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Dear id health science readers...

We are very pleased with the interest in our journal. We would like to thank the scientists who are following our journal and make their valuable criticism and advice. In the following issues, we will reach our readers with a much wider index network. Kindest regards...

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ALTERED CALCIUM DYNAMICS IN HYPERPARATHYROIDISM: CALCULATION OF PMCA PUMP KINETICS IN ERYTHROCYTE GHOSTS

Armagan AKKUS[1*], Yusuf CANER[2], Saim OZDAMAR[3], Aysa HACIOGLU[4], Kursad UNLUHIZARCI[5]

ABSTRACT

Calcium is an important chemical for the metabolic functions of the cell. It is very important in the cellular process that the amount of calcium is balanced both inside and outside the cell. Calcium balance within the cell is achieved through channels and ATP-dependent pumps in the cell membrane. The plasma membrane calcium pump is the most important ATP-dependent pump that maintains calcium balance within the cell by removing calcium from the cell. The maximum calcium removal rate of PMCA (V_{max}) and the amount of calcium released from the pump (K_m) are the parameters that are effective in maintaining the calcium balance within the cell. In this study, the kinetic parameters of the PMCA pump of Hyperparathyroid patients were calculated. For this purpose, erythrocytes isolated from the blood of Hyperparathyroid patients were studied. The hemoglobins in the erythrocytes were emptied. These erythrocytes were closed again and erythrocyte ghosts with only cell membranes were obtained. In this study, erythrocyte ghosts were loaded with a buffer containing Indo 1 FF AM calcium indicator and calcium before being closed again. Calcium concentrations in erythrocyte ghosts were calculated from fluorescence intensities under a fluorescent microscope. From the calcium concentrations measured over time, the kinetic parameters of PMCA were calculated from the Michaelis-Menten equation. In Hyperparathyroid patients, K_m = 0.45 uM, V_{max} = 0.21 uM. According to these results, the PMCA pumice of Hyperparathyroid patients works faster and pumps more Ca out of the cell compared to the control group.

INTRODUCTION

Calcium (Ca^{2+}), which is responsible for cellular events such as muscle contraction, memory, learning, hormone release, gene transcription, fertilization, differentiation and development of cells, necrosis and apoptosis, is seen in most cellular reactions as an intracellular second messenger. The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is 100 nM when the cell is at rest state. When cells are stimulated in various ways, $[Ca^{2+}]_i$ can increase to 1–3 µM (1000–3000 nM). Ca^{2+}_i signaling consists of a transient increase in $[Ca^{2+}]_i$. The signal production function is directly proportional to the increase in cytosolic Ca^{2+} [1]. The increase in Ca^{2+} in the cytosol occurs due to the leakage of Ca^{2+} from organs that store Ca^{2+} within the cell or from the cell nucleus into the cytosol [2].

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As Ca^{2+} increases in the blood, parathyroid hormone (PTH) secretion from the parathyroid glands decreases. Conversely, when the calcium concentration decreases in the extracellular, these pathways are suppressed and PTH secretion is stimulated. Hyperparathyroidism is caused by excessive activity of the parathyroid glands. The parathyroid glands produce excessive amounts of hormones independent of Ca^{2+} in the blood[3].

Plasma Membrane Ca^{2+} ATPases (PMCAs) belong to the type IIB subfamily of P-type ATPases[4]. It is a high-affinity pump responsible for Ca^{2+} export across the cell membrane. The pump contains 10 transmembrane domains, two major cytosolic loops, and a long cytosolic C-terminal tail. The C-terminal tail of PMCAs is crucial for pump regulation[5]. Along with the PMCA pump, the Na/Ca converter is also present in the plasma membrane to ensure calcium balance within the cell. Although the two appear to work together, PMCA activity was found to be sufficient to maintain Ca^{2+} homeostasis in cardiac-specific Na/Ca exchanger knockout mice[6]. Since there is no Na/Ca converter in the erythrocyte membranes, Ca ions leave the erythrocyte vesicles via PMCA pumps. In other words, in our study, there is no other mechanism that can remove Ca ions from erythrocyte vesicles. The most important protein involved in pumping Ca ions out of the PMCA pump in cells is the Calmodulin (CaM) protein[7–9]. The CaM protein complex, which binds with Ca ions, regulates the operation of the PMCA pump by binding to the CaM binding region located at the C terminus of the PMCA pump[10]. The ATP-binding region is located between segments 4 and 5 of the 10-segment structure of the PMCA pump. Terminal C, in the rest state of the PMCA pump, this terminal is connected between segments 2 and 3 of the PMCA pump. Due to this connection, the region where ATP binds is closed. However, when Ca ions bind with CaM, the C terminal opens and ATP can be bound[11].

