E. Lartigau, O. Parise, P. Wibault, and B. Luboinski, "Surgical salvage treatment of T1/T2 glottic carcinoma after failure of radiotherapy," *Am. J. Surg.* , vol. 168, no. 5, pp. 474-475, 1994, doi: 10.1016/S0002-9610(05)80104-7.

[39] M. Makeieff, D. Venegoni, G. Mercante, L. Crampette, and B. Guerrier, "Supracricoid partial laryngectomies after failure of radiation therapy," *Laryngoscope*, vol. 115, no. 2, pp. 353-357, Feb. 2005, doi: 10.1097/01.MLG.0000154751.86431.41.

[40] I. Ganly *et al.* , "Results of surgical salvage after failure of definitive radiation therapy for early-stage squamous cell carcinoma of the glottic larynx," *Arch. Otolaryngol. Head. Neck Surg.* , vol. 132, no. 1, pp. 59-

66, Jan. 2006, doi: 10.1001/ARCHOTOL.132.1.59.

[41] A. J. Ballantyne and G. H. Fletcher, "Surgical management of irradiation failures of nonfixed cancers of the glottic region," *Am. J. Roentgenol. Radium Ther. Nucl. Med.* , vol. 120, no. 1, pp. 164-168, 1974, doi: 10.2214/AJR.120.1.164.

[42] M. Sadri, J. McMahon, and A. Parker, "Management of laryngeal dysplasia: a review," *Eur. Arch. Otorhinolaryngol.* , vol. 263, no. 9, pp. 843-852, Sep. 2006, doi: 10.1007/S00405-006-0078-Y.

[43] M. F. Chen, J. T. C. Chang, N. M. Tsang, C. T. Liao, and W. C. Chen, "Radiotherapy of early-stage glottic cancer: analysis of factors affecting prognosis," *Ann. Otol. Rhinol. Laryngol.* , vol. 112, no. 10, pp. 904-911, Oct. 2003, doi: 10.1177/000348940311201014.

[44] M. S. Benninger, J. Gillen, P. Thieme, B. Jacobson, and J. Dragovich, "Factors associated with recurrence and voice quality following radiation therapy for T1 and T2 glottic carcinomas," *Laryngoscope*, vol. 104, no. 3 Pt 1, pp. 294-298, 1994, doi: 10.1288/00005537-199403000-00009.

[45] W. Steiner, P. Ambrosch, R. M. W. Rödel, and M. Kron, "Impact of anterior commissure involvement on local control of early glottic carcinoma treated by laser microresection," *Laryngoscope*, vol. 114, no. 8, pp. 1485-1491, Aug. 2004, doi: 10.1097/00005537-200408000-00031.

[46] H. E. Eckel, "Local recurrences following transoral laser surgery for early glottic carcinoma: frequency, management, and outcome," *Ann. Otol. Rhinol. Laryngol.* , vol. 110, no. 1, pp. 7-15, 2001, doi: 10.1177/000348940111000102.

[47] L. P. Bron *et al.* , "Treatment of early stage squamous-cell carcinoma of the glottic larynx: endoscopic surgery or cricohyoidoepiglottopexy versus radiotherapy," *Head Neck*, vol. 23, no. 10, pp. 823-829, 2001, doi: 10.1002/HED.1120.

[48] A. Zouhair *et al.* , "Decreased local control following radiation therapy alone in early-stage glottic carcinoma with anterior commissure extension," *Strahlenther. Onkol.* , vol. 180, no. 2, pp. 84-90, Feb. 2004, doi: 10.1007/S00066-004-1164-Y.

[49] N. Agrawal and P. K. Ha, "Management of early-stage laryngeal cancer," *Otolaryngol. Clin. North Am.* , vol. 41, no. 4, pp. 757-769, Aug. 2008, doi: 10.1016/J.OTC.2008.01.014.

[50] G. P. Ledda, N. Grover, V. Pundir, E. Masala, and R. Puxeddu, "Functional Outcomes after CO2 Laser Treatment of Early Glottic Carcinoma," 2006, doi: 10.1097/01.MLG.0000217557.45491.BD.

[51] R. R. Casiano, J. D. Cooper, D. S. Lundy, and J. R. Chandler, "Laser cordectomy for T1 glottic carcinoma: a 10-year experience and videostroboscopic findings," *Otolaryngol. Head. Neck Surg.* , vol. 104, no. 6, pp. 831-837, 1991, doi: 10.1177/019459989110400611.

[52] W. F. Mcguirt *et al.* , "Comparative voice results after laser resection or irradiation of T1 vocal cord carcinoma," *Arch. Otolaryngol. Head. Neck Surg.* , vol. 120, no. 9, pp. 951-955, 1994, doi: 10.1001/ARCHOTOL.1994.01880330035007.

[53] S. M. Cohen, C. G. Garrett, W. D. Dupont, R. H. Ossoff, and M. S. Courey, "Voice-related quality of life in T1 glottic cancer: irradiation versus endoscopic excision," *Ann. Otol. Rhinol. Laryngol.* , vol. 115, no. 8, pp. 581-586, 2006, doi: 10.1177/000348940611500803.

[54] C. D. L. Van Gogh *et al.* , "The efficacy of voice therapy in patients after treatment for early glottic carcinoma," *Cancer*, vol. 106, no. 1, pp. 95-105, Jan. 2006, doi: 10.1002/CNCR.21578.

[55] I. Honocodeevar-Boltežar and M. Žargi, "Voice quality after radiation therapy for early glottic cancer," *Arch. Otolaryngol. Head. Neck Surg.* , vol. 126, no. 9, pp. 1097-1100, 2000, doi: 10.1001/ARCHOTOL.126.9.1097.

[56] C. Y. Yang, P. E. Andersen, E. C. Everts, and J. I. Cohen, "Nodal disease in purely glottic carcinoma: is elective neck treatment worthwhile?," *Laryngoscope*, vol. 108, no. 7, pp. 1006-1008, Jul. 1998, doi: 10.1097/00005537-199807000-00010.

[57] W. M. Mendenhall, J. T. Parsons, T. A. Brant, S. P. Stringer, N. J. Cassisi, and R. R. Million, "Is elective neck treatment indicated for T2N0 squamous cell carcinoma of the glottic larynx?," *Radiother. Oncol.* , vol. 14, no. 3, pp. 199-202, 1989, doi: 10.1016/0167-8140(89)90167-9.

CHAPTER IV

Vaccıne-Induced Immune Thrombotic Thrombocytopenia (Vitt): A Novel Syndrome After Covid-19

İbrahim Ethem PINAR, M.D.[1]

[1] Isparta City Hospital, Department of Hematology, Isparta, Turkey. dr.ethem@hotmail.com, Orcid ID: 0000-0001-9907-1498

ABSTRACT

Coronavirus-2019 disease (also known as “COVID-19”) is still disrupting economic and social activities and has significant morbidity and mortality in many countries worldwide. Many vaccines, employing unique technologies, have been quickly developed against COVID-19. Interestingly, after vaccination against COVID-19, thrombosis of atypical fields has emerged as a rare but generally alarming event accompanied by additional thrombocytopenia in these cases. This article will shed light on the pathophysiology of vaccine-induced immune thrombotic thrombocytopenia (VITT), a novel and infrequent syndrome, and underline the role of interdisciplinary efforts in its treatment.

INTRODUCTION

Coronavirus disease-2019 (also known as “COVID-19”) is a pandemic that spread worldwide, caused by severe acute respiratory syndrome coronavirus 2 (also known as “SARS-CoV-2”), seriously threatening human health and causing significant morbidity and mortality. World Health Organization (WHO) has reported that as of December 8, 2022, more than 642 million people had been diagnosed with COVID-19, and more than 6 million persons had died from the disease worldwide.[1] A breakthrough in treatment has been achieved with the launch of COVID-19 vaccines just nine months after Europe faced its first SARS-CoV-2 infection.[2] The most practical way to limit and curb this epidemic was to provide vaccines to populations. In order to tackle the pandemic, European Medicines Agency (EMA) has issued a conditional marketing authorization for developed vaccines and also has guaranteed continuous and rigorous safety monitoring.[3] After vaccinating more than 400 million persons around the world, no specific safety concerns had been observed, and the incidence of significant adverse events remained extremely low.[4] While a new clinical syndrome, characterized by thrombocytopenia and thrombosis in atypical fields, especially in the cerebral venous sinus and

splanchnic vein, has been rarely observed following the administration of ChAdOx1 nCoV-19 vaccine (Vaxzevria, AstraZeneca),[5] a similar clinical situation has also been reported later with Ad26.COV2.S vaccine (Jcovden, Janssen).[6]

This article aims to focus on the etiological, epidemiological, and pathophysiological issues related to COVID-19 vaccine-induced VITT according to the latest data and to explain the clinical characteristics, diagnosis, and management of VITT.

ETIOLOGY

Rare cases of thrombotic events in life-threatening areas have been observed in combination with a decrease in platelet count following the administration of ChAdOx1 nCoV-19 vaccine.[7, 8] While VITT has been observed in individuals, who had received ChAdOx1 nCoV-19 vaccine, almost no cases of VITT have been seen in individuals, who had received BNT162b2 or mRNA-1273. This suggests that this new syndrome is likely related to the ChAdOx1 nCoV-19 vaccine.[9]

There is currently no evidence of a post-vaccination increase in the risk of developing VITT in patients with a prior history of thrombotic events or with risk factors for the development of a thrombotic event.

Interestingly, it still needs to be fully understood why this immune event-related thrombosis occurs preferentially in cerebral vessels.[10]

EPIDEMIOLOGY

The incidence of VITT is quite rare and has been reported as 1/125,000-1,000,000 in vaccinated cases. More than half of the cases are women, and the age of incidence is between 20-55 years. In any case, the number of reported vaccine-associated VITT cases is also constantly changing.

Considering the association of COVID-19 with increased mortality, EMA has concluded that the clinical benefits of COVID-19 vaccine far outweighed the risk of adverse effects.[11]

PATHOGENESIS

VITT's pathogenesis and pathogenic factors are still unclear, but heparin/PF4 antibodies may be involved in thrombosis.

In a small number of cases, who are exposed to heparin, PF4 antibodies, also known as heparin/PF4 antibodies, may induce thrombotic thrombocytopenia, also called as heparin-induced thrombocytopenia (HIT). This clinical condition suggests that VITT is similar to severe HIT. HIT is a well-understood thrombotic disorder, caused by the recognition of platelet-activating antibodies of a multi-molecular complex, consisting of cationic PF4 and anionic heparin. However, it is interesting that, unlike conventional HIT, VITT patients had had no prior exposure to heparin.

Antibodies to PF4-heparin are more common and initially detected in 6.6% of blood bank donors.[12] However, central sinus vein thrombosis or splanchnic vein thrombosis is rare. These findings suggest that our knowledge on the pathophysiology of VITT has to be revised, as PF4 antibodies may be present before vaccination. Therefore, a causal relationship between PF4 antibodies and VITT is yet to be established. Future studies will also help to provide a direct demonstration that PF4 antibodies could induce thrombus and thrombocytopenia in in-vivo models.

Antibodies against PF4 have also been defined in some severe COVID-19 cases, who had no prior heparin exposure.[13] Therefore, it may be suggested that SARS-CoV-2 might be triggering the production of antibodies against PF4. Furthermore, the administration of ChAdOx1 nCov-19 vaccine causes the

synthesis of particular proteins against SARS-CoV-2. These may be playing an essential role in producing PF4 antibodies through molecular mimicry.[14]

Adenoviruses may cause platelet activation, and adenovirus vectors may also trigger PF4 release, leading to multicellular activation, intense thrombin production, platelet depletion, and thrombus formation.[15, 16]

Finally, the inflammatory response after vaccination may also increase endothelial cell adhesion and tissue factor release, thereby triggering the coagulation pathway, and leading to thrombus formation.

CLINICAL SIGNS AND LABORATORY TESTS

The time from vaccine administration to symptoms associated with VITT is approximately 4-30 days. With the onset of symptoms after the 4th day of vaccination, extreme caution should be exercised, especially if symptoms persist.

Patients with VITT principally present with thrombosis at one or more atypical sites, including cerebral venous sinus thrombosis and splanchnic vein thrombosis. In addition, thromboembolism in the arterial area and signs of thrombotic microangiopathy may also be detected.[17] Symptoms include severe headache, and visual disturbances such as blurred vision. It may be accompanied by persistent abdominal discomfort as well as nausea and vomiting. In addition, shortness of breath can be detected with back and chest pain. Painful or swollen legs, easy bruising, and petechiae may be detected, as well as bleeding that may require immediate medical attention. Patients may also evolve symptoms of disseminated intravascular coagulation and widespread bleeding. Cases with established cerebral venous sinus thrombosis may be complicated by rapidly progressive and often fatal intracranial hemorrhage.[18]

Imaging studies, suggesting thrombosis, which may be complicated by bleeding, focus on cerebral venous sinus thrombosis, splanchnic venous thrombosis, pulmonary artery embolism, and lower extremity venous thrombosis.

Blood routine and peripheral smear indicate thrombocytopenia. Plasma D-dimer is elevated in most patients, fibrinogen is decreased in some patients, and C-reactive protein levels are often significantly elevated.

Antibodies against PF4, detectable by enzyme-linked immunosorbent assay (ELISA), are positive at high levels in nearly all patients in whom this syndrome has been reported.

DIAGNOSIS

American Society of Hematology defines VITT as a clinical syndrome that occurs in individuals within days 4-42 of vaccination and is characterized by all of the following features:

- The development of any arterial or venous thrombosis, more frequently in unusual fields (especially in the cerebral venous sinus and splanchnic venous thrombosis).
- Thrombocytopenia (platelet count $<150 \times 10^9$/L). However, in the early stages of this clinical syndrome, a period in which the platelet count is within the normal range may be observed.
- Antibodies against PF4, as detected by the ELISA test.
- Remarkably high D-dimer (>4 times ULN).

TREATMENT AND MANAGEMENT

Although there are no studies showing that the use of heparin exacerbates thrombosis, hematologists tend to avoid heparin anticoagulation in patients diagnosed with VITT. Therefore, it is recommended to use non-heparin

anticoagulant drugs for anticoagulant treatment in cases diagnosed with VITT. Bivalirudin and argatroban, which are direct thrombin inhibitors, and fondaparinux, apixaban, or rivaroxaban may be used. Patients with thrombosis should receive anticoagulant therapy for at least three months, as in the traditional treatment of venous thromboembolism.

High-dose intravenous immunoglobulin (IVIG) should be administered as soon as possible at a dose of 1 g/kg per day for two days. High-dose IVIG can inhibit FcγR-mediated platelet activation.[19] If patients have severe thrombocytopenia and thrombosis, the use of high-dose IVIG may cause a rapid elevation in the platelet count, thus contributing to the safety of anticoagulant therapy. IVIG can be given urgently to the patients with severe hemorrhage or the patients, requiring surgery. The effect of high-dose IVIG on the immune response to SARS-CoV-2 vaccines is not understood perfectly. However, IVIG, produced from the plasma obtained during the COVID-19 pandemic, might contain neutralizing antibodies to the antigens of the SARS-CoV-2 vaccine, thereby reducing the effectiveness of the vaccine.[20] Therefore, it may be recommended to use IVIG prepared from plasma obtained before the COVID-19 pandemic to prevent vaccine failure. If access to IVIG is not possible, plasma exchange may also be an option.

Platelet transfusion in patients diagnosed with HIT may increase mortality. Platelet replacement should not be performed in patients diagnosed with VITT because of its clinical similarity to HIT. Fibrinogen replacement therapy can be given in patients with bleeding or hypofibrinogenemia, and the fibrinogen count should be increased to 1.5 g/L.

Bruton tyrosine kinase (BTK) inhibitors (such as ibrutinib) are drugs used in the treatment of B-cell lymphoma, that can act on multiple downstream pathways, activated by FcγRIIA with various targets. Studies have shown that

BTK Inhibitors can effectively inhibit FcγRIIA through cross-linking. These may be considered as another plausible treatment option for VITT.[21]

Aspirin should be avoided for treatment and prophylaxis in patients with a diagnosis of VITT. Aspirin is not effective in the inhibition of platelet activation by PF4 antibodies and may improve the risk of hemorrhage in patients with VITT.

If thrombosis in an uncommon site and a decrease in platelet count occurs within the post-vaccination time window, ELISA results for PF4 antibodies should not be expected. Anticoagulation with non-heparin anticoagulants and treatment with high-dose IVIG should be initiated immediately.

CONCLUSION

Despite the risk of thrombosis in vaccination, it is necessary to emphasize the favorable cost-benefit ratio against SARS-CoV-2. However, awareness of VITT should be increased among vaccinated individuals and medical personnel.

Although VITT is rare, it progresses rapidly, is related to a high mortality rate, and requires comprehensive medical attention. Its pathogenesis and methods for monitoring the same require more research.

REFERENCES

1. (WHO), W.H.O. *Coronavirus disease (COVID-19)*. [cited 2022 December 8]; Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
2. Rawat, K., P. Kumari, and L. Saha, *COVID-19 vaccine: A recent update in pipeline vaccines, their design and development strategies.* Eur J Pharmacol, 2021. **892**: p. 173751.
3. (EMA), E.M.A. *Conditional marketing authorisation*. [cited 2022 December 8]; Available from: https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/conditional-marketing-authorisation.
4. Cines, D.B. and J.B. Bussel, *SARS-CoV-2 Vaccine-Induced Immune Thrombotic Thrombocytopenia.* N Engl J Med, 2021. **384**(23): p. 2254-2256.
5. Greinacher, A., et al., *Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination.* N Engl J Med, 2021. **384**(22): p. 2092-2101.
6. Muir, K.L., et al., *Thrombotic Thrombocytopenia after Ad26.COV2.S Vaccination.* N Engl J Med, 2021. **384**(20): p. 1964-1965.
7. Schultz, N.H., et al., *Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination.* New England Journal of Medicine, 2021. **384**(22): p. 2124-2130.
8. Scully, M., et al., *Pathologic Antibodies to Platelet Factor 4 after ChAdOx1 nCoV-19 Vaccination.* New England Journal of Medicine, 2021. **384**(23): p. 2202-2211.
9. Hunter, P.R., *Thrombosis after covid-19 vaccination.* BMJ, 2021: p. n958.
10. Oldenburg, J., et al., *Diagnosis and Management of Vaccine-Related Thrombosis following AstraZeneca COVID-19 Vaccination: Guidance Statement from the GTH.* Hämostaseologie, 2021. **41**(03): p. 184-189.
11. (EMA), E.M.A. *AstraZeneca's COVID-19 vaccine: EMA finds possible link to very rare cases of unusual blood clots with low blood platelets*. [cited 2022 December 11]; Available from: https://www.ema.europa.eu/en/news/astrazenecas-covid-19-vaccine-ema-finds-possible-link-very-rare-cases-unusual-blood-clots-low-blood#:~:text=Cases%20of%20unusual%20blood%20clots%20with%20low%

20platelets%20have%20occurred,help%20recovery%20and%20avoid%20complications.

12. Hursting, M.J., et al., *Platelet factor 4/heparin antibodies in blood bank donors.* Am J Clin Pathol, 2010. **134**(5): p. 774-80.
13. Riker, R.R., et al., *Heparin-induced thrombocytopenia with thrombosis in COVID-19 adult respiratory distress syndrome.* Research and Practice in Thrombosis and Haemostasis, 2020. **4**(5): p. 936-941.
14. Dotan, A. and Y. Shoenfeld, *Perspectives on vaccine induced thrombotic thrombocytopenia.* J Autoimmun, 2021. **121**: p. 102663.
15. Stone, D., et al., *Adenovirus-Platelet Interaction in Blood Causes Virus Sequestration to the Reticuloendothelial System of the Liver.* Journal of Virology, 2007. **81**(9): p. 4866-4871.
16. Othman, M., et al., *Adenovirus-induced thrombocytopenia: the role of von Willebrand factor and P-selectin in mediating accelerated platelet clearance.* Blood, 2007. **109**(7): p. 2832-9.
17. Tiede, A., et al., *Prothrombotic immune thrombocytopenia after COVID-19 vaccination.* Blood, 2021. **138**(4): p. 350-353.
18. See, I., et al., *US Case Reports of Cerebral Venous Sinus Thrombosis With Thrombocytopenia After Ad26.COV2.S Vaccination, March 2 to April 21, 2021.* JAMA, 2021. **325**(24): p. 2448.
19. Greinacher, A., et al., *Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination.* New England Journal of Medicine, 2021. **384**(22): p. 2092-2101.
20. Karnam, A., et al., *Vaccine-induced immune thrombotic thrombocytopenia: Consider IVIG batch in the treatment.* Journal of Thrombosis and Haemostasis, 2021. **19**(7): p. 1838-1839.
21. Von Hundelshausen, P., et al., *Vaccine-Induced Immune Thrombotic Thrombocytopenia (VITT): Targeting Pathomechanisms with Bruton Tyrosine Kinase Inhibitors.* Thrombosis and Haemostasis, 2021. **121**(11): p. 1395-1399.

CHAPTER V

Herpes Zoster Treatment And Pain Management

Parvin PINAR, M.D.[1]

[1] Süleyman Demirel University, Faculty of Medicine, Division of Algology, Isparta, Turkey. pervinyusifova@hotmail.com, Orcid ID: 0000-0003-0324-656X

HERPES ZOSTER – COMMON CLINICAL PICTURE

Herpes zoster (HZ) results from a locally limited, dermatome-related re-activation of a latent infection with *Varicella zoster* virus (VZV). VZV are epidermal and neurotropic DNA (deoxyribonucleic acid) viruses from the group of human herpes viruses. The primary infection usually occurs as an airborne droplet infection mainly of the skin, mostly in childhood, leading to generalized symptoms (varicella, chickenpox).[1, 2] Infection of the epithelium of the upper respiratory tract and T cells of the Waldeyer ring, leads to viremia and consequently, to a disseminated infection of cutaneous epithelial cells. Viral reproduction leads to the classic clinical appearance of the Heubner star map with disseminated skin lesions at various stages. Virus particles are also transported retrogradely from the skin into the neuronal ganglia via sensory nerves. Although the primary infection generally yields persistent immunity, VZV is not completely eliminated from the organism, but remains as a latent infection in the immune system, in hard-to-reach sensory parts of the cranial nerve nuclei and in the dorsal ganglia of the spinal cord.[3]

After years of latent virus persistence in sensory ganglia, reactivation may occur in one or more segments as a result of decreased immune function (e.g., immune aging, iatrogenic or acquired immunodeficiency), but often without any apparent external reasons. Viral particles are subsequently transported along the axons of the sensory nerves, located anterograde to the skin, and viral replication occurs once again in the cutaneous epithelia, albeit only in the affected sensory segment (dermatome). Due to temporally synchronous events, the skin changes of HZ appear in a relatively monomorphic sequence in contrast to the Heubner star map of the Varicella. With an incidence of 6.77 episodes / 1000 inhabitants per year in the general population in Europe, HZ is a common disease.[4] Epidemiological data show a clear connection between the incidence of HZ with the age and gender: while the annual incidences for

persons between 50 and 54 years of age are 3.4 (men) and 5.5 (women) per 1000 persons, for persons over 80 years of age, these figures are 9.9 (men) and 10.7 (women).[4] The incidence of HZ in immunocompromised population, comprising HIV ("human immunodeficiency virus")-positive persons,[5] organ transplant recipients,[6] and persons, who receive immunosuppressive therapies for other reasons,[7] is significantly higher. In patients under treatment for psoriatic arthritis or atopic eczema using Janus kinase (JAK) inhibitors, such as tofacitinib, baricitinib or upadacitinib, HZ is frequently observed as an adverse event.[8, 9] However, the JAK inhibitor therapy is generally resumed without further HZ complications, once HZ is treated.[10] There is also an increased risk of HZ associated with systemic corticosteroids and combinations of biologics with traditional antirheumatic systemic therapies.[8]

There are currently two vaccines in Europe approved for the prevention of herpes zoster: the live attenuated virus vaccine (Zostavax®, MSD Sharp & Dohme GmbH, Munich, Germany) and the adjuvanted, inactivated virus vaccine (Shingrix®, GlaxoSmithKline Biologicals S.A., Rixensart, Belgium), which has been available since 2018. The latter is based on a recombinantly produced subunit of VZV (glycoprotein E, herpes zoster subunit) and a liposome-based adjuvant (AS01B). The vaccination is intended to stimulate the T-cell-dependent specific immune response, which is intended to prevent reactivation of the latent VZV infection and thus the onset of HZ.

Since 2018, the Standing Committee on Vaccination (STIKO) has been recommending the use of the inactivated HZ subunit vaccine (Shingrix®) as a standard vaccination for all persons over the age of 60.[3, 11] In addition, vaccination – also using the inactivated vaccine – is recommended as an indication vaccination for persons over 50 years of age with an immunocompromising underlying disease or therapy (Table 1).

Table 1. Recommendations of the Standing Committee on Vaccination (STIKO)

Indications		**Scheme**
1. General use as standard	Persons >60 years of age	Two doses with adjuvanted inactivated HZ subunit vaccine (Shingrix®) at intervals of at least 2 and at most 6 months
2. Special use	People over 50 years of age with an increased health risk: • Diabetes mellitus • Chronic inflammatory bowel disease • Chronic obstructive pulmonary disease or bronchial asthma • Chronic renal dysfunction • Congenital or acquired immunodeficiency or immunosuppression • HIV infection • Rheumatoid arthritis • Systemic lupus erythematosus	
HZ: Herpes zoster		

The vaccination is administered twice at intervals of 2 to 6 months. Due to almost complete infection of persons over 50 years of age, who had grown up in Germany, it can be avoided in persons, who had a previous varicella disease, as confirmed by anamnestic and serologic history. Persons, who are about to receive a planned immunosuppressive therapy and who are seronegative for VZV constitute the sole exception to this and may receive varicella vaccination instead of zoster vaccination.[3]

A systematic review has shown that that the risk of HZ after vaccination with inactivated vaccine is reduced by approximately %92 in immunocompetent adults of 60 years of age and over.[12] Compared to placebo, vaccination has not caused more frequent severe side effects or autoimmune diseases, but has caused both local side effects (very common: redness, swelling, pain) and systemic flu-like side effects (very common: headache, myalgia, chills, fever, nausea, vomiting, diarrhea).[12, 13]

High efficacy and good safety profile of the inactivated vaccine has also been shown in immunocompromised populations.[14]

On the other hand, the live vaccine is no longer recommended due to its lower and shorter-lasting efficacy, especially in older people.[3]

USUAL VISUAL DIAGNOSIS

The diagnosis of HZ is usually made on the basis of the clinical picture, consisting of unilateral, dermatome-related, herpetiform-grouped vesicles with surrounding erythema and partly polycyclic erosions. Usually, only one dermatome is affected, but several, usually contiguous dermatomes may also be involved. In principle, any dermatome may be affected; the most common location is the thoracic dermatomes, followed by the trigeminal region.

Patients often go through a prodromal phase with general symptoms and abnormal sensations or pain in the affected dermatome even before the appearance of skin changes. The following are dermatome-related skin changes in typical order: first erythematous blemishes appear, on which grouped vesicles form (“grouped vesicles on an erythematous base”). These become increasingly pustular, eroding over time and serous or hemorrhagic crusts develop. Accompanying regional lymphadenopathy can occur. Bacterial superinfections can also occur, especially in older patients or patients with pronounced erosive courses, which are noticeable with yellowish crusts and expansion of the erythema. If the course is uncomplicated, the HZ heals within 7 to 14 days.

If the clinical finding is typical, laboratory confirmation of the diagnosis is not necessary. In case of uncertainty, a lesional swab for VZV DNA PCR (polymerase chain reaction) can be obtained to confirm the diagnosis. VZV-specific antibodies are not conclusive for the diagnosis, since only the detection of a significant increase in Ig(immunoglobulin) G antibody titer allows a

confirmation and both IgM and IgA titers have low sensitivity. An exception is the suspicion of zoster sine herpete (HZ without skin changes), in which the detection of anti-VZV IgG, IgA and IgM antibodies in the serum is sometimes the only diagnostic option. If zoster sine herpete with accompanying facial paralysis is suspected a nasopharynx swab must be obtained to detect VZV DNA.

In any case, when HZ is diagnosed, an examination for indications of complicated courses should be carried out. These include hemorrhagic/necrotizing lesions, aberrant vesicles outside of the affected dermatome, multisegmental or disseminated cutaneous involvement (Zoster generalisatus), as well as meningism, dyspnea and impairment of the general condition as evidence of central nervous or visceral involvement.

If zoster ophthalmicus or oticus is present, ophthalmology or ENT (ear, nose, and throat) specialties must be involved in the diagnosis and therapy. If neurological involvement is suspected (e.g., facial paralysis of Ramsay-Hunt syndrome or meningism), neurology specialty must be involved. In case of neurological symptoms that go beyond the involvement of cranial nerves VII and VIII or in case of clouding of consciousness, an emergency cMRI (cranial magnetic resonance imaging) or cCT (cranial computed tomography) and a lumbar puncture from the cerebrospinal fluid are usually indicated for the detection of VZV DNA.

HZ is an indicator disease for HIV.[15] HIV serology tests should be performed for all HZ patients under the age of 50.[16] HIV diagnostic tests should also be performed for all HZ patients, bearing risk factors for HIV infection (e.g., men engaged in same-sex relationships; i.v. drug users; migrants from regions with high a prevalence of HIV) and all patients regardless of age with atypical manifestations (e.g., multiple dermatomes, necrotizing lesions, prolonged or recurrent course). From a healthcare economy perspective, general HIV testing

is considered cost-effective for all HZ patients and is recommended in the European guidelines on HIV diagnostics.[17]

ACUTE ANTIVIRAL TREATMENT

In immunocompetent individuals, HZ is mostly self-limiting in non-critical localizations. Antiviral treatment is given with the goal of reducing the duration and intensity of cutaneous symptoms and associated pain, thereby improving quality of life. One of the most important goals of treatment is also to reduce the risk of postherpetic neuralgia (PHN), the most common complication of HZ. In addition, particularly in elderly and/or immunodeficient persons, with craniocervical involvement and/or indications of complicated courses, the rate of complications may be reduced.

ONE OF THE MOST IMPORTANT GOALS OF TREATMENT IS TO REDUCE THE RISK OF PHN

There are four nucleoside analogues available for antiviral treatment (acyclovir, valacyclovir, famciclovir, and brivudine). An overview of the route of administration, dosage and frequency of administration can be found in table 2. There are several placebo- and active-controlled, partly randomized studies on antiviral treatment for HZ. These show heterogeneous results in terms of reducing the intensity and duration of cutaneous symptoms and pain. The indications for superiority of brivudine, famciclovir, and valaciclovir over orally administered acyclovir are vague in terms of efficacy, but these have the advantage of a lower administration frequency over oral aciclovir. Only acyclovir is available for parenteral administration.

In contrast to the other therapeutic agents, it is not necessary to adjust the dose of brivudin according to renal function. In patients with renal dysfunction, brivudine is recommended when systemic oral antiviral therapy is indicated. It should be noted that brivudine must not be prescribed if preparations,

containing 5-FU (5-fluorouracil), have been administered systemically or topically within the last four weeks, due to the risk of fatal interactions.

Table 2. Antiviral agents[16]

Agent	Dose	Frequency of use	Duration
Acyclovir p.o.	800 mg[a]	5*1	7 days
Acyclovir i.v.	5–10 mg/kg[a]	3*1	7-10 days
Brivudine p.o.	125 mg[b]	1*1	7 days
Famciclovir p.o.	500 mg[a]	3*1	7-10 days
Valacyclovir p.o.	1000 mg[a]	3*1	7 days
[a]Adjustment according to creatinine clearance required [b]Contraindicated when taking drugs containing 5-FU (5 fluorouracil) or their prodrugs (e.g., flucytosine, capecitabine)			

The indication for initiation of antiviral therapy is based on patient age, location and spread/clinical manifestation as well as comorbidities:[16] while systemic antiviral treatment can be considered for patients under the age of 50 with monosegmental zoster of the extremities or trunk without indications of complicated courses (open recommendation), it should be considered for patients over 50 with craniocervical localization of HZ or symptoms of or an increased risk of complicated courses (strong recommendation). In the case of an increased risk or clinical indications of a complicated course, as well as in immunodeficient patients, treatment with intravenous acyclovir should be initiated. Table 3 presents the recommendations for the implementation and type of systemic antiviral therapy.

If indicated, systemic antiviral therapy should be initiated as early as possible, preferably within 72 hours of the onset of symptoms. According to the guideline,[16] treatment should be maintained as long as new vesicles develop and also if HZ is ophthalmicus or oticus, in immunodeficient patients or if there is clinical evidence of spread (cutaneous, neurological or visceral).

If specific localizations are affected, the treatment should be adapted together with the relevant specialist areas. For example, in the case of acute retinal

necrosis in the context of ophthalmic HZ, prolonged antiviral treatment is recommended. In addition, in Ramsay-Hunt syndrome or HZ oticus with pronounced pain or paralysis of multiple cranial nerves, systemic corticosteroids may also be used.

Table 3. Recommendations[16]

Groups	Recommendation	Medication
1. Met all of the following criteria:	Systemic antiviral therapy may be considered.	Selection of the appropriate medication (acyclovir, brivudine, famciclovir or valacyclovir) taking into account the modalities of administration, contraindications, comorbidities, co-medications, and healthcare-economic aspects
< 50 years of age		
Monosegmental HZ of the extremities or trunk		
No indication of a complicated course		
2. Meets at least one of the following criteria:	Systemic antiviral therapy should be administered.	
≥ 50 years of age		
Head/neck affected		
Moderate to severe pain		
Predisposing skin disorders (e.g., atopic dermatitis)		
Children and adolescents on long-term therapy with topical steroids		
3. Meets at least one of the following criteria:	Systemic antiviral therapy should be administered with intravenously administered acyclovir.	Acyclovir i.v., 5–10 mg/kg 3 times a day (if necessary, adjusted according to renal function), 7 to 10 days
Immunocompromised patients		
Head and neck affected, especially in older patients		
Multi-segmental involvement		
Mucocutaneous infestation		
Hemorrhagic/necrotic lesions		
Aberrant vesicles/satellite lesions		
Generalized HZ (Zoster generalisatus)		
Signs of visceral or central nervous (including vasculitis) involvement		

LOCAL THERAPEUTIC MEASURES

Local antiviral therapies (e.g., acyclovir ointment) are not recommended for cutaneous HZ; instead, a stage-adapted topical therapy that promotes healing, prevents bacterial superinfections and causes subjective relief, should be given. There are no controlled studies on topical therapy for HZ, so recommendations

in this regard are primarily based on the opinion and experience of the guideline committee. In the case of weeping skin changes (blister stage), cooling, anti-inflammatory and/or antiseptic topicals can be helpful (e.g., compresses with NaCl 0.9%, polyhexanide or octenidine solution or black tea). In the crust stage, external agents with a crust-dissolving and/or wound-healing effect (e.g., hydrophilic polyhexanide gel), are recommended. Topical antiviral agents in addition to systemic acyclovir administration and topical corticosteroids, are only recommended in particular situations (e.g., acyclovir eye ointment five times daily for HZ ophthalmicus, additional topical corticosteroids for disciform keratitis, endotheliitis or anterior uveitis).

MANAGEMENT OF PAIN

Pain is a common side effect of HZ. Therefore, all patients should be questioned about the existence of pain and discomfort- for example, using the numerical or visual rating scale for recording pain intensity (0= no pain, 10= worst pain imaginable). Although no controlled studies are available on this issue, the treatment of acute zoster pain serves to reduce the risk of PHN in addition to the immediate pain relief and corresponding improvement in the health-related quality of life.

The treatment of acute pain is based on the WHO (World Health Organization) grading scheme for pain therapy. This includes:

- Use peripherally more effective analgesics (NSAIDs [Non-Steroidal antirheumatic drugs] or other non-opioid analgesics) at lower pain intensity,
- The addition of weakly effective opioids for moderate pain intensity in patients without adequate response to non-opioid analgesics and
- The combination of non-opioid analgesics with potent opioids with severe pain intensity in patients without response to pain therapy.

It is essential to differentiate nociceptive pain, which arises due to local cutaneous tissue damage, from neuropathic pain, which occurs due to neuroinflammation and/or damage to the sensory nerves. Indications of neuropathic pain are prodromal pain and severe, burning-drilling or shooting pain. Tactile allodynia and dysesthesia in the affected and adjacent dermatomes, may also be indicative.

In the case of neuropathic pain, supplementary pain therapy using anticonvulsant adjuvants (gabapentin or pregabalin) is recommended, even during the acute phase. Efficacy in acute and chronic neuropathic pain due to HZ, has been demonstrated in controlled clinical studies.[18, 19] Information on dosing and the most important aspects to be considered in treatment with pregabalin and gabapentin can be found in table 4. If adequate pain control of the neuropathic pain is not achieved within 2 to 4 weeks using the WHO step-by-step scheme and anticonvulsant adjuvant, the therapy should be modified; an antidepressant (e.g., amitriptyline, nortriptyline or desipramine) or local treatment with a capsaicin 8% patch may be additionally used after the healing of skin alterations.

RECOGNITION AND TREATMENT OF PHN

Pain in the affected dermatome, which persists for more than three months after the healing of cutaneous zoster lesions, is defined as PHN. About 10–15% of patients develop PHN after an episode of HZ, with the risk being strongly influenced by age:[20] Just under 7% of persons between 50 to 54 years of age develop PHN, compared to nearly 22% of patients between 80 to 84 years of age. In addition to age, prodromal pain, severe acute pain, pronounced skin changes and ophthalmic HZ are risk factors for PHN.[21]

The symptoms usually improve gradually over time, which may take months to years. Adequate pain therapy is essential to improve the quality of life of those

affected. In addition to the introduction or continuation of the above-mentioned adjuvants for the treatment of neuropathic pain (pregabalin, gabapentin, and possibly additional tricyclic antidepressants), local therapeutic measures such as capsaicin or local anesthetics may also be considered. Due to the complex etiology, in case of persistent pain, it is crucial to involve the pain medicine specialty as a specialized discipline.

Table 4. Supplementary therapy for zoster-associated pain with neuropathic pain component[16]

<table>
<tr><th>Initial dose</th><th>Increase</th><th>Maximum dose</th><th>Things to pay attention</th></tr>
<tr><td colspan="3">Pregabalin</td><td rowspan="4">Dose adjustment based on renal function.
Dizziness, lightheadedness, sleepiness and confusion can lead to a tendency to fall, especially in older patients.
Control of blood glucose in people with diabetes, adjustment of medication if necessary.
Control of the pancreatic enzymes during the dose increase.
May enhance the adverse effects of opioids (e.g., respiratory depression, increased sedation).
Discontinuation: Gradually over at least seven days to avoid withdrawal attacks.</td></tr>
<tr><td>25 mg twice daily p.o.*</td><td>Every 1 to 2 days in 25 mg increments, depending on tolerability *</td><td>300 mg twice daily</td></tr>
<tr><td colspan="3">Gabapentin</td></tr>
<tr><td>300 mg at night or 100 mg 3 times a day **</td><td>After 3 to 4 days, by 300 mg (total dose divided into three single doses per day)**</td><td>1200 mg 3 times a day</td></tr>
<tr><td colspan="4">*According to the product information, the initial daily dose is 150 mg; in 2 to 3 individual doses, the dose is increased after 3 to 7 days to 300 mg daily and further, if necessary, seven days to a maximum of 600 mg per day
**According to the product information, 300 mg once a day on day 1, 300 mg twice daily on day 2, and 300 mg 3 times daily from day 3. Further up-dosing in 300 mg increments every 2 to 3 days to a maximum of 3600 mg per day</td></tr>
</table>

REFERENCES

1. Cohen, J.I., *Herpes Zoster.* New England Journal of Medicine, 2013. **369**(3): p. 255-263.
2. Hayward, A.R. and M. Herberger, *Lymphocyte responses to varicella zoster virus in the elderly.* Journal of Clinical Immunology, 1987. **7**(2): p. 174-178.
3. Impfkommission, S., *Wissenschaftliche Begründung zur Empfehlung einer Impfung mit dem Herpes zoster-subunit-Totimpfstoff.* Epidemiologisches Bulletin, 2018(50): p. 541--567.
4. Curran, D., et al., *Meta-Regression of Herpes Zoster Incidence Worldwide.* Infectious Diseases and Therapy, 2022. **11**(1): p. 389-403.
5. Buchbinder, S.P., et al., *Herpes zoster and human immunodeficiency virus infection.* J Infect Dis, 1992. **166**(5): p. 1153-6.
6. Kwon, D.E., et al., *Incidence of herpes zoster in adult solid organ transplant recipients: A meta-analysis and comprehensive review.* Transpl Infect Dis, 2021. **23**(4): p. e13674.
7. Redeker, I., et al., *Risk of herpes zoster (shingles) in patients with rheumatoid arthritis under biologic, targeted synthetic and conventional synthetic DMARD treatment: data from the German RABBIT register.* Ann Rheum Dis, 2022. **81**(1): p. 41-47.
8. Baumrin, E., et al., *A systematic review of herpes zoster incidence and consensus recommendations on vaccination in adult patients on systemic therapy for psoriasis or psoriatic arthritis: From the Medical Board of the National Psoriasis Foundation.* J Am Acad Dermatol, 2019. **81**(1): p. 102-110.
9. Yun, H., et al., *Risk of Herpes Zoster in Autoimmune and Inflammatory Diseases: Implications for Vaccination.* Arthritis Rheumatol, 2016. **68**(9): p. 2328-37.

10.Choi, W., et al., *Safety of JAK inhibitor use in patients with rheumatoid arthritis who developed herpes zoster after receiving JAK inhibitors.* Clin Rheumatol, 2022. **41**(6): p. 1659-1663.

11.Impfkommission, S., *Empfehlungen der Ständigen Impfkommission (STIKO) beim Robert Koch-Institut 2021*. 2021(34): p. 3--63.

12.Gagliardi, A.M., et al., *Vaccines for preventing herpes zoster in older adults.* Cochrane Database Syst Rev, 2019. **2019**(11).

13.(GSK), G.G.C.K. *Fachinformation: Shingrix*. 2021 September [cited 2022 December, 28]; Available from: https://gskpro.com/content/dam/global/hcpportal/de_DE/produktinformationen/shingrix/FI_Shingrix_Pulver_+_Suspension_zur_Herstellung_einer_Injektionssuspension_092021.pdf.

14.Racine, E., et al., *A systematic literature review of the recombinant subunit herpes zoster vaccine use in immunocompromised 18-49 year old patients.* Vaccine, 2020. **38**(40): p. 6205-6214.

15.Gazzard, B., et al., *Indicator disease-guided testing for HIV--the next step for Europe?* HIV Med, 2008. **9 Suppl 2**: p. 34-40.

16.Gross, G.E., et al., *S2k-Leitlinie zur Diagnostik und Therapie des Zoster und der Postzosterneuralgie.* JDDG: Journal der Deutschen Dermatologischen Gesellschaft, 2020. **18**(1): p. 55-79.

17.Europe, H.i. *HIV indicator conditions: guidance for implementing HIV testing in adults in health care settings*. 2012 [cited 2022 December, 28]; Available from: https://www.eurotest.org/Portals/0/Documents/Guidance.pdf.pdf?ver=2014-01-29-113626-000.

18.Wang, S.L., et al., *The efficacy of pregabalin for acute pain control in herpetic neuralgia patients: A meta-analysis.* Medicine (Baltimore), 2017. **96**(51): p. e9167.

19. Huffman, C.L., et al., *Efficacy and Safety of Once-Daily Controlled-Release Pregabalin for the Treatment of Patients With Postherpetic Neuralgia: A Double-Blind, Enriched Enrollment Randomized Withdrawal, Placebo-Controlled Trial.* Clin J Pain, 2017. **33**(7): p. 569-578.
20. Hillebrand, K., et al., *Incidence of herpes zoster and its complications in Germany, 2005-2009.* J Infect, 2015. **70**(2): p. 178-86.
21. Forbes, H.J., et al., *A systematic review and meta-analysis of risk factors for postherpetic neuralgia.* Pain, 2016. **157**(1): p. 30-54.