Red blood cells are the simplest, most common and well-defined cells in which PMCA has been studied extensively. PMCA activity was best determined in studies with erythrocyte membranes[12]. Erythrocyte ghosts are residues left after hemolysis of red blood cells. It is generally assumed that these residues lack intracellular structure and mainly contain cell membrane. For this reason, ghosts are widely used in the study of red blood cell membrane composition, structure and function[13].

In this study, the kinetic parameters of PMCA pumps of hyperparathyroid patients were measured in the erythrocyte ghosts of them. For this purpose, the erythrocytes of the blood of patients were emptied and Ca^{2+} and the Ca^{2+} indicator Indo 1 FF AM were loaded and sealed again. Kinetic parameters of the PMCA pump were calculated by monitoring Ca^{2+} outputs.

METHOD

Ethics approval

Ethics Committee of Erciyes University School of Medicine. Ethics committee number: 2010/70

Erythrocyte Isolation

In this study, 66 erythrocytes from the blood of untreated patients diagnosed with hyperparathyroidism who came to Erciyes University Faculty of Medicine Hospitals Endocrinology Metabolism outpatient clinics between 2016 and 2017, and 33 erythrocytes from healthy volunteer bloods were studied. As soon as 5 ml peripheral blood samples were taken into EDTA biochemistry tubes from patients diagnosed with hyperparathyroidism and control subjects without any health problems, they were centrifuged at 2000 rpm, 4°C for 5 minutes for erythrocyte isolation. The serum remaining on top was discarded, the remaining portion at the bottom was mixed with 5 ml NaCl solution and centrifuged at 2000 rpm, 4°C for 5 minutes. The supernatant was discarded and the pellet was washed 3 times with NaCl. 2.5 ml erythrocyte suspension was prepared.

Release of Hemoglobin from Erythrocytes

To remove the hemoglobin in the erythrocytes, 2 ul lysis solution ($MgSO_4$ 5 mM, Acetic acid 0.4 mM, pH 4.2) was added to 50 ul erythrocyte suspension. It was gently mixed every 5 minutes and kept for 15 minutes in ice. At the end of 15 minutes, it was centrifuged at 17000xg at 4°C for 20 minutes. The remaining supernatant was discarded. The pellet was resuspended in 1 ml KCl buffer (KCl 160 mM, Hepes 20 mM, pH 7.2). It was centrifuged again at 17000xg at 4°C for 20 minutes. The supernatant was discarded and the pellet was resuspended with 1 ml KCl buffer.

Loading of Erythrocyte Ghost with Ca²⁺ Indicator

Hemoglobin-depleted erythrocytes were loaded with loading solution containing Ca²⁺ indicator (DMN 200 µM, ATP 1 mM, Phosphokeratin 2.5 mM, Phosphokeratin kinase 500 U/l, CaCl 220 µM, Hepes 20 mM, KCl 160 mM, Indo1FF-AM 10 µM). For this, 400 µl of empty erythrocyte solution was taken and resuspended in ice with 100 µl of loading solution. After incubation on ice for 15 minutes, it was incubated at 37°C for 1 hour. After 1 hour, it was centrifuged at 5000 rpm for 10 minutes at room temperature. The upper part was discarded without touching the pellet and 500 µl KCl buffer was added.

Measurement of Fluorescence Intensity Inside Erythrocyte Vesicles

Fluorescence intensity from erythrocyte ghosts was measured by a Nikon Ti Eclipse fluorescence microscope. The erythrocyte ghost suspension was mixed slowly and gently. In a dark environment, 10 µl of this suspension was taken and placed on a clean slide. It was excited with a single wavelength every 5 minutes for 30 minutes and imaged at two emission wavelengths. Excited at approximately 350 nm. The decrease in fluorescence intensity was detected by taking photographs at the emission wavelength of Indo1 FF at ~405 nm when bound to Ca²⁺ and at the emission wavelength of Indo-1 FF at ~485 nm when Ca²⁺ was free.