CHAPTER VI

Clinical Use Of Mesenchymal Stem Cells In Humans

Res. Assist. Dr. Kemal YUCE[1]
Dr. Ahmet İsmail ÖZKAN[2]

[1] Department of Physiology, Faculty of Medicine, Selcuk University Konya, Turkiye. yuce2020@hotmail.com, ORCID ID: 0000-0001-8915-4900
[2] Department of Biology, Faculty of Science, Dicle University Diyarbakır, Turkiye. aiozkan.2015@gmail.com, ORCID ID: 0000-0002-4511-2386

INTRODUCTION

Stem cells can be multipotent stem cells, unipotent stem cells and pluripotent stem cells (PSCs), depend on their development potency. Stem cells have capability to repair/replace degenerative or damaged tissues and advance functional healing in clinical trials and experimental model (Q. W. Liu et al., 2021). Mesenchymal stem cells (MSC) have important potential for regenerative medicine because they have self-renewal and differentiation capability. Cell surface marker profiles, differentiations capacities, cell size, shape, granularity, telomere length, senescence status, trophic factor secretion (secretome), and immunomodulation, should be assessed to use MSC in regenerative medicine. MSC can be administrated as intravenously or by direct injection in tissue. When MSCs are heterogen. So the other names of MSC can be evaluated as mesenchymal stromal cells, mesenchymal progenitor cells, multipotent mesenchymal stromal cells, bone marrow stromal cells, bone marrow-derived MSC, multipotent stromal cells, mesenchymal precursor cells. (Debnath & Chelluri, 2019; Samsonraj et al., 2017).

MSC can be isolated from some kind of tissues including adult tissues (e.g. bone marrow (BM), peripheral blood, adipose tissue (AD), embryonic tissues, and fetal tissues (e.g. Wharton's jelly, amnion, umbilical cord blood (UCB), placenta and amniotic fluid). Adipose tissue derived mesenchymal stem cells are a kind of adipose tissue stem cells (ADSCs). MSC should have capability to adhere to plastic in medium(1), to be positive for CD73, CD105 and CD90 and to be negative for CD45, CD14, CD34, or CD79a, CD11b, and CD19 (2) to differentiate into the osteoblast, adiposit and chondrosit in vitro (3). ADSCs have less human leukocyte antigen class I antigen, mitochondrial energy production, transcriptomic heterogeneity and higher immunosuppression capacity, when compared with the BMSC population (Zhou et al., 2019). BM-MSC isolation have some problems, for example causing infection and pain. used MSCs in clinical trials are usually stem from AD and BM (Becerra-Bayona, Solarte, Alviar Rueda, Sossa, & Arango-Rodriguez, 2022). Because, they are accessible and acceptible as ethic (Zhou et al., 2019).But, HUCMSCs (Human umbilical cord mesenchymal stem cells) have some adventage like painless procedure and beter renewal capability. The problem in here, It can be problem to take ethic approve. MSCs also can be produced as induced

pluripotent stem cells (iPSCs). But, iPSCs aren`t choused because of tumor forming possibility (Ding, Chang, Shyu, & Lin, 2015) Human amniotic mesenchymal stem cells (hAMSCs) are pluripotent stem cells and have neovasculature, angiogenesis, immunomodulatory and immunosuppressive effects and decrease migration of microglia and the activity of inflammatory cells, and prevent mobilization of immune cells to injury place. hAMSCs decrease the oxidative stress backed by the increased antioxidant enzymes and prevent lipid peroxidation. Moreever, hAMSCs increase neurogenesis and neuroprotection through inhibition of inflammation and supporting of neurogenesis. hAMSCs can support the transcription of neurotrophic factors, which prevents death of neurons and increase development of function of neurons. Morever, they stimulate differentiation of progenitor cells to neurons (Abbasi-Kangevari, Ghamari, Safaeinejad, Bahrami, & Niknejad, 2019).But, permission for experiments can be difficult relatively. As result, isolation method, tissue source, and medium composition are significant for rgeneration capability in clinical aplication and isolated organs can be brain, liver, lung, kidney, muscle, thymus, pancreas, skin, bone marrow adipose tissue, fetal tissues, and umbilical cord (Lin et al., 2019; Mushahary, Spittler, Kasper, Weber, & Charwat, 2018).

MESENCHYMAL STEM CELL (MSC) APPLICATIONS

Amyotrophic Lateral Sclerosis (ALS)

ALS is a degenerative disease that has rapid progress. ALS attacks motor neurons (MNs) in the spinal cord, cortex, and brain stem, selectively. It causes death within 3 or 5 years after clinical symptoms (including the weakness of muscle, atrophy of muscle, spasticity, paralysis, and fasciculations) appear. Bone marrow-derived MSCs (BM-MSCs) may lead a new interference to prevent the ALS progression via supplying neurotrophic assistance to the host MNs and via possessing an anti-inflammatory effect. It has been stated that the BM-MSCs (approximately $15 \pm 4.5 \times 10^6$) were administered into the cerebrospinal fluid of patients via lumbar puncture. Those patients have been followed up for 6-months before administration and 18 months after administration. The patients (approximately 30%) have complained of a headache after intrathecal administration of the autologous BM-MSC. It has

also been noted the headache has been a type of headache (mild to moderate) as same as after a standard lumbar puncture. SUSAR (suspected unexpected serious adverse reaction) has not been monitored. After 3 months of the BM-MSC administration, it has been observed a decrease in ALS functional rating scale (ALSFRS) reduction whereas in some cases the decrease in ALSFRS reduction has been sustained for 6 months. The forced vital capacity (FVC) point has been reported to be steady or over 70% in approximately 80% of the patients during 9 months period. Weakness scales (WSs) point has been observed to be steady in 75% of patients after 3 months of following the administration. It has also been reported that intrathecal BM-MSCs administration in ALS patients is a reliable process by retarding disease progress (Syková et al., 2017). In another study, intrathecal autologous adipose-derived MSCs (AD-MSCs) were applied to ALS patients. Although it has been observed that the approach was reliable in the applied doses, the progression of the disease has remained to be continued. Radicular leg and temporary low back pains have been reported as the most observed adverse effects of the top dose level (Staff et al., 2016). In a different ALS study, it has been noted that posterior leg or low back pain corporated with increasing or thickening of lumbar nerve roots has been recorded at the 3 of 4 patients at the highest dose phase. Progression Rate (UMSARS total) has been expressed to be significantly lower against the control group due to the dose-dependent effect. In this study, autologous MSCs were applied intrathecally and it was concluded that this application was effective (Singer et al., 2019).

Multiple Sclerosis (MS)

MS is a chronic inflammatory disease characterized by leukocyte infiltration followed by the formation of sclerosing plaques in brain tissue, demyelinating inflammation and axonal damage. It affects mostly young adults via the central nervous system (CNS) disorder. Pro-inflammatory cytokines have been secreted by T-lymphocytes as a result of being activated. That secretion initiates the MS cascade which led to the neuro-inflammatory. Thus, activated T lymphocytes have an important function in MS pathogenesis. The inflammation occurred during MS by T-lymphocytes causes axonal damage and nerve cell degeneration. It has been noted by the recent studies that MSC has a potentially curative effect (including immune regulatory function and inducing T

regulatory-cell proliferation) on MS-like diseases. In an MSC therapy study, MSCs were administered to the patients and the FoxP3 (T Regulatory cells specific marker) value was measured. Six months after the intrathecal MSC administration, it has been noted that FoxP3 expression was importantly higher than the levels before administration. It has been stated that the significantly increased expression of FoxP3 was related to clinical steady (Cui, Chu, & Chen, 2020; Mohajeri, Farazmand, Mohyeddin Bonab, Nikbin, & Minagar, 2011). Allogeneic 20 x 10^6 umbilical cord MSC (UC-MSC) was administered seven times by intravenous infusion within 7 days for 1 year period. No important unwanted impacts have been reported by those twenty subjects. Headache and fatigue have been expressed as unwanted effects. Symptoms have been recovered after one month of administration. Quality of life has been increased by recoveries in the Expanded Disability Status Scale (EDSS) scores (bowel, sexual dysfunction, and bladder) and in non-dominant hand average scores (walk times and affirmative health progress). After 1 year of administration, inactivated lesions in the brain and cervical spinal cord in 83.3% (15 of 18) of subjects have been observed by MRI scans (Riordan et al., 2018). In another study on MS patients, a common improvement tendency was noticed in all tests, except significantly increased lesion volume. It has been noted that the EDSS was decreased (as 3.5 and 4 points) in two patients. It has also been figured out a connection among the decreased lesion quantity at baseline with the higher content of IL-8, VEGF MSC-CM (mesenchymal stromal cells conditioned media), and IL-6. As a result, it has been highlighted that the transplantation of autologous BM-MSCs and MSC-CM by injecting intrathecally into the patients was reliable, applicable and efficient (Dahbour et al., 2017). Although intrathecally administered autologous BM-MSC-derived neural progenitors (IT BM-MSC-NP) was quite safe and acceptable, it had also a few small adverse effects such as temporary fever and slight headaches that usually recoveries in less than 24 hours. Nevertheless, an EDSS situation was observed at an average level. The sub group of secondary progressive MS (SPMS) patients and ambulant subjects (EDSS $\leq$ 6.5) have showed constantly an affirmative tendency. Consequently, most of the subjects (50% and 70%) showed a healing in both strength of muscle and funtion of bladder after the intrathecally administered MSC-NP (Harris et al., 2018). In a different study, the MSCs transplantation has been administered to fifteen patients. As a result, it has been

observed that the mean value of the EDSS point improved from 6.7 (1.0) to 5.9 (1.6) (Karussis et al., 2010). It has been reported that the MSCs administered to patients tended to decrease mean GEL count (gadolinium-enhancing lesion) at six months period. It was not detected any important change at the secondary endpoints (Llufriu et al., 2014).

Spinal Cord Injury (SCI)

The trauma affecting the spinal cord initially makes the glia and neurons permeable and then initiates the chain of events that lead to cell death and spinal cord injury. The development of cystic cavities and glial wounds leads to difficult repairs. Spinal cord injuries have serious consequences affecting the social, physical, and occupational lives of patients. So, some animal models have been developed to find treatment ways (Ahuja et al., 2017). In a study in which autologous MSCs were applied to ten patients with SCI, all patients experienced different rates of recoveries in susceptibility and motor strength. 2/8 of male patients have been recovered from sexual function. 2/4 of patients with neuropathic ache have been fully recovered after administration. The complaint has been reduced in another patient. It has also been reported that all the bladder and bowel suffering patients have shown significantly recuperated in checks. Two of seven spasm patients have been recovered after administration. Three of nine spasticity patients have been recovered after the cell therapy. However mild rises compared with basal levels have been observed at values of neurotrophic factors (including brain-derived, glial-derived, and ciliary) and neurotrophins (both 3 and 4) those followed-up three treatment of MSCs, no statistically important variation has been monitored. It has been stated that subarachnoid administration of repeated MSCs doses was being well-tolerated method which led to improvements in the life quality of incomplete SCI patients (Vaquero et al., 2017). It has been reported that variable clinical progress (including motor strength, susceptibility, neuropathic ache, spasms, sexual function, spasticity, or sphincter dysfunction) has been observed in patients (to be free from the SCI injury degree or level, age, or duration). The MSC treatment has been administered successfully without any connected unwanted impacts. It has been stated that post micturition residue has been decreased and bladder compliance has been healed in most (66.6%) of the patients in urodynamic studies. It has been revealed in

neurophysiological studies that somatosensory or motor-evoked potentials healed in half (55.5%) of the patients and involuntary muscle contraction and infra-lesional active muscle reinnervation healed in 44.4% of the patients (Vaquero et al., 2018). It has been reported that the highest rate of urinary flow and bladder capacity have been increased in the UC-MSC adminstrated patients. The highest rate of detrusor pressure and urine residue volume decreased in the UC-MSC group. As a result, it has been stated that UC-MSC transplantation after spinal cord injury can effectively improve neurological functions (Cheng et al., 2014). AD-MSCs have been obtained from lipoaspirates of patient subcutaneous adipose tissues. And then the AD-MSCs have been applied (9×10^7 AD-MSCs/patient) intrathecally into the lumbar puncture. It has been stated that the American Spinal Injury Association (ASIA) motor scores healed in 5 patients at 8 months (1-2 degrees in some Myotomes). It has been seen that the healing of anal contraction in 2 patients. ASIA sensory score degeneration has been observed in 1 whereas recovery was in 10. It has been declared that intrathecal administration of AD-MSCs had no significant unwanted effects during 8 months of treatment. It has been noted that small progress was observed in the neurological function of a few patients (Hur et al., 2015). Healing in tactual susceptibility has been observed in all subjects. It has also been reported that motor function of lower limbs improved in 8 subjects, especially in flexors of hip. healing in the urological function was observed in nine subjects. SSEP (Basic somatosensory evoked potentials) changed in a subject after 3 and 6 months of mesenchymal stem cell transplantation. Consequently, autologous MSCs intralesional administration is reliable and applicable for patients with chronic and complete SCI and may support the neurological healings of patients (Mendonça et al., 2014). It has also been observed that scores in BM-MNC and the control groups were lower than the scores of BM-MSC (A, B dimensions of GMFM and A, B, C, D, and E dimensions of FMFM) group after 6 months of the administration. It has been reported that scores in BM-MNC and the control groups were lower than the scores of BM-MSC (A, B, and C dimensions of GMFM and A, B, C, D, and E dimensions of FMFM) group after 12 months of intrathecal cell injections (X. Liu et al., 2017). In a study using neural stem cell-like (NSC-like) cells derived from autologous BM-MSC, it has been noted that the baseline scores were lower than the GMFM scores in the cell implantation group after the 3rd and

6th months of intraspinal infusion. The GMFM scores of the control group have not been importantly increased statistically. Any serious unwanted impacts or difficulties have not been reported in patients. Consequently, it has been shown that NSC-like cells were reliable and powerful therapy in CP originated defects (Chen et al., 2013).

Heart Failure (HF)

Cardiopoietic stem cell therapy (CP-SCT) via autologous MSC cocktail–based priming has been noted to be reliable and applicable treatment without any increase of cardiac or systemic toxicity in heart failure (HF) patients. It was observed that left ventricular ejection fraction (LV-EF) advanced with cells sent by endomyocardial injections. And the end-systolic volume of LV has been decreased with cell therapy. As a result, it has been stated that a clinical score of walking distance (6-minutes) and cardiac parameters (including life quality, physical performance, admission to hospital, and eventless life) improved by cell therapy (Bartunek et al., 2013). In another study, human MSCs (hMSCs) have been applied to the LV sites via transendocardially injection. Serious unwanted impacts have not been observed during the 30-day of administration. It has not been seen serious adverse effects (SAE) for 30 day treatment. It has been reported that the incidence of SAE after 12-month of administration observed in allogeneic-hMSCs (allo-hMSCs) as 28.2% and in autologous-hMSCs (auto-hMSCs) as 63.5%. the EF has been raised in allo-hMSC patients by %8.0 whereas the EF has been raised by %5.4 in auto-hMSCs. Although the 6-minute walk test increased about 37.0 meters in the allo-hMSCs but has stayed at 7.3 meters in the auto-hMSCs. It was found that The Minnesota Living with Heart Failure Questionnaire (MLHFQ) score declined in patients with auto-hMSC and allo-hMSC. Consequently, allo-hMSCs have been largely reduced by tumor necrosis factor-α (TNF-α) compared to auto-hMSCs at 6 months (Hare et al., 2017). It has been reported that administration of allogeneic Wharton's jelly–derived MSCs (WJ-MSCs) with intracoronary infusion healed the heart function and integrated easily into the ischemic cardiac tissues. The exact improvement of lived myocardial cells after an injury and perfusion in the infarct area (IA) after 4 months has been reported to be importantly larger in the WJ-MSC group than the placebo group. The exact improvement of LV-EF after 18 months has also been stated to be importantly larger in the WJ-MSC

group than the placebo group. The exact reductions in volumes of LV end-systolic and end-diastolic after 18 months have been noted to be importantly larger in the WJ-MSC group than the placebo group (Gao et al., 2015). It has been observed that LV systolic function, mainly in anterior wall myocardial segments neighboring to the left anterior descending artery (LAD), and LV-EF improved by implantation of BM-MSC. In these studies, allogeneic or autologous stem cells were administered intracoronary or intravenously and it has been stated that no important adverse impacts about cardiovascular was observed by the administration of MSCs. The implantation of cells has not increased proarrhythmic effects and/or restenosis in-stent. (Bartolucci et al., 2017; Kim et al., 2018; J.-W. Lee et al., 2014).

Osteoarthritis (OA)

OA is a rheumatic disease associated with symptoms such as inflammatory pain, synovitis, joint swelling, and cartilage deterioration. Proinflammatory regulators such as neuropeptides, nitric oxide, cytokines, and prostaglandin E-2 are produced by the catabolic inflamed synovium, altering the balance of repair and degradation of cartilage, and leading to the excessive release of proteolytic enzymes that cause cartilage breakdown (Sellam & Berenbaum, 2010). It has been stated that the healing of intraarticularly autologous BM-MSC implanted patients revealed as reported by visual analog scale (VAS) as dose-dependent (groups of control, low-dose, and high-dose) for assessment and the median value (interquartile range) at 12 months. It has also been shown by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) that the BM-MSC treated the patients were excellent. After 12 months, the healing has been occured by high-dose of BM-MSC administration. Although the control group motion ranges have been kept to be steady, an improvement has been noted after 12 months of BM-MSC administration by the coherent with WOMAC and VAS values. It has been observed by the Whole-Organ Magnetic Resonance Imaging Score (WORMS) protocol that the only high-dose group of BM-MSC caused to decrease of joint damage (Lamo-Espinosa et al., 2016). In an intraarticular AD-MSC treatment study, it has been tested by two groups as either a single dose (100×10^6 AD-MSCs) or two doses (100×10^6 AD-MSCs at baseline and 6 months) of cells for administration. No important unwanted impacts have been reported. After 12 months of the

treatment period, clinically substantial ache healing and functional healing have been revealed for both administered AD-MSCs groups. Because of being a reliable and efficient treatment for knee osteoarthritis (OA), it has been noted that disease development might be prevented by intraarticular autologous AD-MSC administration (Freitag et al., 2019). In another study of OA, the symptomatic knee OA patients were treated with hyaluronic acid (HA) (baseline and 6 months), MSC-1 (UC-MSC single-dose at baseline), or MSC-2 (UC-MSC repeated doses at 6 months and baseline). It has been reported that the substantial ache and functional healing have been observed from the baseline in the intraarticular MSC-implanted group. According to the WOMAC-A (pain subscale), it has been stated that the MSC-2-implanted group had importantly lower ache levels than the HA group at 12 months. It has also been reported that the Pain Visual Analog Scale of the MSC-2 group was importantly lower than the HA group at 12 months (Matas et al., 2019). Although an important change by the control group has not been noted in the WOMAC score at 6 months, an important increasing WOMAC score has been reported by intraarticular AD-MSCs single implantation. It has been stated that both groups have no important unwanted impacts. It has been reported that the cartilage damage in the control group has increased at 6 months however no important change of cartilage defect has been reported in the MSC group by MRI. An effective functional progress and analgesic effect for patients of knee OA have been reported, with no serious adverse events at 6 months period, by intra-articularly implantation of autologous AD-MSCs (W. S. Lee, Kim, Kim, Kim, & Jin, 2019). In an another study on knee OA has been performed on four patients of different ages (54, 55, 57, and 65 years). Firstly, 30 ml of bone marrow has been taken and cultured for MSC growth. After a few weeks (4-5 weeks), autologous MSCs cells have been implanted as a single knee to each patient. It has been noted that the walking period to experience the pain increased for 3/4 of the patients. It has also been reported that the climbing number of stairs and the pain visual analog scale advanced for four of the patients (Davatchi, Abdollahi, Mohyeddin, Shahram, & Nikbin, 2011).

Chronic discogenic low back pain (CDLBP)

Intradiscal implantation of Autologous AD-MSCs has a cartilage differentiation capacity and immunomodulatory influence. Therefore, it has

been considered to have a significant curative ability on chronic discogenic low back pain (CDLBP). In a 1 year study, no major adverse impacts related to cells have been experienced by patients. Although VAS, ODI (the Oswestry Disability Index), and SF-36 (Short Form-36) scores of both low and high doses have been reported to be importantly progressed, no significant difference has been observed among the this two groups (Kumar et al., 2017). It has been noted that allogeneic BM-MSC implanted patients has statistically shown progress compared to baseline in all patients at 12 months. It has been reported that the defects have been exactly filled with regenerative cartilage tissue as reported by MRI and second-look arthroscopies results. Histological analysis revealed hyaline-like regenerations with a high concentration of proteoglycan and type II collagen. It has been stated that the potential of stem cell-induced paracrine mechanisms might had a key role in chondrogenesis and tissue regeneration (de Windt et al., 2017). Differentiation, maturation, and functions of MSCs have been reported to be affected by microRNAs (miRNAs). In such research, it has been stated that the VEGF-A and indoleamine 2,3-dioxygenase (IDO) were decreased in decidua-derived MSCs (dMSCs) of pre-eclampsia patients. Consequently, it has been noted that the miRNAs of dMSCs might have a regulatory role in pre-eclampsia development (Zhao et al., 2014).

Bone Therapy

Bone defects, which are caused by various skeletal disorders, are a clinically common problem. It has been stated that MSC-based treatment might also be an encouraging choice against bone defects. Stem cell Screen–enrich–combine (-biomaterials) circulating system (SECCS) containing autologous MSCs has been designed to cope up with that problem. The SECCS processing has been tested on 42 patients. It has been stated that bone healing was achieved clinically in all patients (Zhuang et al., 2017). It has been stated that an efficient formation of a new bone induction was achieved by the bone marrow (BM) cells. After the culturing *in vitro*, the BM cells was implanted with biphasic calcium phosphate granules into the defect area. No adverse events have been reported when subperiosteally administrated. It has been stated that the new bone formation was stimulated efficiently by autologous MSCs in human subjects (Gjerde et al., 2018). It has been noted that compared to pristine scaffolds, the PCL-MSC ECM [MSC-derived extracellular matrix (ECM) onto

polycaprolactone] scaffolds supported the higher calcium deposition and highly expressed bone-specific genes (especially osteopontin gene). Consequently, it has been stated that the MSC-derived ECM and additive manufacturing (AM)-based scaffolds combination had appropriate effects on the osteogenic differentiation of MSCs (Silva et al., 2020).

REFERENCES

Abbasi-Kangevari, M., Ghamari, S. H., Safaeinejad, F., Bahrami, S., & Niknejad, H. (2019). Potential Therapeutic Features of Human Amniotic Mesenchymal Stem Cells in Multiple Sclerosis: Immunomodulation, Inflammation Suppression, Angiogenesis Promotion, Oxidative Stress Inhibition, Neurogenesis Induction, MMPs Regulation, and Remyelination Stimulation. *Front Immunol, 10*, 238. doi:10.3389/fimmu.2019.00238

Ahuja, C. S., Wilson, J. R., Nori, S., Kotter, M. R. N., Druschel, C., Curt, A., & Fehlings, M. G. (2017). Traumatic spinal cord injury. *Nature Reviews Disease Primers, 3*(1). doi:10.1038/nrdp.2017.18

Bartolucci, J., Verdugo, F. J., González, P. L., Larrea, R. E., Abarzua, E., Goset, C., . . . Khoury, M. (2017). Safety and Efficacy of the Intravenous Infusion of Umbilical Cord Mesenchymal Stem Cells in Patients With Heart Failure. *Circulation Research, 121*(10), 1192-1204. doi:10.1161/circresaha.117.310712

Bartunek, J., Behfar, A., Dolatabadi, D., Vanderheyden, M., Ostojic, M., Dens, J., . . . Terzic, A. (2013). Cardiopoietic Stem Cell Therapy in Heart Failure. *Journal of the American College of Cardiology, 61*(23), 2329-2338. doi:10.1016/j.jacc.2013.02.071

Becerra-Bayona, S. M., Solarte, V. A., Alviar Rueda, J. D., Sossa, C. L., & Arango-Rodriguez, M. L. (2022). Effect of biomolecules derived from human platelet-rich plasma on the ex vivo expansion of human adipose-derived mesenchymal stem cells for clinical applications. *Biologicals, 75*, 37-48. doi:10.1016/j.biologicals.2021.11.001

Chen, G., Wang, Y., Xu, Z., Fang, F., Xu, R., Wang, Y., . . . Liu, H. (2013). Neural stem cell-like cells derived from autologous bone mesenchymal stem cells for the treatment of patients with cerebral palsy. *Journal of Translational Medicine, 11*, 21-21. doi:10.1186/1479-5876-11-21

Cheng, H., Liu, X., Hua, R., Dai, G., Wang, X., Gao, J., & An, Y. (2014). Clinical observation of umbilical cord mesenchymal stem cell transplantation in treatment for sequelae of thoracolumbar spinal cord injury. *J Transl Med, 12*, 253.

Cui, L.-Y., Chu, S.-F., & Chen, N.-H. (2020). The role of chemokines and chemokine receptors in multiple sclerosis. *International Immunopharmacology, 83*, 106314. doi:10.1016/j.intimp.2020.106314

Dahbour, S., Jamali, F., Alhattab, D., Al-Radaideh, A., Ababneh, O., Al-Ryalat, N., . . . Awidi, A. (2017). Mesenchymal stem cells and conditioned media in the treatment of multiple sclerosis patients: Clinical, ophthalmological and radiological assessments of safety and efficacy. *CNS Neuroscience & Therapeutics, 23*(11), 866-874. doi:10.1111/cns.12759

Davatchi, F., Abdollahi, B. S., Mohyeddin, M., Shahram, F., & Nikbin, B. (2011). Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. *Int J Rheum Dis, 14*(2), 211-215. doi:10.1111/j.1756-185X.2011.01599.x

de Windt, T. S., Vonk, L. A., Slaper-Cortenbach, I. C. M., van den Broek, M. P. H., Nizak, R., van Rijen, M. H. P., . . . Saris, D. B. F. (2017). Allogeneic Mesenchymal Stem Cells Stimulate Cartilage Regeneration and Are Safe for Single-Stage Cartilage Repair in Humans upon Mixture with Recycled Autologous Chondrons. *STEM CELLS, 35*(1), 256-264. doi:10.1002/stem.2475

Debnath, T., & Chelluri, L. K. (2019). Standardization and quality assessment for clinical grade mesenchymal stem cells from human adipose tissue. *Hematol Transfus Cell Ther, 41*(1), 7-16. doi:10.1016/j.htct.2018.05.001

Ding, D. C., Chang, Y. H., Shyu, W. C., & Lin, S. Z. (2015). Human umbilical cord mesenchymal stem cells: a new era for stem cell therapy. *Cell Transplant, 24*(3), 339-347. doi:10.3727/096368915X686841

Freitag, J., Bates, D., Wickham, J., Shah, K., Huguenin, L., Tenen, A., . . . Boyd, R. (2019). Adipose-derived mesenchymal stem cell therapy in the treatment of knee osteoarthritis: a randomized controlled trial. *Regen Med, 14*(3), 213-230. doi:10.2217/rme-2018-0161

Gao, L. R., Chen, Y., Zhang, N. K., Yang, X. L., Liu, H. L., Wang, Z. G., . . . Hu, X. (2015). Intracoronary infusion of Wharton's jelly-derived mesenchymal stem cells in acute myocardial infarction: double-blind, randomized controlled trial. *BMC Medicine, 13*(1). doi:10.1186/s12916-015-0399-z

Gjerde, C., Mustafa, K., Hellem, S., Rojewski, M., Gjengedal, H., Yassin, M. A., . . . Layrolle, P. (2018). Cell therapy induced regeneration of severely atrophied mandibular bone in a clinical trial. *Stem Cell Research & Therapy, 9*(1). doi:10.1186/s13287-018-0951-9

Hare, J. M., DiFede, D. L., Rieger, A. C., Florea, V., Landin, A. M., El-Khorazaty, J., . . . Heldman, A. W. (2017). Randomized Comparison of Allogeneic Versus Autologous Mesenchymal Stem Cells for Nonischemic Dilated Cardiomyopathy. *Journal of the American College of Cardiology, 69*(5), 526-537. doi:10.1016/j.jacc.2016.11.009

Harris, V. K., Stark, J., Vyshkina, T., Blackshear, L., Joo, G., Stefanova, V., . . . Sadiq, S. A. (2018). Phase I Trial of Intrathecal Mesenchymal Stem Cell-derived Neural Progenitors in Progressive Multiple Sclerosis. *EBioMedicine, 29*, 23-30. doi:10.1016/j.ebiom.2018.02.002

Hur, J. W., Cho, T.-H., Park, D.-H., Lee, J.-B., Park, J.-Y., & Chung, Y.-G. (2015). Intrathecal transplantation of autologous adipose-derived mesenchymal stem cells for treating spinal cord injury: A human trial. *The Journal of Spinal Cord Medicine, 39*(6), 655-664. doi:10.1179/2045772315y.0000000048

Karussis, D., Karageorgiou, C., Vaknin-Dembinsky, A., Gowda-Kurkalli, B., Gomori, J. M., Kassis, I., . . . Slavin, S. (2010). Safety and

Immunological Effects of Mesenchymal Stem Cell Transplantation in Patients With Multiple Sclerosis and Amyotrophic Lateral Sclerosis. *Archives of Neurology, 67*(10). doi:10.1001/archneurol.2010.248

Kim, S. H., Cho, J. H., Lee, Y. H., Lee, J. H., Kim, S. S., Kim, M. Y., . . . Kim, H. S. (2018). Improvement in Left Ventricular Function with Intracoronary Mesenchymal Stem Cell Therapy in a Patient with Anterior Wall ST-Segment Elevation Myocardial Infarction. *Cardiovascular Drugs and Therapy, 32*(4), 329-338. doi:10.1007/s10557-018-6804-z

Kumar, H., Ha, D.-H., Lee, E.-J., Park, J. H., Shim, J. H., Ahn, T.-K., . . . Han, I.-B. (2017). Safety and tolerability of intradiscal implantation of combined autologous adipose-derived mesenchymal stem cells and hyaluronic acid in patients with chronic discogenic low back pain: 1-year follow-up of a phase I study. *Stem Cell Research & Therapy, 8*(1). doi:10.1186/s13287-017-0710-3

Lamo-Espinosa, J. M., Mora, G., Blanco, J. F., Granero-Moltó, F., Nuñez-Córdoba, J. M., Sánchez-Echenique, C., . . . Prósper, F. (2016). Intra-articular injection of two different doses of autologous bone marrow mesenchymal stem cells versus hyaluronic acid in the treatment of knee osteoarthritis: multicenter randomized controlled clinical trial (phase I/II). *Journal of Translational Medicine, 14*(1). doi:10.1186/s12967-016-0998-2

Lee, J.-W., Lee, S.-H., Youn, Y.-J., Ahn, M.-S., Kim, J.-Y., Yoo, B.-S., . . . Hong, M. K. (2014). A Randomized, Open-Label, Multicenter Trial for the Safety and Efficacy of Adult Mesenchymal Stem Cells after Acute Myocardial Infarction. *Journal of Korean Medical Science, 29*(1), 23. doi:10.3346/jkms.2014.29.1.23

Lee, W. S., Kim, H. J., Kim, K. I., Kim, G. B., & Jin, W. (2019). Intra-Articular Injection of Autologous Adipose Tissue-Derived Mesenchymal Stem

Cells for the Treatment of Knee Osteoarthritis: A Phase IIb, Randomized, Placebo-Controlled Clinical Trial. *Stem cells translational medicine, 8*(6), 504-511. doi:10.1002/sctm.18-0122

Lin, W., Huang, L., Li, Y., Fang, B., Li, G., Chen, L., & Xu, L. (2019). Mesenchymal Stem Cells and Cancer: Clinical Challenges and Opportunities. *Biomed Res Int, 2019*, 2820853. doi:10.1155/2019/2820853

Liu, Q. W., Huang, Q. M., Wu, H. Y., Zuo, G. S., Gu, H. C., Deng, K. Y., & Xin, H. B. (2021). Characteristics and Therapeutic Potential of Human Amnion-Derived Stem Cells. *Int J Mol Sci, 22*(2). doi:10.3390/ijms22020970

Liu, X., Fu, X., Dai, G., Wang, X., Zhang, Z., Cheng, H., . . . An, Y. (2017). Comparative analysis of curative effect of bone marrow mesenchymal stem cell and bone marrow mononuclear cell transplantation for spastic cerebral palsy. *Journal of Translational Medicine, 15*(1). doi:10.1186/s12967-017-1149-0

Llufriu, S., Sepúlveda, M., Blanco, Y., Marín, P., Moreno, B., Berenguer, J., . . . Saiz, A. (2014). Randomized Placebo-Controlled Phase II Trial of Autologous Mesenchymal Stem Cells in Multiple Sclerosis. *PLoS ONE, 9*(12), e113936. doi:10.1371/journal.pone.0113936

Matas, J., Orrego, M., Amenabar, D., Infante, C., Tapia-Limonchi, R., Cadiz, M. I., . . . Espinoza, F. (2019). Umbilical Cord-Derived Mesenchymal Stromal Cells (MSCs) for Knee Osteoarthritis: Repeated MSC Dosing Is Superior to a Single MSC Dose and to Hyaluronic Acid in a Controlled Randomized Phase I/II Trial. *Stem cells translational medicine, 8*(3), 215-224. doi:10.1002/sctm.18-0053

Mendonça, M. V., Larocca, T. F., de Freitas Souza, B. S., Villarreal, C. F., Silva, L. F., Matos, A. C., . . . dos Santos, R. R. (2014). Safety and neurological assessments after autologous transplantation of bone

marrow mesenchymal stem cells in subjects with chronic spinal cord injury. *Stem Cell Res Ther, 5*(6), 126.

Mohajeri, M., Farazmand, A., Mohyeddin Bonab, M., Nikbin, B., & Minagar, A. (2011). FOXP3 gene expression in multiple sclerosis patients pre- and post mesenchymal stem cell therapy. *Iran J Allergy Asthma Immunol, 10*(3), 155-161.

Mushahary, D., Spittler, A., Kasper, C., Weber, V., & Charwat, V. (2018). Isolation, cultivation, and characterization of human mesenchymal stem cells. *Cytometry A, 93*(1), 19-31. doi:10.1002/cyto.a.23242

Riordan, N. H., Morales, I., Fernández, G., Allen, N., Fearnot, N. E., Leckrone, M. E., . . . Paz Rodriguez, J. (2018). Clinical feasibility of umbilical cord tissue-derived mesenchymal stem cells in the treatment of multiple sclerosis. *Journal of Translational Medicine, 16*(1). doi:10.1186/s12967-018-1433-7

Samsonraj, R. M., Raghunath, M., Nurcombe, V., Hui, J. H., van Wijnen, A. J., & Cool, S. M. (2017). Concise Review: Multifaceted Characterization of Human Mesenchymal Stem Cells for Use in Regenerative Medicine. *Stem Cells Transl Med, 6*(12), 2173-2185. doi:10.1002/sctm.17-0129

Sellam, J., & Berenbaum, F. (2010). The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. *Nature Reviews Rheumatology, 6*(11), 625-635. doi:10.1038/nrrheum.2010.159

Silva, J. C., Carvalho, M. S., Udangawa, R. N., Moura, C. S., Cabral, J. M. S., L. da Silva, C., . . . Linhardt, R. J. (2020). Extracellular matrix decorated polycaprolactone scaffolds for improved mesenchymal stem/stromal cell osteogenesis towards a patient-tailored bone tissue engineering approach. *Journal of Biomedical Materials Research Part B: Applied Biomaterials, 108*(5), 2153-2166. doi:10.1002/jbm.b.34554

Singer, W., Dietz, A. B., Zeller, A. D., Gehrking, T. L., Schmelzer, J. D., Schmeichel, A. M., . . . Low, P. A. (2019). Intrathecal administration of

autologous mesenchymal stem cells in multiple system atrophy. *Neurology, 93*(1), e77-e87. doi:10.1212/wnl.0000000000007720

Staff, N. P., Madigan, N. N., Morris, J., Jentoft, M., Sorenson, E. J., Butler, G., . . . Windebank, A. J. (2016). Safety of intrathecal autologous adipose-derived mesenchymal stromal cells in patients with ALS. *Neurology, 87*(21), 2230-2234.

Syková, E., Rychmach, P., Drahorádová, I., Konrádová, Š., Růžičková, K., Vořišek, I., . . . Bojar, M. (2017). Transplantation of Mesenchymal Stromal Cells in Patients with Amyotrophic Lateral Sclerosis: Results of Phase I/IIa Clinical Trial. *Cell Transplantation, 26*(4), 647-658. doi:10.3727/096368916x693716

Vaquero, J., Zurita, M., Rico, M. A., Aguayo, C., Bonilla, C., Marin, E., . . . Rodríguez, B. (2018). Intrathecal administration of autologous mesenchymal stromal cells for spinal cord injury: Safety and efficacy of the 100/3 guideline. *Cytotherapy, 20*(6), 806-819. doi:10.1016/j.jcyt.2018.03.032

Vaquero, J., Zurita, M., Rico, M. A., Bonilla, C., Aguayo, C., Fernández, C., . . . Fernández, M. V. (2017). Repeated subarachnoid administrations of autologous mesenchymal stromal cells supported in autologous plasma improve quality of life in patients suffering incomplete spinal cord injury. *Cytotherapy, 19*(3), 349-359. doi:10.1016/j.jcyt.2016.12.002

Zhao, G., Zhou, X., Chen, S., Miao, H., Fan, H., Wang, Z., . . . Hou, Y. (2014). Differential expression of microRNAs in decidua-derived mesenchymal stem cells from patients with pre-eclampsia. *J Biomed Sci, 21*(1), 81.

Zhou, W., Lin, J., Zhao, K., Jin, K., He, Q., Hu, Y., . . . Ouyang, H. (2019). Single-Cell Profiles and Clinically Useful Properties of Human Mesenchymal Stem Cells of Adipose and Bone Marrow Origin. *Am J Sports Med, 47*(7), 1722-1733. doi:10.1177/0363546519848678

Zhuang, Y., Gan, Y., Shi, D., Zhao, J., Tang, T., & Dai, K. (2017). A novel cytotherapy device for rapid screening, enriching and combining mesenchymal stem cells into a biomaterial for promoting bone regeneration. *Scientific Reports, 7*(1). doi:10.1038/s41598-017-15451-0

CHAPTER VII

HIV-Associated Neuropathies

Parvin PINAR, M.D.[1]

[1] Süleyman Demirel University, Faculty of Medicine, Division of Algology, Isparta, Turkey. pervinyusifova@hotmail.com, Orcid ID: 0000-0003-0324-656X

INTRODUCTION

Since the use of highly active antiretroviral therapy (HAART) in 1996, the incidence of HIV (human immunodeficiency virus)-related dementia and opportunistic diseases of the nervous system have decreased significantly.[1] However, the incidence and prevalence of HIV-associated sensory neuropathies (HIV-SN) have increased.[1] It is now the most common neurological complication caused by HIV disease and, in addition, one of the leading causes of neuropathy worldwide. This suggests that HIV in patients with polyneuropathy should be part of every clinical neurologist's differential diagnostic repertoire. According to the CDC (Centers for Disease Control) classification, HIV-associated polyneuropathies represent a broad spectrum of individual sub-forms with the main common feature of being consistently linked to specific stages of HIV disease.

The combination of anamnesis, clinical findings, neurophysiology, and immune status allows a suspected targeted diagnosis in the majority of patients. Due to the comorbidities of HIV-infected people and numerous interactions between the drugs used to treat polyneuropathy and antiretroviral substances, further treatment in specialized neurological centers is recommended.

DIAGNOSIS

Laboratory Tests

A current immune status should be present to assess whether it is more likely after a spontaneously remitting early manifestation, such as Guillain-Barré syndrome (GBS), or rather after a late presentation, such as, e.g., an opportunistic polyradiculopathy.

Due to the increasing age of the patients, comorbidities are becoming increasingly relevant. In addition to HIV infection, the entire spectrum of

diseases that can induce polyneuropathy should be considered. Table 1 shows laboratory tests that are useful for clarifying the cause of polyneuropathies.

CSF (cerebrospinal fluid) analysis is trend-setting in acute and chronic inflammatory, demyelinating polyneuropathy, and inflammatory pathogen-related polyneuropathies. Cell counts and proteins should be analyzed. CSF cytology and, if necessary, PCR (polymerase chain reaction, e.g., polyradiculitis caused by cytomegalovirus) are indicated. If there is a suspicion of tumorous infiltration of nerve roots, the detection of neoplastic cell forms is shown.

Electrophysiology

Neurophysiological examinations are indicated as basic diagnostics to assess an axonal or demyelinating type and to objectify a subclinical involvement of large-caliber nerve fibers in clinically predominant small-fiber involvement. Analysis of the somatosensory evoked potentials may be necessary to differentiate a parallel HIV-associated myelopathy present.

Table 1. Laboratory examinations for the clarification of polyneuropathies

Basic Diagnostics	
ESR, CRP, complete blood count, electrolytes, liver and kidney parameters, protein and immune electrophoresis, TSH, oral glucose load test, HbA1c, vitamin B12	
Advanced Examinations	
Vasculitis	Rheumatoid factors, ANA, p-ANCA, c-ANCA, cryoglobulins, serology, eosinophils
Malabsorption	Vitamin B1, B6, folic acid, possibly Schilling test for vitamin B12 deficiency
Paraproteinemia	Immune electrophoresis, immune fixation, Bence Jones proteins in 24-hour urine, bone X-ray, bone marrow biopsy
Sarcoidosis	Chest X-ray, ACE determination
Paraneoplasia	Autoantibodies against neural tissue, bone marrow, extended search for primary tumor (lung, gastrointestinal and urogenital tract), whole-body PET
ESR: Erythrocyte sedimentation rate, CRP: C reactive protein, TSH: Thyroid stimulating hormone, HbA1c: Hemoglobin A1c, ANA: Antinuclear antibody, p-ANCA: Perinuclear anti-neutrophilic cytoplasmic antibodies, c-ANCA: Cytoplasmic anti-neutrophil cytoplasmic antibodies, ACE: Angiotensin-converting enzyme, PET: Positron emission tomography	

Tests to Assess Small Fiber Morphology and Function

Small fiber neuropathy (SFN) is formed by the involvement of the thinly myelinated and unmyelinated nerve fibers (A-delta and C fibers), while thickly myelinated nerve fibers are relatively unaffected. Distally marked pain, burning sensations, or the inability to tolerate a bed cover are symptoms that make SFN likely. The diagnosis can be clinically problematic because, in addition to the abnormal sensations described by the patient, there are no additional sensory or motor deficits (weakening of reflexes, paresis), and the electrophysiology is also physiological.

Investigations using pain-evoked potentials through electrical stimulation of the A-Delta and C-fibers in the skin have shown that neuropathy can be objectified in 74% of the cases, in contrast to 31% with conventional neurography methods.[2] The diagnostic alternative is a skin punch biopsy. The extent of the loss of distal nerve endings can be determined, and the diagnosis of SFN is confirmed by immunohistochemical imaging of the epidermal innervation.[3]

Special Forms of HIV-associated Neuropathies

Polyneuropathies during HIV infection can be classified based on clinical, neurophysiological, and histological bases. Table 2 provides an overview.

Table 2. Types of HIV-associated neuropathies

HIV-associated Sensory Neuropathies (DSP and ATN)
Inflammatory Polyneuropathies (AIDP and CIDP)
Mononeuritis Multiplex
Autonomic Neuropathy
Neuropathy in Diffuse Infiltrative Lymphocytosis
Neuropathy Associated with Opportunistic Infections
DSP: Distal sensory peripheral neuropathies, ATN: Antiretroviral toxic neuropathies, AIDP: Acute inflammatory demyelinating polyneuropathy, CIDP: Chronic Inflammatory Demyelinating Polyneuropathy

HIV-ASSOCIATED SENSORY NEUROPATHIES

The most common neurological problem; is HIV-associated sensory neuropathy (HIV-SN), which is detected in the course of the disease in HIV-infected persons.[4, 5] A distinction is made between two subtypes that cannot be clinically separated from one another: direct HIV-associated distal symmetric polyneuropathy (HIV-DSP), which occurs primarily in the advanced stage of HIV disease, and antiretroviral toxic neuropathy (ATN), which is caused by certain medications used to treat HIV, such as dideoxynucleosides.

Epidemiology

According to available extensive cohort studies, more than half of all HIV patients show signs of neuropathy.[6, 7] Epidemiological studies in patients with advanced HIV disease in the pre-HAART period revealed that the one-year incidence of symptomatic HIV-SN was 36%, while this rate decreased to 21% in the post-HAART period.[7, 8] Risk factors for the development of HIV-SN are age >40 years, CD4 cells <50 cells/mm^3, an initial viral load >10,000 copies/ml, and concomitant diabetes mellitus.[9, 10] In a 3-year case-control study with 509 subjects, subjects who were positive for mitochondrial haplotype T, developed polyneuropathy significantly more frequently.[11] Whether this is indeed a risk factor needs to be confirmed by further studies.