Calculation of Fluorescence Intensity

Fluorescence intensity was measured with the Fiji 64 version of the Image J program from photographs taken every 5 minutes. A single erythrocyte was selected for each measurement. Fluorescence intensity was calculated from the area values and background values of the selected erythrocytes with the following formula:

Fluorescence intensity=Integrated intensity – (Area x Background average gray value)   (1)

Calculation of Ca²⁺ Concentration in Erythrocyte Ghosts

Analyzes using fluorescent ion indicators can be performed using ratiometric or non-ratiometric methods. Ca²⁺ concentration in cells loaded with indicators that can be both stimulated and measured with a single wavelength is calculated by non-ratiometric methods. The ratio between two fluorescence intensities is calculated by ratiometric measurements. In ratiometric measurements there is either an emission shift or an excitation spectrum shift. Since Indo1 FF is a ratiometric Ca2+ indicator, the Ca2+ concentration in erythrocyte ghosts was calculated with the ratiometric calculations of Tsien et al[14].

What all ratiometric methods have in common is that the intensity of the emitted light is measured twice and a ratio (R) of these intensities is calculated. A ratio value is calculated for each pixel of two simultaneously acquired images. Calcium concentration is calculated from formula 2.

$$\left[Ca^{+2}\right] = K_d \frac{\left(R - R_{\min}\right)}{\left(R_{\max} - R\right)} \frac{S_{f2}}{S_{b2}} \qquad (2)$$

$$R_{max} = \frac{S_{b1}}{S_{b2}} \qquad R_{min} = \frac{S_{f1}}{S_{f2}} \qquad R = \frac{F_1}{F_2}$$

Sf1, measured concentration of free Ca²⁺ indicators in λ1, S_{f2}, measured concentration of free Ca²⁺ indicators in λ2, S_{b1}, measured concentration of Ca²⁺ indicators bound with Ca²⁺ ions in λ1, S_{b2}, measured Ca²⁺ indicators bound with Ca²⁺ ions in λ2 is the measured concentration of indicators. Kd=Indo1 FF is the dissociation constant of the FF indicator. Indo1 FF dissociation constant is 230 nM.

Calculation of K_m and V_{max} of PMCA from Ca²⁺ Concentration

After calculating the Ca²⁺ concentration, the kinetic parameters of PMCA were calculated from these values. In these calculations, the enzyme-substrate relationship was taken as a model in the working principle of PMCA. The kinetics of PMCA were adapted according to the principles of enzyme kinetics. In enzyme kinetics, reaction rate expresses the change in concentration

of a product per unit time. The reaction rate in PMCA kinetics expresses the change in Ca^{2+} concentration per unit time.

Reaction Rate:

$$v = \frac{\Delta[Ca^{2+}]}{\Delta t} \qquad (3)$$

Its unit is mole/litre.time

$$v = \frac{V\,max[Ca^{2+}]}{Km + [Ca^{2+}]} \qquad (4)$$

Vmax refers to the maximum outflow rate of Ca^{2+} ion and K^m refers to the Ca^{2+} concentration corresponding to half the rate of V^{max}.

Statistical Analysis

Michaelis Menten graphs were drawn and V^{max} and Km values were calculated using Graph Path 8 software. T-student test was applied for statistical analysis of PMCA K^m and V^{max} values between the patient group and the control group. $p < 0.05$ was considered statistically significant.

FINDINGS

Calibration of Erythrocyte Ghosts

Erythrocytes were isolated from the blood and hemoglobin was removed from erythrocytes. The important thing here was that the bursting erythrocytes closed after loading. Various experiments were made for temperature and incubation time and the most suitable time was determined. When the hemoglobin in the erythrocyte ghosts is emptied and incubated in the loading solution at 37°C for 1 hour, a complete closure of the erythrocytes is observed as in Figure 1.

Figure 1. *Erythrocyte ghosts. During the preparation phase, some erythrocytes could not reseal after being discharged. Under the microscope, lysed erythrocytes are easily distinguished from others.*
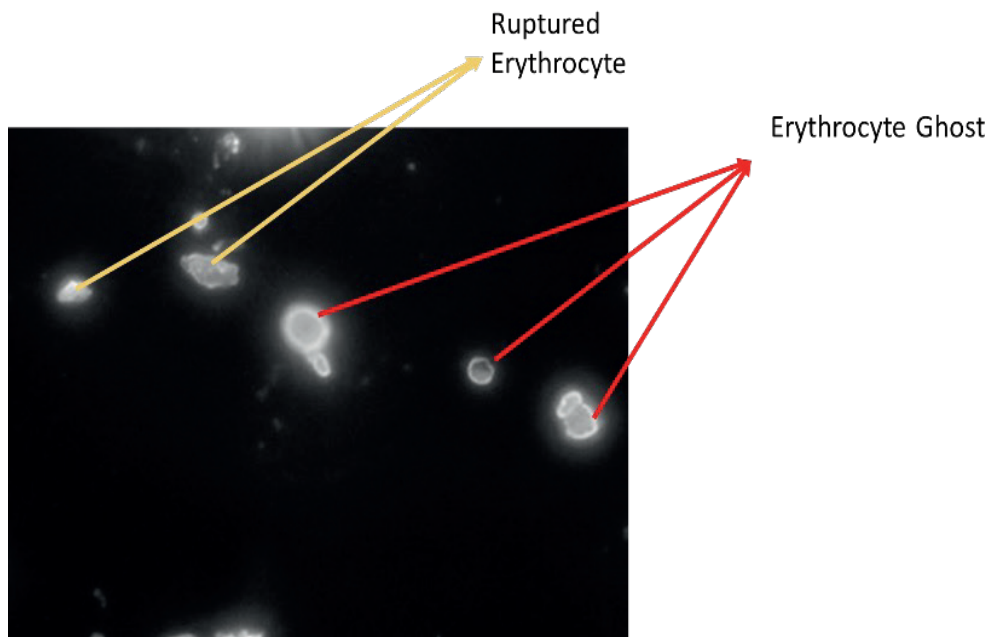
### Changes in Ca Concentrations From Fluorescence Intensities Over Time in Erythrocyte Ghosts

The loaded erythrocyte ghosts were photographed under a fluorescent microscope on DAPI and FITC filters at 5-minute intervals for 30 minutes. Accordingly, 924 images obtained from 66 erythrocyte ghosts of Hyperparathyroid patients and 464 images of healthy people's erythrocyte ghosts were analyzed in Image J software. Fluorescence intensities of erythrocyte ghosts were calculated over time in photographs taken with DAPI and FITC filters. Erythrocyte ghosts were evaluated one by one. The area and integrated density of the selected erythrocyte ghost were measured. A region was selected in the background area and the area and mean gray value of this region were measured. These values are the values measured in Image-J software for the erythrocyte ghost we selected. With these values, fluorescence intensity values were calculated over time from DAPI and FTIC images of erythrocyte ghosts from Formula 1.

Since Indo1-FF is a ratiometric indicator, ratio values (R) are required to calculate the $[Ca^{2+}]_i$ in erythrocyte ghosts. The R value was calculated by dividing the fluorescence intensity of erythrocyte ghosts measured at 405 nm wavelength by the fluorescence intensity measured at 485 nm. $[Ca^{2+}]_i$ change over time was measured from formula 2. It was observed that the $[Ca^{2+}]_i$ of the control group decreased statistically significantly over time ($p<0.001$) and had a perfectly negative decrease over time ($r=0.9646$) (Figure 2.A). Likewise, $[Ca^{2+}]_i$ in the ghosts of Hyperparathyroid patients has a negative correlation with time ($r=0.8585$) and decreased statistically significantly ($p<0.05$) (Figure 2.B). The change in Ca^{2+} concentration in the Hyperparathyroid group over time was found to be blunt with the change in Ca^{2+} concentration in the control group and was significant ($r=0.9537$, $p<0.001$). According to this result, the Ca^{2+} level of the patient group decreases together with that of the control group (Figure 2.C).