Typical substances that cause ATN are the dideoxynucleosides stavudine, didanosine, and zalcitabine. According to recent data, taking the protease inhibitors indinavir, saquinavir and ritonavir are also related to a substantially increased risk of developing ATN.[12]

Clinic

The two forms are clinically indistinguishable. HIV-SN is a typical length-dependent neuropathy. Initial symptoms are dysesthesia, usually in the toes,

radiating to the ankle or calf in a sock-like pattern, and often having a painful, burning character. In the course of the disease, the fingers or hands can also be affected by the glove-shaped design. At the same time, there are often sensory deficits such as hypesthesia or hypalgesia. Significant pareses should raise doubts about the suspected diagnosis. Weakened or absent Achilles tendon reflexes are common. If hyperreflexia is present, parallel myelopathy should be considered.

Diagnosis

Electrophysiologically, a sensitive axonal lesion pattern is often found. Approximately one-fifth have normal neurography findings due to the isolated involvement of small-caliber nerve fibers.[13] These patients typically show a reduction in intraepidermal nerve fiber density in the skin punch biopsy.[3, 14] The reduction in nerve fiber density correlated with low CD4 positive cell counts and a high HIV RNA viral load, as well as pain intensity.[7, 9, 10]

Pathology/Pathogenesis

The HIV-DSP and the ATN are based on different pathogenetic mechanisms, which are only partially known to us. A reliable animal model of HIV infection and polyneuropathy is not available.

HIV-DSP: Data available to date conclude that it is a length-dependent neuropathy with the characteristics of a "dying-back axonopathy." This is supported by the morphological pathological investigations, which show a distal degeneration of myelinated and unmyelinated axons,[15] as well as no or only minimal neuronal cell death in the spinal ganglion.[16-18] This assumption is supported by observations in the skin punch biopsy, which show an increase in the loss of epidermal nerve fibers from proximal to distal.[3] In addition, it has recently been shown that patients with HIV disease have a reduced capacity for nerve regeneration of distal epidermal nerve fibers after

standardized lesions in skin punch biopsy, even before clinical signs of HIV-SN appear.[19]

The mechanism of damage is still controversial. There is currently very little evidence that HIV directly infects the neurons in the spinal ganglion, so this is, at best, a secondary mechanism.[15] HIV-infected perivascular macrophages in the spinal ganglion seem to play an essential role in pathogenesis. Morphological studies have revealed multiple macrophages infiltrates in the spinal ganglion,[15, 16] which activates and expresses MHC (major histocompatibility complex) antigens and proinflammatory cytokines.[15] The severity of DSP correlates with the degree of macrophage infiltration.[20] Two pathogenetic mechanisms have been suggested: the direct virus-induced neurotoxic effect of HIV or its proteins, such as gp120 on dorsal spinal ganglion cells,[21-23] and the indirect neurotoxic effect, which is the byproduct of HIV-infected macrophages.[20]

ATN: In general, the toxicity caused by HAART is mediated by mitochondrial dysfunction. Prolonged survival in patients diagnosed with AIDS ("acquired immunodeficiency syndrome") results in remarkably higher doses of retroviral drugs. The dideoxnucleosides stavudine, didanosine, and zalcitabine also have mitochondrial toxic effects in tissue cultures. Exposure of PC12 lineage cells to didanosine and zalcitabine resulted in structural changes in the mitochondria and increased lactate production.[24] The effects are related to the concentration of these substances. The first changes in the mitochondrial ultrastructure appear after just a few days. The toxic potential to induce these changes is organized in the following order: zalcitabine >stavudine >didanosine.[24] The mitochondrial DNA content in subcutaneous adipose tissue correlates well with NRTI (nucleoside reverse transcriptase inhibitor) use, but not with the incidence of ATN.[25]

Keswani et al. showed that the neurotoxicity mediated by zalcitabine, didanosine, and stavudine was mediated through a mitochondrial mechanism independent of mitochondrial DNA polymerase γ.[26] Dalakas et al. identified structural abnormalities in axonal mitochondria and Schwann cells in HIV-infected patients with ATN.[27] In contrast to HIV-DSP, where the site of attack appears to be the dorsal spinal ganglion, the toxicity of ATN may also occur directly on the axon.

Causal Therapy

HIV-DSP: Safe treatment for the cause is not available. There is a prospective study that was able to show an improvement in quantitative sensory testing under HAART over eight months.[28] Epidemiological studies by Schifitto et al. show that the one-year incidence of symptomatic DSP decreased from 36% pre-HAART period to 21% post-HAART period, simply a similar responsiveness to HAART.[7, 8]

ATN: If ATN is suspected, the continuation or modification of antiretroviral medication should be discussed in an interdisciplinary manner; as far as possible, neurotoxic preparations should be discontinued. Remission may last from four weeks to about six months and is incomplete due to HIV-DSP often existing in parallel. The same considerations apply to the other neurotoxic drugs (e.g., vincristine, isoniazid, ethambutol, metronidazole, doxorubicin, and dapsone)used in HIV-infected patients.

Symptomatic Pain Therapy

The focus of the treatment is often the symptomatic therapy of the pain. General considerations of the treatment of painful polyneuropathies apply. Due to interactions, typical substances such as carbamazepine should only be used with extreme caution since interactions with protease inhibitors or NNRTI

(non-nucleoside reverse transcriptase inhibitors) often increase the viral load, which entails resistance to these substances.

Prospective controlled studies in patient groups with HIV-SN have shown the effectiveness of gabapentin,[29] lamotrigine in ATN,[30] acetylcarnitine (intramuscular administration of 2 x 500 mg),[31] and cannabis (inhalatively over five days).[32] There are promising data from the treatment of painful diabetogenic PNP (polyneuropathy) for the selective serotonin and norepinephrine reuptake inhibitor duloxetine.[33] Efficacy data for painful HIV-SN are expected in the future. Substances such as peptide T (6 mg/day intranasally),[34] mexiletine (maximum daily dose 600 mg),[35] amitriptyline (daily dose of 75–100 mg),[35] memantine,[36] prosaposin,[37] topical administration of 5% lidocaine[38] or 0.075% capsaicin[39] did not lead to any significant effect compared to placebo.

Gabapentin, lamotrigine, amitriptyline and pregabalin are widely used in the clinic. Table 3 summarizes the substances used in the clinic. A combination therapy for substances with different mechanisms of action seems possible and pathophysiologically sensible, although there is no data on HIV-SN in this regard.

Table 3. Symptomatic Therapy of HIV-SN

Substance	Effective dose (maximum dose)	Adverse effects	Interactions with antiretroviral therapy
1. Simple Physical Measures Avoid tight socks and shoes, standing for long periods, and contrast showers. Caution: no hot water bottles if there is a lack of protection for sensitivity!			
2. If the pain persists, acute intervention with conventional analgesics (if possible no longer than 14 days)			
Paracetamol	3 times 500 mg		
Naproxen	2 times 250 mg		
3. If symptoms persist, long-term therapy			
Gabapentin	900 - 2400 mg (3600) in 3 divided doses	Drowsiness, nausea, rarely pancreatitis	Pancreatic toxicity
Pregabalin	150 mg (600 mg) in 2 divided doses	Drowsiness, nausea, weight gain	No relevant interactions
Lamotrigine in ATN	100 – 200 mg (300 mg) in 2 divided doses, Caution: increase dosage very slowly!	Rash, headache, nausea	Caution: Abacavir and NNRTI can also cause a skin rash - do not start simultaneously!
Amitriptyline	75 – 150 mg (300 mg) evenings	Anticholinergic side effects, sedation, tiredness Caution: AV block, glaucoma	Interaction with protease inhibitors
Tramadol	50 – 100 mg (titration 400 mg)	Initial sedation, initial nausea, hypotension	
Morphine	Initially 2 times 10 mg (titration 200 mg)	Initial sedation and nausea, constipation, micturition disorder, accumulation in RI	Caution: sedating effect when combined with efavirenz
Duloxetine	30 mg (60 mg) as a single dose in the morning	Off-label good tolerability, nausea, dry mouth, constipation, hepatotoxicity	Interaction with ritonavir and abacavir
NNRTI: Non-nucleoside reverse transcriptase inhibitors (nevirapine, efavirenz, delavirdine), RI: Renal impairment			

INFLAMMATORY POLYNEUROPATHIES

Equivalent to the non-HIV-associated forms, HIV-associated acute inflammatory demyelinating polyneuropathy (AIDP; Guillain-Barré syndrome, GBS) is differentiated from HIV-associated chronic inflammatory demyelinating polyneuropathy (CIDP).[40, 41]

HIV-AIDP: The disease is rare (approx. 1%) and occurs mainly during seroconversion or in stage 1 of the HIV infection when CD4 cells >500/µl.[41] The clinical picture is indistinguishable from that of HIV-uninfected patients. An exception is a slightly increased CSF cell count in patients with HIV infection (CSF cell count up to 150/µL in approximately 50% of patients). Polyneuroradiculitis/myelitis caused by *cytomegalovirus, Varicella-zoster virus, Herpes simplex virus, and Epstein-Barr virus* infection must be considered in the differential diagnosis. Paraplegic symptomatology with a sensitive level and reflex jump in the clinical examination and higher cell counts in the cerebrospinal fluid is not compatible with the diagnosis, so magnetic resonance imaging is also indicated. Acute HIV-AIDP is primarily progressive; if the diagnosis is made early and therapy is started promptly, there is usually a good recovery.

HIV-CIDP: The rare chronic demyelinating polyneuropathy of HIV-infected patients is characterized by progressive symmetrical paresis and sensory disturbances that progress from distal to proximal over weeks to months. The diagnosis can be made with sufficient certainty by demonstrating increased protein in the cerebrospinal fluid and typical neurophysiological findings. Electroneurography shows prolonged distal motor latencies, markedly slowed nerve conduction velocities, and delayed F-wave latencies. Electromyography usually shows low spontaneous activity.[41] The pathological mechanism of HIV-CIDP has not been elucidated. It is believed to be an autoimmune process triggered by HIV infection. Differential diagnoses are similar to HIV-AIDP.

Therapy: There are not enough studies on the treatment of HIV-associated inflammatory neuropathies. At this point, the same therapy principles are applied to the non-HIV-associated forms. This means for the HIV-AIDP immunoglobulins (0.4 g/kg body weight/day for 5 days) or plasmapheresis.[4, 40, 42, 43] The high risk of infection and high technical effort are limiting factors for plasmapheresis.

For HIV-CIDP, immunoglobulins (0.4 g/kg body weight/day for 5 days) are used for temporary immune replacement. Alternatively, 5 to 6 plasmapheresis treatments are administered for 10 to 14 days, and usually, 2 to 3 plasmapheresis treatments need to be repeated every 6 to 10 weeks. If the immune status is good, treatment with prednisolone (1 mg/kg body weight/4 weeks) followed by a reduction to 10-20 mg for 10 to 14 weeks as maintenance therapy is also possible.

MONONEURITIS MULTIPLEX

Multiplex-type mononeuropathy and mononeuritis are rare. Clinically, the disease is characterized by the mono or multifocal, asymmetric cranial nerve (mainly the facial nerve) or peripheral nerve involvement that is localized to single nerves in the early stages of HIV infection. There are sometimes rapidly progressive, occasionally also slow, self-limiting courses over months.[4, 14, 43]

The cause is often vasculitis.[4, 14] CSF analysis and imaging procedures should be used generously for clarification. Neurophysiologically, there are predominantly signs of axonal lesions.[44, 45] The sural nerve biopsy can play a crucial role in the differential diagnosis. Perivascular inflammatory infiltrates of CD8 cells, which may spread to the vessel walls, clearly allow the diagnosis of HIV-induced neuritis or vasculitis.

Therapy: Since the data are minimal, there is no generalized treatment scheme. Primarily immunoglobulins (0.4 g/kg body weight/day for 5 days) or prednisolone 100 mg/day for two weeks and then a gradual dose reduction. However, intensive immunotherapy can suppress the immune system to the extent that opportunistic infections occur.

AUTONOMIC NEUROPATHY

There is controversy as to whether it makes sense to consider an autonomic polyneuropathy in isolation, especially since autonomic involvement is often seen in the context of the polyneuropathies mentioned above and no separate, isolated polyneuropathy affecting only the autonomic nervous system in patients with HIV infection is known.[4] Studies examining autonomic functions in HIV patients also found inconsistent results about dysautonomia,[46] so controlled longitudinal studies are urgently needed.

POLYNEUROPATHY IN DIFFUSE INFILTRATIVE LYMPHOCYTOSIS

Diffuse infiltrative lymphocytosis (DILS) is a very rare systemic disease in HIV-infected people with moderate to advanced HIV disease (CD4 cells <500/µL). Typical characteristics are CD8 hyperlymphocytosis (>1200/µL) with infiltration of visceral organs and, in some cases, peripheral nerves.[47-51] Clinically, the disease resembles Sjögren's syndrome. Neurologically, a mostly symmetrical, rarely multifocal, painful sensorimotor axonal polyneuropathy may occur acutely to sub-acutely. Histologically, there is evidence of the HIV genome as well as angiocentric infiltrations of CD8 cells in the epi- and endoneurium.[48, 50]

Therapy: Larger therapy studies are not available due to the rarity of the disease. Minor case series report an improvement under antiretroviral treatment with zidovudine and steroids.[50] Therefore, the general recommendation is the

initiation of HAART and prednisolone 1 mg/kg body weight for four weeks and transition to maintenance therapy with 10–20 mg after three months.[43]

NEUROPATHY ASSOCIATED WITH OPPORTUNISTIC INFECTIONS

In the case of advanced immunosuppression, mono neuritis multiplex can also occur as an expression of a pathogen-related infection, which then usually progresses rapidly and can lead to quadriplegia. The incidence of HIV infection is less than one percent. In principle, the clinical findings do not differ from those in the other forms presented. Polyneuroradiculitis caused by pathogens usually manifests as flaccid, rapidly progressive paraparesis of the lower extremities with breeches anesthesia and bladder emptying disorders.

Electroneurography shows axonal damage. CSF shows pleocytosis and increased total protein, albumin, and immunoglobulins. A PCR to detect the pathogen can secure the suspected diagnosis and be used quickly. Treatment is based on pathogen detection.[42] In up to 80% of cases, the cytomegalovirus can be proven to be in the CSF, with other organ manifestations often occurring in the course,[4, 14] and usually leading to confirmation of the suspected diagnosis more quickly (retina, urine). Other opportunistic pathogens (e.g., *Mycobacterium tuberculosis, Cryptococcus neoformans, Varicella-zoster virus, Epstein-Barr virus, Herpes simplex virus*) can also cause poly-neuro radiculitis. A meningeal lymphoma or a syphilis infection should also be considered.

Therapy: The therapy depends on the pathogen. In the case of cytomegalovirus polyradiculitis, ganciclovir is effective,[52-54] and foscarnet can be used in case of resistance.[55]

REFERENCES

1. Wright, E.J., *Neurological disease: the effects of HIV and antiretroviral therapy and the implications for early antiretroviral therapy initiation.* Curr Opin HIV AIDS, 2009. **4**(5): p. 447-52.
2. Katsarava, Z., et al., *Pain related potentials by electrical stimulation of skin for detection of small-fiber neuropathy in HIV.* J Neurol, 2006. **253**(12): p. 1581-4.
3. Lakritz, J.R., et al., *Loss of intraepidermal nerve fiber density during SIV peripheral neuropathy is mediated by monocyte activation and elevated monocyte chemotactic proteins.* J Neuroinflammation, 2015. **12**: p. 237.
4. Verma, A., *Epidemiology and clinical features of HIV-1 associated neuropathies.* J Peripher Nerv Syst, 2001. **6**(1): p. 8-13.
5. Weniger, B.G., et al., *Workshop report: Nucleic acid delivery devices for HIV vaccines: Workshop proceedings, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA, May 21, 2015.* Vaccine, 2018. **36**(4): p. 427-437.
6. Motwani, L., et al., *Neuropathy in Human Immunodeficiency Virus: A Review of the Underlying Pathogenesis and Treatment.* Cureus, 2022. **14**(6): p. e25905.
7. Schifitto, G., et al., *Incidence of and risk factors for HIV-associated distal sensory polyneuropathy.* Neurology, 2002. **58**(12): p. 1764-8.
8. Schifitto, G., et al., *Markers of immune activation and viral load in HIV-associated sensory neuropathy.* Neurology, 2005. **64**(5): p. 842-8.
9. Asahchop, E.L., et al., *HIV-associated sensory polyneuropathy and neuronal injury are associated with miRNA-455-3p induction.* JCI Insight, 2018. **3**(23).
10. McArthur, J.C., B.J. Brew, and A. Nath, *Neurological complications of HIV infection.* Lancet Neurol, 2005. **4**(9): p. 543-55.

11.Roda, R.H., et al., *Large Mitochondrial DNA Deletions in HIV Sensory Neuropathy.* Neurology, 2021. **97**(2): p. e156-e165.

12.Pettersen, J.A., et al., *Sensory neuropathy in human immunodeficiency virus/acquired immunodeficiency syndrome patients: protease inhibitor-mediated neurotoxicity.* Ann Neurol, 2006. **59**(5): p. 816-24.

13.Tagliati, M., et al., *Peripheral nerve function in HIV infection: clinical, electrophysiologic, and laboratory findings.* Arch Neurol, 1999. **56**(1): p. 84-9.

14.Keswani, S.C., et al., *HIV-associated sensory neuropathies.* AIDS, 2002. **16**(16): p. 2105-17.

15.Pardo, C.A., J.C. McArthur, and J.W. Griffin, *HIV neuropathy: insights in the pathology of HIV peripheral nerve disease.* J Peripher Nerv Syst, 2001. **6**(1): p. 21-7.

16.Bradley, W.G., et al., *Morphometric analysis of the peripheral neuropathy of AIDS.* Muscle Nerve, 1998. **21**(9): p. 1188-95.

17.Esiri, M.M., C.S. Morris, and P.R. Millard, *Sensory and sympathetic ganglia in HIV-1 infection: immunocytochemical demonstration of HIV-1 viral antigens, increased MHC class II antigen expression and mild reactive inflammation.* J Neurol Sci, 1993. **114**(2): p. 178-87.

18.Griffin, J.W., et al., *Peripheral nerve disorders in HIV infection. Similarities and contrasts with CNS disorders.* Res Publ Assoc Res Nerv Ment Dis, 1994. **72**: p. 159-82.

19.Hahn, K., et al., *Impaired reinnervation in HIV infection following experimental denervation.* Neurology, 2007. **68**(16): p. 1251-6.

20.Weichseldorfer, M., et al., *Identifying CCR5 coreceptor populations permissive for HIV-1 entry and productive infection: implications for in vivo studies.* J Transl Med, 2022. **20**(1): p. 39.

21.Apostolski, S., et al., *Complement dependent cytotoxicity of sensory ganglion neurons mediated by the gp120 glycoprotein of HIV-1.* Immunol Invest, 1994. **23**(1): p. 47-52.

22.Apostolski, S., et al., *The gp120 glycoprotein of human immunodeficiency virus type 1 binds to sensory ganglion neurons.* Ann Neurol, 1993. **34**(6): p. 855-63.

23.Keswani, S.C., et al., *Schwann cell chemokine receptors mediate HIV-1 gp120 toxicity to sensory neurons.* Ann Neurol, 2003. **54**(3): p. 287-96.

24.Medina, D.J., et al., *Comparison of mitochondrial morphology, mitochondrial DNA content, and cell viability in cultured cells treated with three anti-human immunodeficiency virus dideoxynucleosides.* Antimicrob Agents Chemother, 1994. **38**(8): p. 1824-8.

25.Cherry, C.L., et al., *Exposure to dideoxynucleosides is reflected in lowered mitochondrial DNA in subcutaneous fat.* J Acquir Immune Defic Syndr, 2002. **30**(3): p. 271-7.

26.Keswani, S.C., et al., *FK506 is neuroprotective in a model of antiretroviral toxic neuropathy.* Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society, 2003. **53**(1): p. 57-64.

27.Dalakas, M.C., C. Semino-Mora, and M. Leon-Monzon, *Mitochondrial alterations with mitochondrial DNA depletion in the nerves of AIDS patients with peripheral neuropathy induced by 2'3'-dideoxycytidine (ddC).* Lab Invest, 2001. **81**(11): p. 1537-44.

28.Martin, C., et al., *Antiretroviral therapy may improve sensory function in HIV-infected patients: a pilot study.* Neurology, 2000. **54**(11): p. 2120-7.

29.Hahn, K., et al., *A placebo-controlled trial of gabapentin for painful HIV-associated sensory neuropathies.* J Neurol, 2004. **251**(10): p. 1260-6.

30.Simpson, D.M., et al., *Lamotrigine for HIV-associated painful sensory neuropathies: a placebo-controlled trial.* Neurology, 2003. **60**(9): p. 1508-14.

31.Youle, M., M. Osio, and A.S. Group, *A double-blind, parallel-group, placebo-controlled, multicentre study of acetyl L-carnitine in the symptomatic treatment of antiretroviral toxic neuropathy in patients with HIV-1 infection.* HIV Med, 2007. **8**(4): p. 241-50.

32.Abrams, D.I., et al., *Cannabis in painful HIV-associated sensory neuropathy: a randomized placebo-controlled trial.* Neurology, 2007. **68**(7): p. 515-21.

33.Wernicke, J.F., et al., *A randomized controlled trial of duloxetine in diabetic peripheral neuropathic pain.* Neurology, 2006. **67**(8): p. 1411-20.

34.Simpson, D.M., et al., *Peptide T in the treatment of painful distal neuropathy associated with AIDS: results of a placebo-controlled trial. The Peptide T Neuropathy Study Group.* Neurology, 1996. **47**(5): p. 1254-9.

35.Kieburtz, K., et al., *A randomized trial of amitriptyline and mexiletine for painful neuropathy in HIV infection. AIDS Clinical Trial Group 242 Protocol Team.* Neurology, 1998. **51**(6): p. 1682-8.

36.Schifitto, G., et al., *A placebo-controlled study of memantine for the treatment of human immunodeficiency virus-associated sensory neuropathy.* J Neurovirol, 2006. **12**(4): p. 328-31.