**Figure 2.** *Change of Ca^{2+} concentration in erythrocyte ghosts over time*
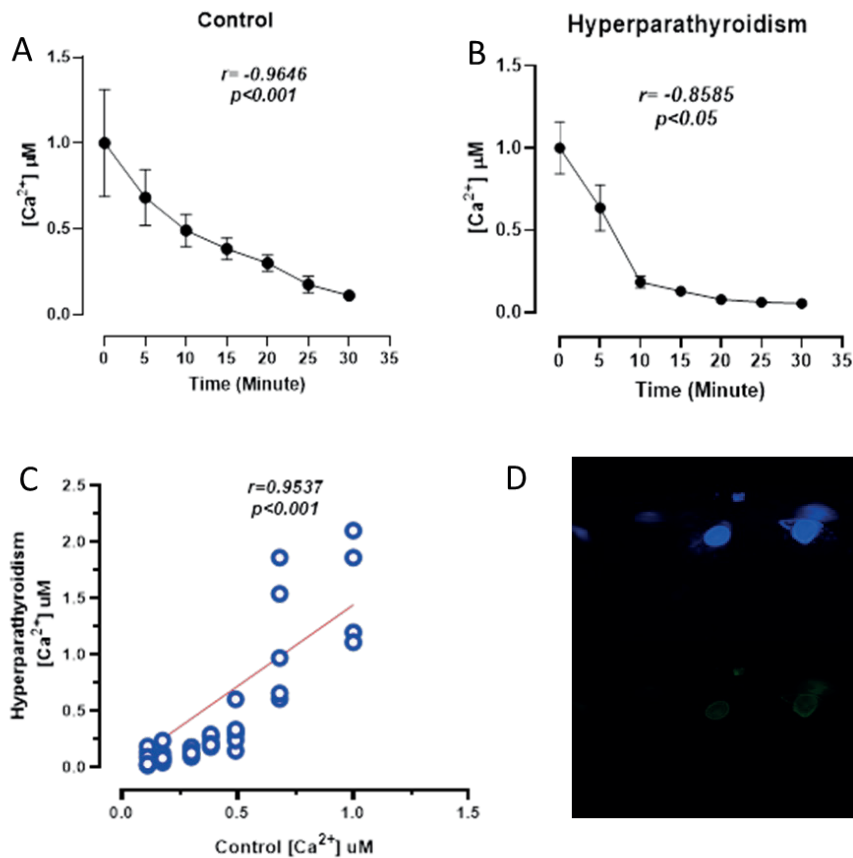
Ca^{2+} concentrations were calculated from the fluorescence intensity of the Indo1 FF AM Ca^{2+} indicator loaded on the erythrocyte ghosts of the control (A) and patient (B) groups. C). Graph showing the correlation of Ca2+ over time in the Hyperparathyroid and control groups. The Hyperparathyroid patient group has a strong positive correlation with the control group. D) Image of erythrocyte ghosts loaded with Indo 1 FF AM Ca^{2+} indicator under fluorescence microscope.

Ca^{2+} bound to the indicator (top, blue) Ca^{2+} not bound to the indicator (bottom, green).

Ca^{2+} was calculated as a percentage based on the concentration at minute 0 in both groups (Figure 3). While Ca^{2+} decreased to 76.4% after 5 minutes in the control group, it decreased to 56.2% in the patient group. There was a statistically significant decrease between the 0 minute and the 5 minutes of the patient group ($p<0.05$).

Figure 3. *The percentage of Ca^{2+} concentration values between the Control and the Patient groups*
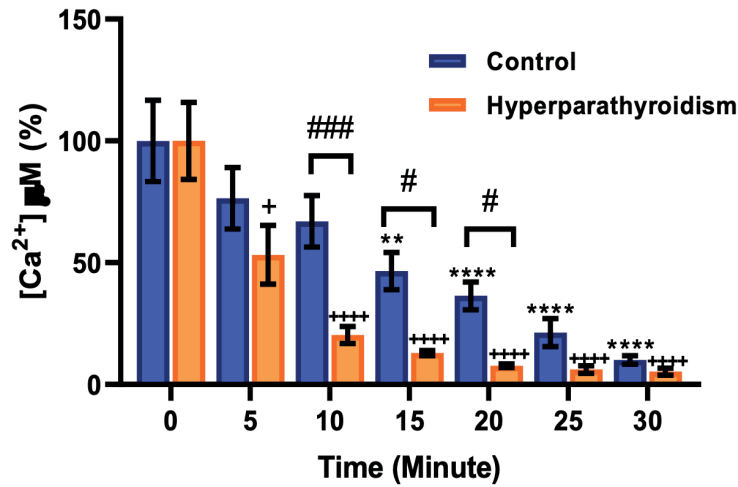
Percentage concentrations were calculated according to the Ca^{2+} concentration values measured at minute 0 of the control and patient groups over time, and the control group and patient groups were evaluated statistically according to the decrease amounts. ****,++++ $p<0.0001$, ### $p<0.001$, ** $p<0.01$, +,# $p<0.05$.

Kinetic Parameters of PMCA

To calculate the amount (K_m) and removal rate (V_{max}) of calcium ions in the erythrocyte ghosts of PMCA of Hyperparathyroid patients, a speed graph was drawn according to the calcium concentration in all erythrocytes (Figure 4). From these graphs, the calcium removal rate of the pump and the released [Ca^{2+}] were calculated. K_m is [Ca2+], which is half of V_{max}.

Figure 4. *Michaelis-Menten graphs of the Hyperparathyroid patient group and the control group*
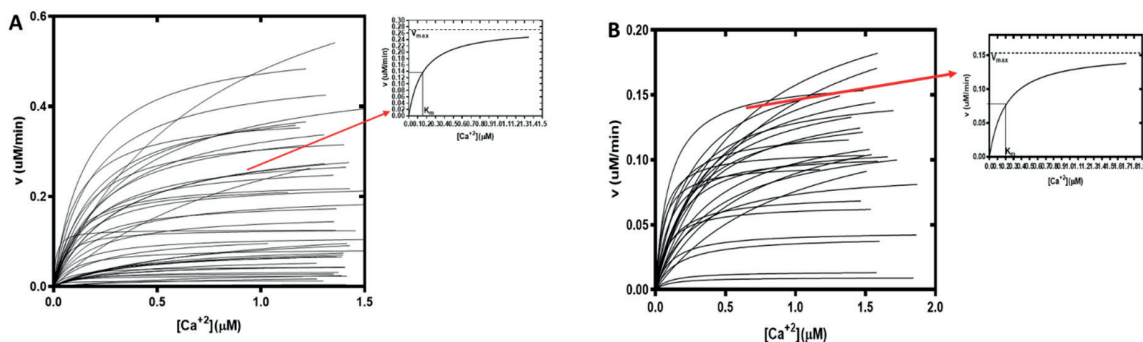
In Figure 4; A. Michaelis-Menten graphs of Hyperparathyroid patient groups. From the velocity graph relative to [Ca^{2+}], V_{max}, the rate of calcium ions leaving the pump, and K_m, the amount of calcium ions leaving the pump, were calculated. $K_m = V_{max}/2$. B. Control group Michaelis-Menten plots. Velocity curves relative to [Ca^{2+}] in control group erythrocytes.

When the K_m values of the Hyperparathyroid and control groups were compared with each other, the average K_m value of the PMCA pumps of the Hyperparathyroid patients was found to be 0.4541±0.069 uM, and the average K_m value of the PMCA pumps of the control group was found to be 0.2428±0.045 uM. A statistically significant difference was found between the amount of calcium released from the PMCA pump of Hyperparathyroid patients and the amount of calcium released from the PMCA pump of the control group ($p < 0.05$).

Figure 5. *The difference between the K_m and V_{max} mean values of PMCA in the Hyperparathyroid and control groups*
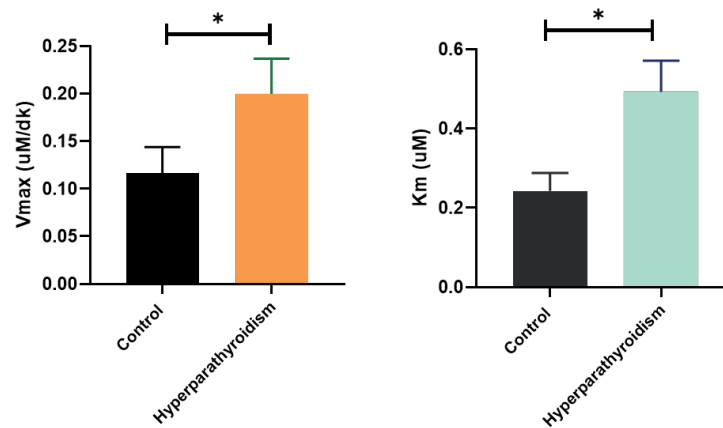
The V_{max} value calculated from the Michaelis-Menten graphs is higher and statistically significant in the Hyperparathyroid patient group than in the control group ($p < 0.05$). The calcium concentration rising at $V_{max}/2$ rate is higher and statistically significant in the patient group than in the control group ($p < 0.05$).