37.Evans, S.R., et al., *A randomized trial evaluating Prosaptide for HIV-associated sensory neuropathies: use of an electronic diary to record neuropathic pain.* PLoS One, 2007. **2**(6): p. e551.

38.Estanislao, L., et al., *A randomized controlled trial of 5% lidocaine gel for HIV-associated distal symmetric polyneuropathy.* J Acquir Immune Defic Syndr, 2004. **37**(5): p. 1584-6.

39. Paice, J.A., et al., *Topical capsaicin in the management of HIV-associated peripheral neuropathy.* J Pain Symptom Manage, 2000. **19**(1): p. 45-52.

40. Brannagan, T.H., 3rd and Y. Zhou, *HIV-associated Guillain-Barre syndrome.* J Neurol Sci, 2003. **208**(1-2): p. 39-42.

41. Cornblath, D.R., et al., *Inflammatory demyelinating peripheral neuropathies associated with human T-cell lymphotropic virus type III infection.* Ann Neurol, 1987. **21**(1): p. 32-40.

42. DNAA, D.N.-A.-A., *Erkrankungen des peripheren Nervensystems und der Muskulatur der HIV-Infektion.* Der Nervenarzt, 2000. **71**: p. 442-450.

43. Wulff, E.A., A.K. Wang, and D.M. Simpson, *HIV-associated peripheral neuropathy: epidemiology, pathophysiology and treatment.* Drugs, 2000. **59**(6): p. 1251-60.

44. Schielke, E., H.W. Pfister, and K.M. Einhaupl, *Peripheral facial nerve palsy associated with HIV infection.* Lancet, 1989. **1**(8637): p. 553-4.

45. Robinson-Papp, J. and D.M. Simpson, *Neuromuscular diseases associated with HIV-1 infection.* Muscle Nerve, 2009. **40**(6): p. 1043-53.

46. Robinson-Papp, J., et al., *Sympathetic function and markers of inflammation in well-controlled HIV.* Brain Behav Immun Health, 2020. **7**: p. 100112.

47. Franco-Paredes, C., et al., *Diagnosis of diffuse CD8+ lymphocytosis syndrome in HIV-infected patients.* AIDS Read, 2002. **12**(9): p. 408-13.

48. Gherardi, R.K., et al., *Neuropathy in diffuse infiltrative lymphocytosis syndrome: an HIV neuropathy, not a lymphoma.* Neurology, 1998. **50**(4): p. 1041-4.

49. Kazi, S., et al., *The diffuse infiltrative lymphocytosis syndrome. Clinical and immunogenetic features in 35 patients.* AIDS, 1996. **10**(4): p. 385-91.

50.Moulignier, A., et al., *Peripheral neuropathy in human immunodeficiency virus-infected patients with the diffuse infiltrative lymphocytosis syndrome.* Ann Neurol, 1997. **41**(4): p. 438-45.

51.Itescu, S., L.J. Brancato, and R. Winchester, *A sicca syndrome in HIV infection: association with HLA-DR5 and CD8 lymphocytosis.* Lancet, 1989. **2**(8661): p. 466-8.

52.Fuller, G.N., et al., *Ganciclovir for lumbosacral polyradiculopathy in AIDS.* Lancet, 1990. **335**(8680): p. 48-9.

53.Miller, R.G., J.R. Storey, and C.M. Greco, *Ganciclovir in the treatment of progressive AIDS-related polyradiculopathy.* Neurology, 1990. **40**(4): p. 569-74.

54.Kameda, K., et al., *Cytomegalovirus polyradiculopathy in three Japanese patients with AIDS.* Intern Med, 2015. **54**(5): p. 513-8.

55.Anders, H.J., et al., *Ganciclovir and foscarnet efficacy in AIDS-related CMV polyradiculopathy.* J Infect, 1998. **36**(1): p. 29-33.

CHAPTER VIII

Power Of A Study And Effect Size

Assist. Prof. Dr. Mustafa Çağatay BÜYÜKUYSAL[1]

[1]Zonguldak Bülent Ecevit Üniversitesi, Tıp Fakültesi, Biyoistatistik Anabilim Dalı, Zonguldak, Türkiye. cbuyukuysal@gmail.com, Orcid ID: 0000-0001-9810-5633

One of the most basic purposes of statistical methods is to make parameter estimations of a population using statistics from samples and to reach a consistent decision by testing the hypotheses (1). After a hypothesis is written, the researcher will never know whether his hypothesis is true or not. Also will never know whether he has made a correct decision when he accepts or rejects the hypothesis as a result of hypothesis testing. After hypothesis testing, one of the 4 possible states in the table below occurs (Table-1).

- Yazar Adı Soyadı kısmından önce mutlaka unvan eklenmelidir.
- Orcid ve E posta bilgisi yazar dipnotunda mutlaka bulunmalıdır.

Table 1. True decision situations and error types in statistical tests

Decision about null hypothesis (H_0)	**Reality**	
	H_0 True	**H_0 False**
H_0 Not reject	Correct Decision *(True negative)* *(probability = 1 - α)*	Tip-II Error *(False negative)* *(probability = β)*
H_0 Reject	Tip-I Error *(False positive)* *(probability = α)*	Correct Decision *(True positive)* *(probability = 1 - β);* ***(Power)***

Power of a study

As indicated in Table-1, there are 2 types of errors (Type-I and Type-II errors) that the researcher may make as a result of a hypothesis test. The error that occurs when the correct null hypothesis (H0) is rejected is called α (alpha) error or Type-I error. However, the error that occurs when the false hypothesis H0 is accepted is called β (beta) error or Type-II error. The probability of accepting the true hypothesis H0 is (1 - α) and this probability is called the confidence level while the probability of rejecting the null hypothesis H0 is (1 - β). This probability is also called the power of the test. In other words, power is the probability that the hypothesis test correctly captures the existing difference (2). At most studies, whether it's in the health or social field, an alternative method being tested to an existing practice. In this case, since failing to detect a

clinically significant difference between the two methods (accepting H0 when it is actually false) would be a serious error, it is very important for the reliability of the study that the Type-II error is low, in other words, that the power of the study is high. In a hypothesis test, α is usually 0.05, 0.01 or 0.001, while the power of the test (1 - β) is required to be at least 0.80. The power of a significance test is a probability that varies with effect size, α (alpha) value and sample size. A calculation without taking any of these values into account would not be very valid.

Significance Level (p value)

The value known as the p-value is the amount of error if the null hypothesis (H0) is rejected and also is called as Type-I error. When comparing two independent groups, if a p-value of a hypothesis test is smaller than the selected alpha level (usually this value is taken as 0.05), it will be assumed as a significant difference between the groups. In statistical comparisons where the sample size is very large, the p-value will almost always be smaller than the chosen alpha level. In other words, a statistically significant difference will be found. In this way, however, the statistical significance resulting from a large sample size may not have clinical relevance. Most researchers will find it difficult to explain the statistical significance they obtain when they overlook this fact. Statistical significance depends on the sample size as well as the effect size. However, the effect size is not affected by the sample size. For example, in a study comparing control and treatment groups in terms of walking test score; if clinical significance will occur when there is a difference of at least 6 points in the treatment group while the average walking test score is 30 points in the control method, the effect size for the comparison between the two groups is calculated for the 6-point difference between the groups. Therefore, even if the sample size is large, a difference of less than 6 points between the groups will not be statistically significant in the comparisons to be made considering

the effect size. Therefore, reaching a conclusion based only on sample size and p-value without considering the effect size will often not give accurate results.

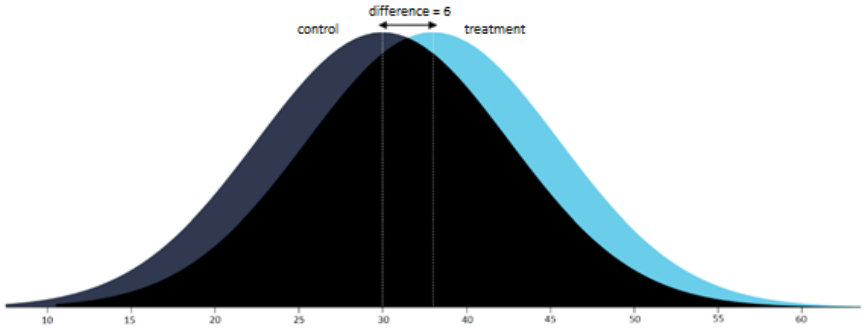


Figure 1. Expected difference between control and treatment groups

Effect Size

Especially when different treatment groups are compared in health studies, whether there is a significant difference between the treatment groups can be explained by a p-value, while how effective a treatment is, i.e. which method is superior, can be explained by an effect size (3). In the simplest terms, effect size can be defined as the clinically significant difference between the means of two compared groups (4). Before calculating the effect size, there are some situations that the researcher should pay attention to. The first one is which standard deviation the researcher will use if comparing two groups. Although it is generally recommended to use the standard deviation of placebo or control groups that have not been exposed to any factor in research in this field, it would be more accurate to use the pooled standard deviation, which averages the standard deviation of the two groups (5). The combined standard deviation is the average of the standard deviations of the experimental and control groups. As can be seen in Equation 1, the pooled standard deviation proposed here is not a standard deviation value calculated by adding all values in the groups together. For example, if the standard deviation of both groups is very low but

the group means are quite different, the true pooled mean (equation-1) will be lower than the standard deviation calculated by pooling all values (5).

$$\sigma_{pooled} = \sqrt{\frac{(n_1-1)s_1^2+(n_2-1)s_2^2}{n_1+n_2-2}} \qquad (1)$$

Where n1 and n2 are the number of subjects in the experimental and control groups. However, the use of the pooled standard deviation is somewhat biased, although it is a better estimator. Helgens and Olkin (1984) proposed a correction formula for this bias (6).

$$\sigma[d] = \frac{\mu_D-\mu_K}{SD_{pooled}}\ x\ \left(\frac{N-3}{N-2,25}\right) x\ \sqrt{\frac{N-2}{N}} \qquad (2)$$

Where N is the total number of people in the study. Another situation that the researcher should pay attention to is to check the assumption that the groups fit the normal distribution in calculations related to effect size. If one or all of the groups in the study do not fit to the normal distribution, it will be difficult to calculate and interpret an effect size.

Parametric Effect Size Measures for Two Independent Groups

Cohen d Effect Size

This criterion, proposed by Jacob Cohen in 1962, is calculated as the ratio of the difference between the group means to the standard deviations of the groups under the assumption that the variances of the two groups are homogeneous. Accordingly, Cohen's d effect size is shown as in Equation 3.

$$d = \frac{\overline{\mu}_1-\overline{\mu}_2}{\sigma_{pooled}} \qquad (3)$$

The standard deviation indicated here is the population standard deviation. However, since it is not possible to know this value in studies, the standard deviation of either group or a "pooled" standard deviation from both groups is

obtained (equation 1). In this equation, s1 and s2 indicate the standard deviations of the groups and n indicates the number of samples (4). The Cohen's d effect size can also be calculated from the t-test value of the difference between groups in two different ways (equations 4 and 5).

$$d = \frac{2t}{\sqrt{df}} \qquad (4)$$

$$d = \frac{t(n_1+n_2)}{\sqrt{df}\sqrt{n_1 n_2}} \qquad (5)$$

In this equation, df indicates the degrees of freedom for the t-test and n indicates the number of samples in the groups. Although Jacob Cohen has generally defined small (d=0.2), medium (d=0.5) and large (d=0.8) effect sizes for effect size, this classification is relatively subjective and relying only on this classification may bring some risks in practice.

Glass Delta Effect Size

The Glass Delta effect size is a measure obtained by taking the control group as the standard and proportioning the difference between the means of the experimental and control groups to the standard deviation of the control group (equation 6).

$$\Delta_s = \frac{\bar{x}_c - \bar{x}_e}{s_c} \qquad (6)$$

Here $\bar{x}_c$ ve $\bar{x}_e$ are the means of the control and experimental groups, while sc is the standard deviation of the control group.

Hedge g Effect Size

Hedges and Olkin (1982) proposed an effect size measure obtained by adding a small correction term to the Cohen's d effect size when the sample size is below 20. As shown in Equation-7, although the formula is similar to the

Cohen's d effect size, a correction factor was added at the end of the formula to reduce bias due to small sample size (n<50).

$$g = \frac{\bar{\mu}_1 - \bar{\mu}_2}{\sigma_{pooled}} \left(\frac{n-3}{n-2{,}25}\right) \sqrt{\frac{n-2}{n}} \qquad (7)$$

Here n is equal to n1 + n2.

Nonparametric Effect Size Measures for Two Independent Groups

Cliff Delta Effect Size

Nonparametric effect size measures are used when the measurement variable is not continuous and the data do not fit to normal distribution. The Cliff Delta effect size measure estimates the probability that a selected value in one group is greater than the corresponding value in another group. With x1 and x2 being the scores within groups, the Cliff Delta effect size measure is calculated as in equation 8 (8).

$$Cliff\ Delta = \frac{\#(x_1 > x_2) - \#(x_1 < x_2)}{n_1 n_2} \qquad (8)$$

Where "#" is the number of events that satisfy the relevant condition. Cliff Delta value takes a value between -1 and +1. Values of -1 and +1 indicate that there is no overlap between the two groups, while 0 indicates that the group distributions completely overlap (6).

VDA (Vargha &Delaney A) Effect Size

The VDA effect size measure refers to the sum of the probability that a randomly selected score from group 1 is greater than a randomly selected score from group 2 and the probability that a randomly selected score from group 2 is equal to a randomly selected score from group 1 times 0.5 (6, 9).

$$A_{12} = P(X_1 > X_2) + 0{,}5P(X_1 = X_2) \qquad (9)$$

Here, the indices below the criterion A indicate the groups compared. Although the VDA effect size measure is not continuous, it can be used at least in data measured with ordinal scale. Values of 0.56, 0.64 and 0.71 in the VDA effect size correspond to small, medium and large effect sizes, respectively (9).

Rank-Biserial Correlation Coefficient Criterion

Another nonparametric effect size measure is the rank-biserial correlation measure. This criterion is calculated for the Mann Whitney U test, for which 3 formulas have been proposed. In 1953, Cureton developed the definitions of agreement (agreement, P) and inversion (inversion, Q) pairs when comparing two groups. Where P is the number of eligible pairs and Q is the number of ineligible pairs, the relevant effect size measure is shown as in Equation 10 (10).

$$r = \frac{P-Q}{P_{max}} \qquad (10)$$

Conversions between Effect Sizes

Effect sizes calculated for different studies or different hypothesis tests can be converted to another effect size. df to indicate the degrees of freedom (11),

Transformation of Cohen's d effect size into Hedges' g statistic can be done as in Equation 11.

$$g = d\sqrt{\left(\frac{df}{n_1+n_2}\right)} \qquad (11)$$

Conversion of Cohen's d effect size to r (equations-12 and 13),

$$n_1 \neq n_2 \; ise \; r = \frac{d}{\sqrt{d^2+\frac{(n_1+n_2)^2}{n_1 n_2}}} \qquad (12)$$

$$n_1 = n_2 \; ise \; r = \frac{d}{\sqrt{d^2+4}} \qquad (13)$$

Transforming Hedges' g statistic into Cohen's d effect size (equation-14),

$$d = \frac{g}{\sqrt{df_{hata}}} = g\sqrt{\left(\frac{n_1+n_2}{df}\right)} \qquad (14)$$

Conversion of Hedges' g statistic to r (equation-15),

$$r = \sqrt{\frac{g^2 n_1 n_2}{g^2 n_1 n_2+(n_1+n_2)df}} \qquad (15)$$

Transforming the Glass Δ statistic into Cohen's d effect size (equation-16),

$$d = \frac{\Delta}{\sqrt{df_{hata}}} \qquad (16)$$

Transforming the Glass Δ statistic into r (equation-17),

$$r = \sqrt{\frac{\Delta^2 n_1 n_2}{\Delta^2 n_1 n_2+(n_1+n_2)df}} \qquad (17)$$

Transforming r into Hedges' g statistic (equation-18),

$$g = \frac{r}{\sqrt{(1-r^2)}}\sqrt{\frac{df(n_1+n_2)}{n_1 n_2}} \qquad (18)$$

Conversion of r to Cohen's d effect size (equation-19),

$$d = \frac{2r}{\sqrt{(1-r^2)}} \qquad (19)$$

Effect size refers to the size of the effect in the clinic and is very important in pre-study power analysis to determine the required sample sizes in planned studies. As mentioned at the beginning of the paper, the power of a statistical test (1 - β) is the probability of rejecting a false H0 hypothesis. Since the

statistical power will increase as the effect size increases, it is very important for the researcher to estimate the possible effect size or to decide on the minimum effect size of interest for the proposed research (6).

REFERENCES

1. Kazım Özdamar. SPSS ile Biyoistatistik. Kaan Kitabevi, 5. Baskı, 2003. ISBN: 975-6787-07.4
2. Reha Alpar. Spor, Sağlık ve Eğitim Bilimlerinden Örneklerle Uygulamalı İstatistik ve Geçerlik-Güvenirlik. Detay Yayıncılık. 2. Baskı, Eylül 2012.
3. Gail M. Sullivan, Richard Feinn. Using Effect Size – or Why the p Value Is Not Enough. Journal of Graduate Medical Education, September 2012.
4. Jacop Cohen. Statistical Power Analysis for the Behavioral Sciences, Lawrence Erlbaum Associates Publishers, 2nd Edition, 1988.
5. Robert Coe. It's the Effect Size, Stupid. What effect size is and why it is important. British Educational Research Association annual conference, Exeter, 2002.
6. Ayşegül Yabacı Tak. Comparison of effect size methods. Bu Bursa Uludag University, Institute of Health Sciences, Master Thesis, Bursa, 2021.
8. Norman Cliff. Dominance statistics: Ordinal analyses to answer ordinal questions. Psychological Bulletin, 114(3), 1993.
9. Andras Vargha, Harold D. Delaney. A Critique and Improvement of the CL Common Language Effect Size Statistics of McGraw and Wong. Journal of Educational and Behavioral Sciences, 25(2), 2000.
10. Dave S. Kerby. The simple difference formula: an approach to teaching nonparametric correlation. Comprehensive Psychology, Volume:3, 2014.
11. Mehmet Onur Kaya. Comparison of the mantel haenszel and peto methods used in combining odds ratio. Bursa Uludag University, Institute of Health Sciences, Master Thesis, Bursa, 2016.

CHAPTER IX

Extracellular Vesicles And Clinical Studies Review

Gizem SOLAK

Faculty of Medicine, Uludag University, Bursa, Turkey 011800398@ogr.uludag.edu.tr

Introduction

Extracellular vesicles (EVs) facilitate intercellular communication by fusion to the plasma membrane or by endocytosis into recipient cells. Pan and Johnstone were the first researchers to describe EVs in 1983. Although the first researchers claim that EVs were a way to discard unwanted material from cells, the last studies find out that EVs are a way for intercellular communication in physiologic or pathologic situations. Extracellular vesicles are secreted by many types of living cells such as immune cells, red blood cells, kidney cells, tumor cells, etc. They are in different sizes, cargo, and surface markers. It contains growth factors, cytokines, lipids, RNAs, metabolites, and receptors. Microvesicles bud through the outward of the plasma membrane. Multivesicular bodies consist of budding of the endosomal inner membrane. (1) Isolation methods of EVs should be compared to generate a gold standard method to detect them specifically. To compare the results for better isolation, publications on EVs should be explained and made clear purification of EVs methods. (2) EVs can be absorbed via endocytosis or membrane fusion and then they release their contents into the cell. (3) Vesicles might also be released from nanotubular bodies extending from the plasma membrane.

(4) EVs include, phosphatidylserine ceramide ganglioside GM3, sphingomyelin, cholesterol, and disaturated lipids. (5,6) In a study of astrocytes and glioblastoma cells, DNA has been seen with the inclusion of genomic and mitochondrial DNA.(7) mRNAs, miRNAs, and rRNAs, fragments of tRNA, long non-coding RNA short non-coding RNA, piwi-interacting RNA, Y RNA, vault RNA have been detected in EVs.(8-14).

Extended studies have been performed to analyze the content of EVs and three databases collected from lots of studies have been made. Three databases are accessible openly: Exocarta, Vesiclepedia, and EVpedia. (1) EVs are detected in blood, tears, saliva, extracellular fluids, and milk. (15) EVs are classified into the following three types: exosomes are less than 150 nm. MVs/shedding particles are 100~1000 nm, and apoptotic bodies are more than 1000 nm.

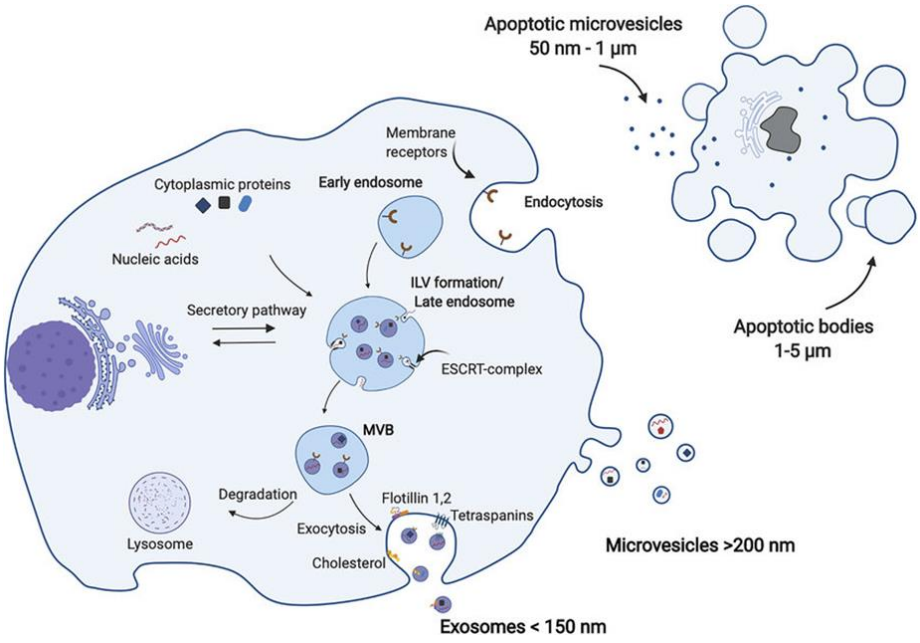


Figure 1. Biogenesis of Evs (37)

Cells uptake EVs through either fusion of plasma membrane or endocytosis. Endocytosis can be classified into the types of endocytotic processes, including clathrin-mediated endocytosis, caveolin-mediated endocytosis, lipid raft-mediated endocytosis, macropinocytosis, and phagocytosis. (6) EVs take away their contents to recipient cells as information. They function as long-distance cell-to-cell communication.

Extracellular vesicles can bind target cells by receptor–ligand activation and delivering their contents. EVs take part in intercellular signaling, coagulation, inflammation, and cellular homeostasis. (18) The content of EVs cause them to play a role in cancer and neurodegenerative (19), infectious, and autoimmune diseases. EVs are promising in diagnosis, treatment, and prognosis in clinical approach. Extracellular vesicles (cancer-derived) affect the tumor microenvironment and provide cancer progression. When the levels of these extracellular vesicles increase in blood and urine, cancer progress. (20, 21)

Isolation Methods

Prominent methods for EVs isolation: Ultracentrifugation, size exclusion chromatography, gel filtration, immunoblotting techniques, immunomagnetic extraction, density gradient centrifugation, nanomembrane ultrafiltration. (15) Ultracentrifugation, has some centrifugation stages to displace cells, large vesicles, debris, and precipitate exosomes. Therefore, this method is low-cost and not a hard application, but contaminants are risky. Ultrafiltration membranes are run to distinguish exosomes from other macromolecules. It is a fast and low-cost method but alteration of EVs structure and contamination problem is a risk. Density gradient centrifugation incorporates ultracentrifugation with sucrose or iodixanol. This method is good to purify the subpopulation of EVs but it is a laborious and time-consuming method. Immunoaffinity is a method using antibodies. Isolation of subpopulation and high precision are advantages of it. Exosome precipitation, the technique glycol changes compound polymerization precipitation exosome solubility. (16) Techniques characterization of

exosomes: TEM/SEM, dynamic light scattering, Bradford protein assay, exosome tracking, western blotting, flow cytometry. (17)

Clinical Studies

Extracellular vesicle cargo can use as a diagnostic marker. Patients with glioblastoma have correlated miRNA levels in their cerebrospinal fluid. (22) Another patient group with a high profile of extracellular miRNA levels in cerebrospinal fluid correlates with the status of Alzheimer's and Parkinson's diseases. (23) İn the 2016 breast cancer study miR-21, and miR-1246 revealed higher levels in the plasma samples' exosomes as compared to healthy group samples. It is promising for detecting breast cancer in the future. (24) In the study of de Gonzalo-Calvo and colleagues, lncRNA was studied as a biomarker of subclinical cardiac abnormalities in type 2 diabetes, and lncRNA was found a predictor of diastolic dysfunction in type 2 diabetes patients. (25) According to Momen Heravi et al, some miRNA levels were found high in animal models and humans with alcoholic hepatitis. Especially, according to ROC analyses, miRNA-192, miRNA-122, and miRNA-30a are diagnostic to define alcohol-induced liver injury. miRNA-192 was shown high potential for the diagnosis of Alcoholic hepatitis. (26) Recent studies indicate that exosomes regulate sensitivity to chemotherapeutical agents by containing noncoding RNAs. MicroRNAs (miRs), long non-coding RNAs, and circular RNAs facilitate the metastasis of tumor cells and escape from apoptosis. (27) Pasini et al, evaluated the immunologic responses in a group of patients who are nonsmall cell lung cancer and monitoring the clinical results. It gives hope for future immunotherapy studies. (28, 29) Extracellular vesicles (cancer-derived) affect the tumor

microenvironment and support cancer progression. When the levels of these extracellular vesicles increase in blood and urine, cancer progress. (30, 31) In the 2022 study, EVs can ensure the molecular fingerprint of the original cells, this attribute makes it our potential biomarker. Also, this shows us important pathways for cancer progression which is important for pharmaceutically. Some studies showed that the interaction of melanoma EVs with surrounding tissue can affect tumor metastasis progression at angiogenesis, tumor-stroma interactions, and lymphangiogenesis. (36) In the cerebral ischemia study, microRNAs 9, 124, 134, 152-3p, 223 are related to the seriousness of ischemia. MicroRNAs 152-3p levels are decreased in acute ischemic stroke. MicroRNAs 134 and 223 are related to poor prognosis. MicroRNAs 9, 124, and 134 are associated with infarct size. MicroRNAs 9, 124, and 134 with interleukin-6 levels are highly detected. (14, 32-35)

Conclusion

The review summarizes what EVs are and their clinical applications. Lots of studies have been published about EVs. Studies have revealed that EVs with high sensitivity and specificity can be used as liquid biopsies in the diagnosis and prognosis of patients' follow-up and responses to cure. It can be possible to have more processes when we have better isolation and classification method and techniques for EVs. They have valuable contents to carry to the recipient cells. It means that we have a “message” from cells. We just need to clarify the foreign language “message” to understand the cells. In the future, EVs should be clearer to develop immunotherapeutic techniques and use for cancer and some diseases as a biomarker. We believe that, in the near future, the

progression of high and quality performance detection techniques for exosomes will show us revolutionary databases that can be applied for EVs analyses for both clinical applications and research studies.

References

1) Kim DK, Kang B, Kim OY, Choi DS, Lee J, Kim SR, Go G, Yoon YJ, Kim JH, Jang SC, Park KS, Choi EJ, Kim KP, Desiderio DM, Kim YK, Lötvall J, Hwang D, Gho YS. EVpedia: an integrated database of high-throughput data for systemic analyses of extracellular vesicles. J Extracell Vesicles. 2013 Mar 19;2. doi: 10.3402/jev.v2i0.20384. PMID: 24009897; PMCID: PMC3760654.

2) Momen-Heravi F, Balaj L, Alian S, Tigges J, Toxavidis V, Ericsson M, Distel RJ, Ivanov AR, Skog J, Kuo WP. Alternative methods for characterization of extracellular vesicles. Front Physiol. 2012 Sep 7;3:354. doi: 10.3389/fphys.2012.00354. PMID: 22973237; PMCID: PMC3435888.

3) Mulcahy LA, Pink RC, Carter DR. Routes and mechanisms of extracellular vesicle uptake. J Extracell Vesicles. 2014 Aug 4;3. doi: 10.3402/jev.v3.24641. PMID: 25143819; PMCID: PMC4122821.

4) Gould SJ, Raposo G. As we wait: coping with an imperfect nomenclature for extracellular vesicles. J Extracell Vesicles. 2013 Feb 15;2. doi: 10.3402/jev.v2i0.20389. PMID: 24009890; PMCID: PMC3760635.

5) Llorente A, Skotland T, Sylvänne T, Kauhanen D, Róg T, Orłowski A, Vattulainen I, Ekroos K, Sandvig K. Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. Biochim Biophys Acta. 2013 Jul;1831(7):1302-9. doi: 10.1016/j.bbalip.2013.04.011. PMID: 24046871.

6) Abels ER, Breakefield XO. Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. Cell Mol Neurobiol. 2016 Apr;36(3):301-12. doi: 10.1007/s10571-016-0366-z. Epub 2016 Apr 6. PMID: 27053351; PMCID: PMC5546313.

7) Guescini M, Genedani S, Stocchi V, Agnati LF. Astrocytes and Glioblastoma cells release exosomes carrying mtDNA. J Neural Transm (Vienna). 2010 Jan;117(1):1-4. doi: 10.1007/s00702-009-0288-8. Epub 2009 Aug 13. PMID: 19680595.

8) Crescitelli R, Lässer C, Szabó TG, Kittel A, Eldh M, Dianzani I, Buzás EI, Lötvall J. Distinct RNA profiles in subpopulations of extracellular vesicles: apoptotic bodies, microvesicles and exosomes. J Extracell

Vesicles. 2013 Sep 12;2. doi: 10.3402/jev.v2i0.20677. PMID: 24223256; PMCID: PMC3823106.

9) Cheng L, Sun X, Scicluna BJ, Coleman BM, Hill AF. Characterization and deep sequencing analysis of exosomal and non-exosomal miRNA in human urine. Kidney Int. 2014 Aug;86(2):433-44. doi: 10.1038/ki.2013.502. Epub 2013 Dec 18. PMID: 24352158.

10) Huang X, Yuan T, Tschannen M, Sun Z, Jacob H, Du M, Liang M, Dittmar RL, Liu Y, Liang M, Kohli M, Thibodeau SN, Boardman L, Wang L. Characterization of human plasma-derived exosomal RNAs by deep sequencing. BMC Genomics. 2013 May 10;14:319. doi: 10.1186/1471-2164-14-319. PMID: 23663360; PMCID: PMC3653748.

11) Ogawa Y, Taketomi Y, Murakami M, Tsujimoto M, Yanoshita R. Small RNA transcriptomes of two types of exosomes in human whole saliva determined by next generation sequencing. Biol Pharm Bull. 2013;36(1):66-75. doi: 10.1248/bpb.b12-00607. PMID: 23302638.

12) Xiao D, Ohlendorf J, Chen Y, Taylor DD, Rai SN, Waigel S, Zacharias W, Hao H, McMasters KM. Identifying mRNA, microRNA and protein profiles of melanoma exosomes. PLoS One. 2012;7(10):e46874. doi: 10.1371/journal.pone.0046874. Epub 2012 Oct 9. PMID: 23056502; PMCID: PMC3467276.

13) Nolte-'t Hoen EN, Buermans HP, Waasdorp M, Stoorvogel W, Wauben MH, 't Hoen PA. Deep sequencing of RNA from immune cell-derived vesicles uncovers the selective incorporation of small non-coding RNA biotypes with potential regulatory functions. Nucleic Acids Res. 2012 Oct;40(18):9272-85. doi: 10.1093/nar/gks658. Epub 2012 Jul 19. PMID: 22821563; PMCID: PMC3467056.

14) Li CC, Eaton SA, Young PE, Lee M, Shuttleworth R, Humphreys DT, Grau GE, Combes V, Bebawy M, Gong J, Brammah S, Buckland ME, Suter CM. Glioma microvesicles carry selectively packaged coding and non-coding RNAs which alter gene expression in recipient cells. RNA Biol. 2013 Aug;10(8):1333-44. doi: 10.4161/rna.25281. Epub 2013 Jun 17. PMID: 23807490; PMCID: PMC3817155.

15) Bano R, Ahmad F, Mohsin M. A perspective on the isolation and characterization of extracellular vesicles from different biofluids. RSC Adv. 2021 Jun 1;11(32):19598-19615. doi: 10.1039/d1ra01576a. PMID: 35479207; PMCID: PMC9033677.

16) Jia G, Sowers JR. Targeting endothelial exosomes for the prevention of cardiovascular disease. Biochim Biophys Acta Mol Basis Dis. 2020 Aug 1;1866(8):165833. doi: 10.1016/j.bbadis.2020.165833. Epub 2020 May 4. PMID: 32380265.

17) Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH. Review of the Isolation, Characterization, Biological Function, and Multifarious Therapeutic Approaches of Exosomes. Cells. 2019 Apr 3;8(4):307. doi: 10.3390/cells8040307. Erratum in: Cells. 2021 Feb 22;10(2): PMID: 30987213; PMCID: PMC6523673.

18) Boriachek K, Islam MN, Möller A, Salomon C, Nguyen NT, Hossain MSA, Yamauchi Y, Shiddiky MJA. Biological Functions and Current Advances in Isolation and Detection Strategies for Exosome Nanovesicles. Small. 2018 Feb;14(6). doi: 10.1002/smll.201702153. Epub 2017 Dec 28. PMID: 29282861.

19) Howitt J, Hill AF. Exosomes in the Pathology of Neurodegenerative Diseases. J Biol Chem. 2016 Dec 23;291(52):26589-26597. doi: 10.1074/jbc.R116.757955. Epub 2016 Nov 16. PMID: 27852825; PMCID: PMC5207170.

20) Grange C, Tapparo M, Collino F, Vitillo L, Damasco C, Deregibus MC, Tetta C, Bussolati B, Camussi G. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. Cancer Res. 2011 Aug 1;71(15):5346-56. doi: 10.1158/0008-5472.CAN-11-0241. Epub 2011 Jun 13. PMID: 21670082.

21) De Palma G, Sallustio F, Curci C, Galleggiante V, Rutigliano M, Serino G, Ditonno P, Battaglia M, Schena FP. The Three-Gene Signature in Urinary Extracellular Vesicles from Patients with Clear Cell Renal Cell Carcinoma. J Cancer. 2016 Sep 27;7(14):1960-1967. doi: 10.7150/jca.16123. PMID: 27877211; PMCID: PMC5118659.

22) Akers JC, Hua W, Li H, Ramakrishnan V, Yang Z, Quan K, Zhu W, Li J, Figueroa J, Hirshman BR, Miller B, Piccioni D, Ringel F, Komotar R, Messer K, Galasko DR, Hochberg F, Mao Y, Carter BS, Chen CC. A cerebrospinal fluid microRNA signature as biomarker for glioblastoma. Oncotarget. 2017 Jun 1;8(40):68769-68779. doi: 10.18632/oncotarget.18332. PMID: 28978155; PMCID: PMC5620295.

23) Burgos K, Malenica I, Metpally R, Courtright A, Rakela B, Beach T, Shill H, Adler C, Sabbagh M, Villa S, Tembe W, Craig D, Van Keuren-Jensen K. Profiles of extracellular miRNA in cerebrospinal fluid and serum from patients with Alzheimer's and Parkinson's diseases correlate with disease status and features of pathology. PLoS One. 2014 May 5;9(5):e94839. doi: 10.1371/journal.pone.0094839. PMID: 24797360; PMCID: PMC4010405.

24) Hannafon BN, Trigoso YD, Calloway CL, Zhao YD, Lum DH, Welm AL, Zhao ZJ, Blick KE, Dooley WC, Ding WQ. Plasma exosome microRNAs are indicative of breast cancer. Breast Cancer Res. 2016 Sep 8;18(1):90. doi: 10.1186/s13058-016-0753-x. PMID: 27608715; PMCID: PMC5016889.

25) de Gonzalo-Calvo D, Kenneweg F, Bang C, Toro R, van der Meer RW, Rijzewijk LJ, Smit JW, Lamb HJ, Llorente-Cortes V, Thum T. Circulating long-non coding RNAs as biomarkers of left ventricular diastolic function and remodelling in patients with well-controlled type 2 diabetes. Sci Rep. 2016 Nov 22;6:37354. doi: 10.1038/srep37354. PMID: 27874027; PMCID: PMC5118808.

26) Momen-Heravi F, Saha B, Kodys K, Catalano D, Satishchandran A, Szabo G. Increased number of circulating exosomes and their microRNA cargos are potential novel biomarkers in alcoholic hepatitis. J Transl Med. 2015 Aug 12;13:261. doi: 10.1186/s12967-015-0623-9. PMID: 26264599; PMCID: PMC4533956.

27) Słomka A, Kornek M, Cho WC. Small Extracellular Vesicles and Their Involvement in 1) 10.3390 / hücreler11182913. PMID: 36139487; PMCID: PMC9496799.

28) Pasini L, Ulivi P. Extracellular Vesicles in Non-Small-Cell Lung Cancer: Functional Role and Involvement in Resistance to Targeted Treatment and Immunotherapy. Cancers (Basel). 2019 Dec 21;12(1):40. doi: 10.3390/cancers12010040. PMID: 31877735; PMCID: PMC7016858.

29) Morse MA, Garst J, Osada T, Khan S, Hobeika A, Clay TM, Valente N, Shreeniwas R, Sutton MA, Delcayre A, Hsu DH, Le Pecq JB, Lyerly HK. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. J Transl Med. 2005 Feb 21;3(1):9. doi: 10.1186/1479-5876-3-9. PMID: 15723705; PMCID: PMC551593.

30) Grange C, Tapparo M, Collino F, Vitillo L, Damasco C, Deregibus MC, Tetta C, Bussolati B, Camussi G. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. Cancer Res. 2011 Aug 1;71(15):5346-56. doi: 10.1158/0008-5472.CAN-11-0241. Epub 2011 Jun 13. PMID: 21670082.

31) De Palma G, Sallustio F, Curci C, Galleggiante V, Rutigliano M, Serino G, Ditonno P, Battaglia M, Schena FP. The Three-Gene Signature in Urinary Extracellular Vesicles from Patients with Clear Cell Renal Cell Carcinoma. J Cancer. 2016 Sep 27;7(14):1960-1967. doi: 10.7150/jca.16123. PMID: 27877211; PMCID: PMC5118659.

32) Ji Q, Ji Y, Peng J, Zhou X, Chen X, Zhao H, Xu T, Chen L, Xu Y. Increased Brain-Specific MiR-9 and MiR-124 in the Serum Exosomes of Acute Ischemic Stroke Patients. PLoS One. 2016 Sep 23;11(9):e0163645. doi: 10.1371/journal.pone.0163645. PMID: 27661079; PMCID: PMC5035015.

33) Chen Y, Song Y, Huang J, Qu M, Zhang Y, Geng J, Zhang Z, Liu J, Yang GY. Increased Circulating Exosomal miRNA-223 Is Associated with Acute Ischemic Stroke. Front Neurol. 2017 Feb 27;8:57. doi: 10.3389/fneur.2017.00057. PMID: 28289400; PMCID: PMC5326773.

34) Song P, Sun H, Chen H, Wang Y, Zhang Q. Decreased Serum Exosomal miR-152-3p Contributes to the Progression of Acute Ischemic Stroke. Clin Lab. 2020 Aug 1;66(8). doi: 10.7754/Clin.Lab.2020.200106. PMID: 32776748.

35) Pluta R, Jabłoński M. Exosomes in Post-Ischemic Brain. In: Pluta R, editor. Cerebral Ischemia [Internet]. Brisbane (AU): Exon Publications; 2021 Nov 6. Chapter 6. PMID: 34905312.

36) Lattmann E, Levesque MP. The Role of Extracellular Vesicles in Melanoma Progression. Cancers (Basel). 2022 Jun 23;14(13):3086. doi: 10.3390/cancers14133086. PMID: 35804857; PMCID: PMC9264817.

37) Host-derived extracellular vesicles for antimicrobial defense - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Biogenesis-of-extracellular-vesicles-Extracellular-vesicles-are-produced-through-a_fig1_350953972 [accessed 12 Dec, 2022]

CHAPTER X

Zinc Oxide Green of nanoparticles Synthesis, Characterization and Anti-Bacterial effects made using Punica Granatum (Pomegranate) Extract.

Assoc. Prof. Dr. Sevim AKCAGLAR[1]

[1] Department of Medical Microbiology, Faculty of Medicine, Bursa Uludag Uni. Bursa /TURKEY e.mail:akcaglar@uludag.edu.tr Orcid ID: 0000-0002-4759-9634

INTRODUCTION

Nanotechnology, also known as control of matter at the atomic, molecular and supramolecular level; It has emerged as one of the basic disciplines with developments in various disciplines such as technological chemistry, physics, biology, environment, materials science, medicine and pharmacy, and advanced research support. [1] These features are; it enabled the preparation of antimicrobial, anticancer, anti-inflammatory surfactant, drug carrier and pharmacological products. Biotechnologically produced metallic nanoparticles (NPs) are of interest in scientific applications and technology because they are common in applications in the biomedical and physicochemical fields.

One of the most important public health problems in recent years is increasing antibiotic resistance. Side effects of drug-resistant bacteria create medical problems caused by the use of synthetic drugs. Zinc oxide nanoparticles (ZnO-NPs) are made using plant biomass/extract. These nanoparticles are suitable for use in medical and industrial fields. It is necessary to develop environmentally friendly, non-toxic methods for their synthesis.

Biological synthesis, also known as green synthesis; high pressure is a practical method used to obtain NPs easily and ecologically without the need for high temperature values and toxic chemicals. Plant-mediated biosynthesis of metallic NPs containing organic functional groups found in the plant occurs through biomolecules (such as proteins, vitamins, amino acids, enzymes, polysaccharides, citrates, and organic acids). [2,3]

Antibiotics are the most typical treatment method for bacterial pathogens, which has been going on for years. Antibiotics are basically pharmacological agents produced by any microorganism to kill or stop another microorganism from multiplying. Antibiotic production provides a selective advantage for the microorganism that produces it. In this process, the membrane structure of the

bacteria is disrupted and the cell goes into apoptosis. Recent studies have shown that bacterial pathogens show resistance to various antibiotics [4] due to increased unconscious consumption of antibiotics, thus limiting the effectiveness of these agents. This has led to the importance of new combined drug therapies.

The ever-increasing bacterial resistance formation among pathogenic bacteria, caused by inappropriate or misuse of antibiotics [5], is becoming an increasingly serious problem. In addition, coupled with the recent increase in biofilm-associated infections in humans, more effective agents and strategies have begun to be sought to combat antimicrobial resistance [5,6].

As a result of the literature review, it has been seen that metals and metal oxides have been used in the treatment of infections and diseases in the past years [8]. Effect of metals; its antimicrobial powers are selectively disrupting the process necessary for cell growth. In this way, it can act on Gram-positive and Gram-negative bacteria The basis of the antimicrobial mechanism; the action of metal oxides is based on the release of metal ions, which are absorbed by the microbial cell. [9-10].

In the biosynthesis of nanoparticles, plant extracts act as reducing, stabilizing and sealing agents [11]. It is reported that the pomegranate plant has important biomolecules and metabolites, including organic acids, polyphenols, flavonoids, anthocyanins, alkaloids, fatty acids and vitamins [12-14]. Pomegranate is a fruit with a high phenolic content. In this way, it can show antimicrobial, anthelmintic, anti-inflammatory and antioxidant properties [15].

Current studies provide information that zinc oxide nanoparticles can be used successfully in microorganism control and inhibition of infections caused by Staphlococcus Aureus, Escherichia Coli and C.albicans [16]. As extreme diseases are spreading around the world, it is crucial that this work be carried

over to replace antibiotics with leaf sap. This would not only save people from enduring major diseases, but also reduce belief in man-made antibiotics. Therefore, it is important to carry on this research to develop a new vaccine for specific diseases using plant extracts.