DISCUSSION

Ca^{2+} were labeled with a fluorescent marker and visualized in erythrocyte ghosts under a fluorescence microscope. A ratiometric Ca^{2+} indicator was used for this. In fluorescence measurements, side effects such as quenching of fluorescence and optical denstiy differences that affect the accuracy of the measurement are not taken into account in such ratiometric indicators. Because ratiometric Ca^{2+} indicators, when bound to Ca^{2+}, their fluorescence properties change and they are excited or radiate at different wavelengths. Therefore, they become normalized within themselves[15]. The fact that the PMCA pump can be best studied in erythrocyte membranes was proven by the first discovery of PMCA pumps in erythrocytes[16]. Generally, loading a Ca^{2+} marker into a cell is achieved by making the cell membrane permeable to the Ca^{2+} marker[17]. The Ca^{2+} marker was loaded into the erythrocytes by evacuating the hemoglobin and other proteins in the erythrocyte. The resealing erythrocytes without anything other than the chemicals loaded inside them means that the cell membrane will be activated only according to these chemicals[16].

There was any question of whether keeping it at low temperature for a long time or at high temperature for a short time during the closing phase would be more suitable for resealing. After the loading phase of erythrocyte ghosts, it was kept at 4°C overnight, as Teorell et al. tried. It was determined that the erythrocyte membranes were deformed and the resealable erythrocyte membranes were few. Schwoch et al. reported in their publication that resealing the erythrocyte membranes incubated at 37°C for 1 hour gave

better results[13]. Thereupon, after the erythrocyte ghosts were loaded, they were incubated for 1 hour at 37°C. Resealed erythrocytes appeared to retain their vesicle state without being damaged or reopened during subsequent washing steps.

Fluorescence intensity differed in images taken through two different filters. The DAPI filter revealed Ca^{2+} markers bound with Ca^{2+} within the erythrocyte ghosts. When PMCA takes Ca^{2+} out of the erythrocyte ghost, it cannot be detected with the DAPI filter because it releases Ca^{2+} markers. Therefore, the fluorescence intensities determined on the DAPI filter decreased over time. Since the Ca^{2+} marker remaining in the ghost was not bound with the Ca^{2+}, the emission wavelengths changed and were viewed with a FITC filter on the fluorescence microscope. Therefore, the measured fluorescence intensities of erythrocyte ghosts imaged with the FITC filter increased with time. Intracellular Ca^{2+} imaging studies performed in different tissues were also used[15,18–20]. After measuring the fluorescence intensities with two different filters, the Ca^{2+} concentration was calculated by ratiometric analysis[21–23].

Time-dependent Ca^{2+} concentration was evaluated between the Hyperparathyroid patient group and the control group. The amount of Ca^{2+} that decreased over time was found to be different between the two groups. While the Ca^{2+} concentration in the control group did not decrease significantly during the 5-minute period, the Ca^{2+} concentration in the Hyperparathyroid patient group decreased significantly in the first 10 minutes of the 5-minute time period. In healthy individuals, the decrease in Ca^{2+} inside and outside the cell over time varies from cell to cell and from person to person[1,24]. Since it is very important that the intracellular Ca^{2+} concentration is in balance, the decrease in the intracellular Ca^{2+} concentration over time should be in a way that does not disrupt this balance[25].

The kinetics of the active PMCA pump may be altered in Hyperparathyroid patients. Therefore, by calculating the kinetic parameters of the PMCA pump, it was determined numerically that this pump, located in the cell membrane, was affected by the calcium increase caused by hyperparathyroidism. The PMCA pump, which works with ATP phosphorylated by the enzyme, is suitable for the enzyme model, and this model has been supported by various studies[12,26].

In this model, the Ca^{2+} in the erythrocyte ghosts was assumed to be the substrate. The PMCA pump acts as an enzyme in this case. Therefore, the pump has a K_m constant and V_{max} speed depending on the enzyme kinetics[27]. Here, although K_m is a fixed value for each enzyme, since it varies depending on the substrate, the K_m values of the PMCA pumps of the erythrocyte ghosts obtained in the study were found to be different for each erythrocyte ghosts. K_m value was found to be different between the patient group and the control group compared to the control. K_m value is the substrate concentration required for the enzyme to perform its function. If K_m has a high value, the bond between the enzyme and the substrate is weak. In this case, the enzyme needs a large amount of substrate to reach its maximum speed[28].

In our study, the K_m value of PMCA pumps of Hyperparathyroid patients was found to be higher than the control group. This means that the PMCA pump needs to extract more calcium to activate. Only then does the pump remove Ca^{2+} at an increasing rate until it reaches a certain maximum value. When the maximum speed reaches V_{max}, the removal rate remains constant even if the Ca^{2+} removed increases. At high K_m value, it reaches this maximum speed at very high Ca^{2+} concentration[29]. In the study, a significant difference was found between the V_{max} values calculated in the patient and control groups compared to the control. The rate of removal of Ca^{2+} by PMCA pumps in Hyperparathyroid patients was found to be higher than in the control group. The fact that K_m and V_{max} values were different in Hyperparathyroid patients compared to the control group proved numerically that the PMCA pump works kinetically differently in patient tissues.