MATERIALS AND METHODS

Zinc nitrate [$Zn(NO_3)_2 \cdot 6H_2O$] 98% as a source, of $Zn2^+$, sodium hydroxide, and ethanol were bought, off Sigma, Mueller–Hinton agar (Sigma-Aldrich, St. Louis, MO, USA). All mixes were made using dilute aquatic. The bacterial family used in this research was American Type Culture Collection strains bought from BioMérieux. Pomegranate peels collecting the peels of the pomegranate fruit (PP) were purchased from a regional fair. [17] Later prepared unwater, the pomegranate peels were powdered into a mix of powder using a mechanical mortar. Fifty grams of the powdered plant materials were immersed in 500 mL flasks include, including 200 mL of deionized aquatic, and warmed to a warm plate at 50 ◦C for 30 min. The plates were then incubated at 25 ◦C for 24 h below magnetic mixing; thereafter, the extracts were strained using a Whatman grade 1 filter wrapper. The herb extracts were kept in a freezer at 4 ◦C for furthermore usage.

Synthesis of Zinc Oxide Nanoparticles

The zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) 0.1 M liquor was composed by melting 6.58 g in 300 mL distilled aquatic. To make the mixed constitution, ten milliliters of the waters pomegranate essences were more and more joined dropwise into the dilution while being mixed magnetically at 60 °C for around two hours. Later, mix, and preserve, in a dark department for 72 hours of investigation of the 72-hour color investigation, from reddy brown to dark brown. Then mıxed at 10.000 rpm for 10 min and the pellets were gathered. The isolated pellets were protected in airproof flasks for further investigation

and later dehydrated in a bakery at 80 C for 8 timers. On the contrary [18]. a brown to dark brown color change was observed.

Characterization of the Synthesized Metal Oxide Nanoparticles

The resulting synthesized metal oxide nanoparticles were designated by UV–visible spectroscopyX-ray diffraction (XRD, scanning electron microscopy (SEM), and Fourier transform infrared spectrophotometry (FTIR). The XRD analysis was appraised to provide knowledge related to the naivety, and crystal form of the defined granules. FTIR analysis was evaluated to describe the surface functional groups, in the leaf extract and the nanoparticle specimens (Tugarova et al., 2018). The microstructure and the composite, of the synthesized zinc oxide nanoparticles, were accomplished application of SEM analysis [17,18].

Antimicrobial sensitivity test

The antibacterial property of P. granatum/ZnO-NPs specimens was evaluated using the broth microdilution method joined to Clinical and Laboratory Standards Institute (CLSI) reports. Only, a colony of Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria were isolated from Mueller-Hinton agar (MHA) plates and inoculated into Mueller-Hinton broth (MHB). The culture was ıncubated 24 hours at 37°C. Subsequently, the bacterial condensation was systematized to an optical density (OD) of 1.0 at 600 nm (about 8 108 CFU/mL) with MHB. Two-fold series dilutions of P. granatum/ZnO-NPs were composed in 96- well plates to be the last test concentrations of 7, 15, 31, 62, 125, 250, 500, and 1000 mg/mL per well. 10 mL of bacterial suspension equal to 8 106 CFU/mL of exponentially increased bacterial cells were joined to the wells. The dishes were incubated at 35 ± 2 C for 18 h. After the at-night incubation, the plate was then evaluated for absorbance usage with a microplate reader (GloMax Discover Instrument, Promega) to define, the MIC that prohibited a

50% increase of the isolates, and MIC50 values. Positive control, imipenem antibiotics and fluconazole antifungal (1 mg/mL), and negative controls (blank, without microbial inoculum) were contained in all essays.

RESULTS AND DISCUSSION

P. granatum F. peel distill includes plenty of phytochemical composites that made major act decrease and stabilizing factors for the accomplished, capacity of ZnO-NPs. These compounds, punicalagin and gallic acid perform up regarding 73% of P. granatum distill, with a percent of 41% and 32% respectively [19]. Therefore, it is significant in the reducing proses.

Ultraviolet-Visible (UV–Vis) Spectroscopy

The recognition of the production of zinc oxide particles was characterized by research with UV–Vis spectroscopic work with a wavelength range of 200–800 nm. UV–Vis sorption spectra for zinc oxide nanoparticles synthesis from pomegranate leaf (ZnO-NPs-PL) indicated an absorbance peak at 290 nm and a dissimilar hard peak at 350 nm, while in the case of zinc oxide nanoparticle stimulation reported from (ZnO-NPs-PF), a stimulation sorption peak was considered at 345 nm (Fig1). [6-9]. The sorption summit provided demonstrates the formation of zinc oxide nanoparticles come after the green synthesis process, and so shows a combined shake of electrons of the particles with the light waves. The peak ratings provided are near the typical, line of the light sorption stage of zinc oxide nanoparticles at 360–380 nm. A parallel consequence was described by Ezealisiji et al. [15], where a potent peak at 359 nm was found. Lega where nanorod-image zinc oxide nanoparticles with a nanoparticle size area of 20–100 nm were synthesized, a similar sorption peak at 354 nm was provided. Magnifera Rajesh Kumar et al. found the absorption peak capacity of 355 nm [14-17].

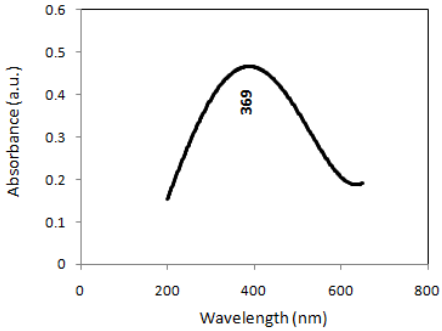


Figure 1. UV spectrum of ZnO NPs

X-ray Diffraction (XRD) Analysis

Specification of synthesized zinc oxide nanoparticles via pomegranate leaf distills was investigated by X-ray diffraction. This was made to aspecific the pure and crystal formation of the metal oxide nanoparticles [49, 50]. The provided diffraction peaks at 2values, a s demonstrated in Figure 2, were 31.71, 34.34, 36.25, 47.54, 56.62, 62.76, 68.00, 69.10, 72.43 and 72.43 for zinc 56.53, 62.84, 67.93, 69.05 and 72.69 for the pomegranate extract-intermediate zinc oxide nanoparticles. These peak values connect to the crystal or lattice plane of (100), (002), (101), (102), (110), (103), (200), (112), (201), and (202), with respect to the Joint Committee on Powder Diffraction Studies Standards (JCPDS These peak amounts relate to the crystal or lattice plane of (100), (002), (101), (102), (110), (103), (200), (112),(201) and (202), with respect to to the Joint Committee on Powder Diffraction Studies Standards (JCPDS card number 008, 82–1042 and 5–0664)

The correspondent plane, also known as Bragg's reflection line, recommends that the synthesized metal oxide nanoparticles might be of globular crystalline structure. Parallel, reactions were formed by Fu and Fu. [15], who biosynthesized zinc oxide nanoparticles in pomegranate leaf extracts.

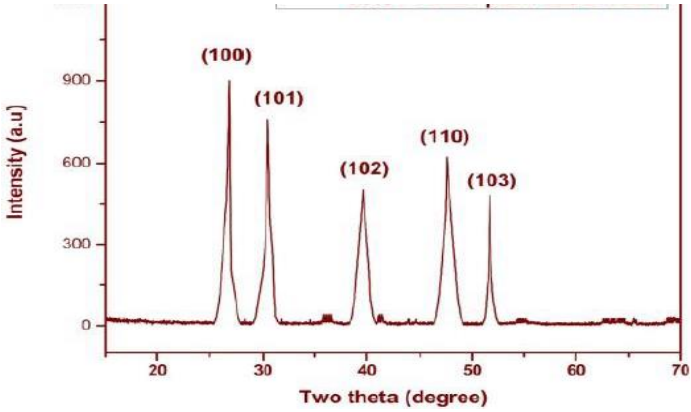


Fıgure2. X-ray Diffraction (XRD) Analysis

Fourier Transform Infrared (FT-IR) Spectroscopy

The composition and synthesis of functional groups in synthesized ZnO are shown by FT-IR. The spectrums of the samples were identified using a wavelength area of 4000–400 cm^{-1}, as investigated in Figure (3). Peaks are shown in 3340.50, 2990.01, 1615.5, 14010.05, 1091.05, 570.23, 455.361, and 429.084 cm^{-1}. The peak at (3340.50) cm^{-1} correlates with to N–H(Amine) link shaking of amine or amide groups. The less-than-wavenumber absorption peaks (453.001) cm^{-1} and the peak at 2990.01 cm^{-1} are determined to be the C=O stretch, which defines the chemical as a ketone. the peak at (1615.50) cm^{-1} corresponding to (C–N) strain vibrations of aromatic amines. Peak 1410.05 cm-1 corresponds to(C-H) incline in alkanes. the peak at (1091.05) cm^{-1} and (570.23)cm^{-1} verify C–X extend in alkyl halides. Finally, the band at (453.01cm^{-1}) and (429.054cm^{-1}) were due to C-N-C bending in amines. In order to produce ZnO, which was seen as a band and an efficient capping agent, the biomolecules in the plant extract are in charge of tendency zinc ions. This aids in the generation of NPs. These results whole declared the existence of effective groups in the biosynthesized ZnONPs, which is significant for their utilization and application.

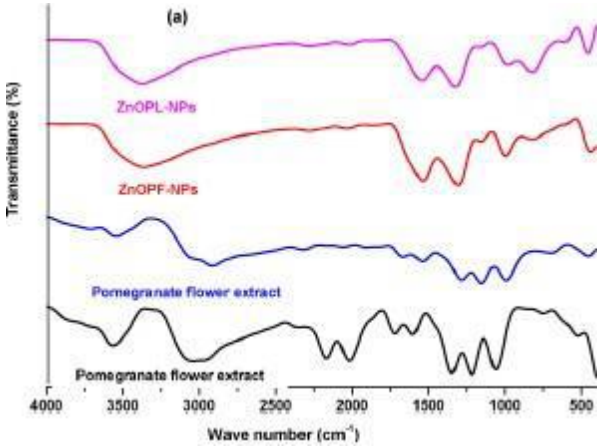


Figure 3. FTIR spectrum of the PL extract, PF extract, (ZnO–NPs–PL) and (ZnO–NPs–PF)

Biosynthesis spesification of ZnONPs

Today, the green production of zinc oxide nanoparticles (ZnONPs) using plant extracts is becoming more common every day. It has been shown that metal ions and biological systems interact to reduce them to metallic nanoparticles The use of these NPs produced by biosynthesis in different medical applications has been determined in many studiesThe green synthesis used the bioactive ingredient from the pomegranate extract in the present work and served taken into account to be a basic pase in the formation the increased color intensity of incubated of NPs, as a decrease metal ion and close agent detected in their investigation that the raised color density of incubated NPs was a requirement to start step in the production of NPs Nowadays researchs, SEM analysis was utilized to defined the structure and dimension of the biogenic zinc oxide nanoparticles (ZnO NPS) (SEM)) The dimension of metallic nanoparticles affects their spesifications and function (Fig5). In this example, our NPS was compatible and comparable to those of the others [20].

Figure 5.SEM image of pomegranate leaves synthesized with ZnO NPs

The crystalline nature of the biosynthesized zinc oxide nanoparticles ZnONPs, which are produced as a result of the reduction of metal ions (Zn2+) by the bioactive chemicals included in the plant extract, is depicted by the XRD pattern (Figure 2). Furtheremore, detected the current wide dimension of the biosynthesized nanoparticles. The offered XRD patterns are in agreement with first reports on microstructures His crystallites are nanoscale and have an elevated crystallinity, with respect to cording to the strong peaks

This investigates supports the findins made public by Abdelghany S al [20]. Whole, the findings of the current research and the liquid pomegranate extract green synthesis with respect to the mentioned key features are considered the correct approach for manufacturing stable ZnONPs rapidly Figure shows the visible peak in the UV-Vis spectra at 370 nm after 72 hours of incubation as illustrate in Figure (1).

The FT-IR data (Figure 4) demonstrated that the NPs of the pomegranate extract contained a variety of functional groups.Alkanes Phenols, alcohols,and aldehydes were the compounds with these functional groups[18,19]. Green Synthesis of Zno Nanoparticles Using Pomegranate.

Ultraviolet-Visible (UV-VIS) Spectroscopy

ZnO NPs' existence was verified by a substantial absorption band with a maximum of 370 nm in the 320–400 nm range. Green Synthesis of Zno Nanoparticles Using Pomegranate, absorption peak at 370 nm Figure (1).

Evaluation of antibacterial effects of P. granatum/ZnO-NPs

Based on several research papers, ZnO-NPs of particle sizes less than 37 nm were successfully synthesized in temperatures lower than 500 C .MIC value for these ZnO-NPs against E. coli and E. faecalis were reported to be 32 mg/mL and 16 mg/mL, respectively Smaller sizes of ZnO-NPs produce lower MIC value as compared to bigger-sized ZnO-NPs. The exact mechanism of ZnO-NPs killing of bacteria is still being debated and explored, but there are a few proposed antibacterial mechanisms usually discussed among scholars.

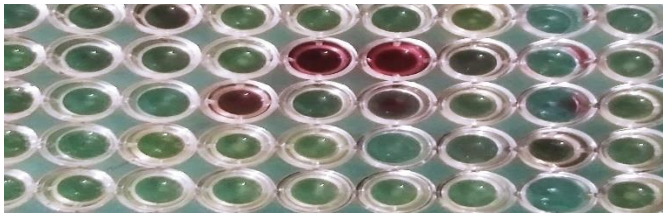


Figure6. Evaluation of antibacterial effects of P. granatum/ZnO-NPs

It is suggested that NPs directly interact with the bacterial cell wall or membrane by releasing metal ions that disrupt the cell permeability, causing damage to the first layer of defence [19]. Upon entry into the cells, theNPs will affect the bacteria's biochemical processes by causing damage to DNA and proteins denaturation [18]. This will finally trigger apoptosis or cell death as the bacteria fails to replicate normally.It can also be noted that Gram-positive bacteria, Gram-negative is more susceptible to as lower concentration of is needed to inhibit 50% E. faecalis growth compared to Gram-negative bacteria, E. Coli. This finding is similar to previous publications that reported presence of an outer membrane in Gram-negative bacteria might cause them to be more resistant to antimicrobials [20]. Cytotoxicity and antibacterial activities A Review on Antibacterial Activity of GreenSynthesis Zinc Oxide

CONCLUSION

Latterly disseminated literature has been seriously reviewed in this paper on the antibacterial analysis of green synthesis zinc oxide nanoparticle. At the present time, environmentally friendly resolvent, systems and eco-friendly reducing and covering factors have been used to form "Green" nanoparticles. The aim of the available processing was to investigate, the manufacture of zinc oxid nanoparticles using pomegranate fruit extract as a reducing agent. In UV-vis spectra, the prominent surface plasmon sorption peak of zinc at 370 nm served as proof that nanoscale zinc had formed. Scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy were utilized to analyze the morphology and crystalline nature of the produced zinc molecule.

In addition, the existence of the inhibition zone for nanoparticles implies that zinc oxide nanoparticles can be used as antibacterial agents. Green Synthesis of Zno Nanoparticles Using Pomegranate Fruit Extract Hence, it could be concluded that pomegranate peel extract is a good candidate for biosynthesis of ZnO-NPs and the utilized green method is effective It is expected that the synthesis of new generation NP-based drugs will be realized with the ongoing and newly planned studies, thus contributing to the development of new targeted treatment protocols.

References

1. Adnan Alnehia ,1,2 Abdel-Basit Al-Odayni ,3,4 Annas Al-Sharabi ,2 A. H. Al-Hammadi ,1 and Waseem Sharaf Saeed 3 Pomegranate Peel Extract-Mediated Green Synthesis of ZnO-NPs:Extract Concentration-Dependent Structure, Optical, andAntibacterial Activity Hindawi Journal of ChemistryVolume 2022, Article ID 9647793, 11 pages https://doi.org/10.1155/2022/9647793

2.Fauzul Adzim Azlan1, Ainun Rahmahwati Ainuddin @ Nordin1 RPMME A Review on Antibacterial Activity of Green Synthesis Zinc Oxide NanoparticleHomepage: http://publisher.uthm.edu.my/periodicals/index.php/rpmme e-ISSN : 2773-4765 DOI: https://doi.org/10.30880/rpmme.2022.03.01.043

3.Hemra Hamrayev 1, Kamyar Shameli 1,*, Serdar Korpayev Green Synthesis of Zinc Oxide Nanoparticles and Its Biomedical Applications: A Review Journal of Research in Nanoscience and Nanotechnology 1, Issue 1 (2021) 62-74

4.Mohamad Sukri, S. N. A., Shameli, K., Mei-Theng Wong, M., Teow, S.-Y., Chew, J., & Ismail, N. A. (2019). Cytotoxicity and antibacterial activities of plant-mediated synthesized zinc oxide (ZnO) nanoparticles using Punica granatum (pomegranate) fruit peels extract. Journal of Molecular Structure. doi:10.1016/j.molstruc.2019.04.026

5.Ifeanyichukwu, U. L., Fayemi, O. E., & Ateba, C. N. (2020). Green Synthesis of Zinc Oxide Nanoparticles from Pomegranate (Punica granatum) Extracts and Characterization of Their Antibacterial Activity. Molecules, 25(19), 4521. doi:10.3390/molecules25194521

6.Jun Xu1 , Yubo Huang1, Shihui Zhu1,Nedra Abbes2 , Xiaoning Jing1 and Liang ZhangA review of the green synthesis ofZnO nanoparticles using plant extracts

and their prospects for application inantibacterial textiles

7. Harun Ciftci Bioactive Nanoparticles Synthesized By Green Method Yeşil Yöntemle Sentezlenen Biyoaktif Nanopartiküller Black Sea Journal of Engineering and Sciencedoi: 10.34248/bsengineering.816084

8.Antibacterial Activities of Green Synthesized SilverNanoparticles from Punica granatum Peel ExtractLale Rozykulyyeva1, a), Suryani Dyah Astuti2, b), Andi Hamim Zaidan3, c), Anak Agung Surya Pradhana4, d), Putri Suryaning Puspita3,

9. Mohamed Taha Yassin * , Ashraf Abdel-Fattah Mostafa, Abdulaziz Abdulrahman Al-Askar and Fatimah O. Al-OtibiFacile Green Synthesis of Zinc Oxide Nanoparticles withPotential Synergistic Activity with Common Antifungal Agents against Multidrug-Resistant Candidal Strains Botany and Microbiology Department, College of Science, King Saud University, Riyadh

10.Ifeanyichukwu, U. L., Fayemi, O. E., & Ateba, C. N. (2020). Green Synthesis of Zinc Oxide Nanoparticles from Pomegranate (Punica granatum) Extracts and Characterization of Their Antibacterial Activity. Molecules, 25(19), 4521. doi:10.3390/molecules25194521

11.Soner Dönmez*Green Synthesıs And Characterızatıon Of Zınc Oxıde Nanopartıcles By Usıng Rhododendron Pontıcum L. Leaf Extract Turkish Journal Of Health Science And Life 021, Vol.4, No.1, 54-57.

12.Hala M. Abdelmigid 1 , Nahed Ahmed Hussien 2,* , Amal Ahmed Alyamani 1 , Maissa M. Morsi 2,Noha Moslah AlSufyani 2 and Hanan Abdulaziz kadi.,Green Synthesis of Zinc Oxide Nanoparticles Using Pomegranate Fruit Peel and Solid Coffee Grounds vs. ChemicalMethod of Synthesis, with Their Biocompatibility and Antibacterial Properties Investigation Molecules 2022, 27, 1236https://doi.org/10.3390/molecules27041236

13.Farjana Rahman1, Md Abdul Majed Patwary1,*, Md. Abu Bakar Siddique2, Muhammad Shahriar Bashar3, Md. Aminul Haque4, Beauty Akter1, Rimi Rashid5, Md. Anamul Haque6, A. K. M. Royhan Uddin1 Green

synthesis of ZnO nanoparticles using Cocos nucifera leaf extract: Characterization, antimicrobial, antioxidant, and photocatalytic activity https://doi.org/10.1101/2022.10.27.514023; this

14. Yassin, M.T.; Mostafa, A.A.F.; Al-Askar, A.A.; Al-Otibi, F.O. Facile Green Synthesis of Silver Nanoparticles Using Aqueous Leaf Extract of Origanum majorana with Potential Bioactivity against Multidrug Resistant Bacterial Strains. Crystals 2022, 12, 603.
15. He, G.; Huang, B.; Lin, Z.; Yang, W.; He, Q.; Li, L. Morphology transition of ZnO nanorod arrays synthesized by a two-step aqueous solution method. Crystals 2018, 8, 152. [CrossRef]
16. Priyadharshini, S.S.; Shubha, J.P.; Shivalingappa, J.; Adil, S.F.; Kuniyil, M.; Hatshan, M.R.; Shaik, B.; Kavalli, K. Photocatalytic Degradation of Methylene Blue and Metanil Yellow Dyes Using Green Synthesized Zinc Oxide (ZnO) Nanocrystals. Crystals 2021, 12, 22. [CrossRef
17. Mariappan Rajapriya., Sundararaj Aruna Sharmili., Raju Baskar.,Ravichandran BalajiNaiyf S. Alharbi4Shine Kadaikunnan.i,Jamal M. Khaled.,Khalid F. Alanzi.,, Baskaralingam Vaseeharan Synthesis and Characterization of Zinc Oxide Nanoparticles Using-Cynara scolymus Leaves: Enhanced Hemolytic, AntimicrobialAntiproliferative, and Photocatalytic Activity Journal of ClusterScience(2020)31:791–801https://doi.org/10.1007/s10876-019-0166
18. Rozykulyyeva, L., Astuti, S. D., Zaidan, A. H., Pradhana, A. A. S., & Puspita, P. S. (2020). Antibacterial activities of green synthesized silver nanoparticles from Punica granatum peel extract. The 2nd Internatıonal Conference On Physıcal Instrumentatıon And Advanced Materıals 2019. doi:10.1063/5.0034126
19. Barzinjy, A., Esmaeel, M., Aydin, S., Hussain, F., & Hamad, S. (2020). Biosynthesis and Characterization of Zinc Oxide Nanoparticles from

Punica Granatum (Pomegranate) Juice extract and its Application in Thin Films Preparation by Spin-Coating Method. Micro & Nano Letters. doi:10.1049/mnl.2019.0501

20. Abdelghany S. Shaban1 · Medhat E. Owda2 · Mostafa M. Basuoni1 · Mohamed A. Mousa1 Ahmed A. Radwan1 · Ahmed K. Saleh3 Punica granatum peel extract mediated green synthesis of zinc oxide nanoparticles: structure and evaluation of their biological applications Biomass Conversion and Biorefinery https://doi.org/10.1007/s13399-022-03185-7

ISBN: 978-625-6404-35-9