The V_{max} and K_m values of the normal human PMCA pump, calculated by Delgado et al. using erythrocyte ghosts, are 33 nmol/min and 2μM, respectively [30]. In studies conducted on liver cells, V_{max} values were found to be between 33 and 1,300 nmol/min, and K_m was between 25 nM and 1.6 μM[30]. In another study similar in method to our study, it was conducted with normal human erythrocytes and K_m for the PMCA pump was found to be 24±14 μM and V_{max} was 1.0±0.6 μM/s[27]. In our study, the K_m value of the PMCA pumps of the normal human group was found to be 0.2428±0.045 μM and the Vmax value was 0.1037±0.025 μM/min.

CONCLUSION

As a result, PMCA pumps, which are responsible for maintaining intracellular calcium balance, have important intracellular functions. It is at a key point in the survival of the cell and in the apoptosis of tumor cells. Therefore, disruption of the kinetics of the PMCA pump means disruption of the calcium balance of a healthy cell. In our study, it was shown that the kinetics of PMCA pumps are different in Hyperparathyroid cells, a type of disease in which the calcium balance is disrupted, compared to healthy cells. This difference may be due to various reasons. One of these reasons is that the calcium concentration circulating in the plasma is high in Hyperparathyroid patients, and therefore the amount of calcium entering the cells will be high. The PMCA pump will work faster to remove this calcium within the cell. Another reason may be that the PMCA pump in Hyperparathyroid patients is expressed more than normal.

Conflict of interest statement

The authors declare that they have no conflicts of interests.

Acknowledgements

REFERENCES

1. Berridge, M. J., Bootman, M. D. & Lipp, P. Calcium--a life and death signal. *Nature* 395, 645–648 (1998).

2. Montero, M. *et al.* Chromaffin-cell stimulation triggers fast millimolar mitochondrial Ca2+ transients that modulate secretion. *Nat. Cell Biol.* 2, 57–61 (2000).

3. Hall, J. E. & Guyton, A. C. *Guyton Medical Physiology,14 th Edition*. (2021).

4. Axelsen, K. B. & Palmgren, M. G. Evolution of substrate specificities in the P-type ATPase superfamily. *J. Mol. Evol.* 46, 84–101 (1998).

5. Krebs, J. Structure, Function and Regulation of the Plasma Membrane Calcium Pump in Health and Disease. *Int. J. Mol. Sci.* **23**, (2022).

6. Pott, C., Goldhaber, J. I. & Philipson, K. D. Genetic manipulation of cardiac Na+/Ca2+ exchange expression. *Biochem. Biophys. Res. Commun.* 322, 1336–1340 (2004).

7. Gietzen, K., Tejcka, M. & Wolf, H. U. Calmodulin affinity chromatography yields a functional purified erythrocyte (Ca+ + Mg2+)-dependent adenosine triphosphatase. *Biochem. J.* 189, 81–88 (1980).

8. Niggli, V., Penniston, J. T. & Carafoli, E. Purification of the (Ca2+-Mg2+)-ATPase from human erythrocyte membranes using a calmodulin affinity column. *J. Biol. Chem.* 254, 9955–9958 (1979).

9. Holton, M. L., Wang, W., Emerson, M., Neyses, L. & Armesilla, A. L. Plasma membrane calcium ATPase proteins as novel regulators of signal transduction pathways. *World J. Biol. Chem.* 1, 201 (2010).

10. Wuytack, F. & Raeymaekers, L. The Ca2+-transport ATPases from the plasma membrane. *J. Bioenerg. Biomembr.* 24, 285–300 (1992).

11. Osborn, K. D., Zaidi, A., Mandal, A., Urbauer, R. J. B. & Johnson, C. K. Single-Molecule Dynamics of the Calcium-Dependent Activation of Plasma-Membrane Ca2+-ATPase by Calmodulin. *Biophys. J.* 87, 1892 (2004).

12. Bredeston, L. M. & Rega, A. F. Pre-steady-state phosphorylation and dephosphorylation of detergent-purified plasma-membrane Ca2+-ATPase. *Biochem. J.* 361, 355 (2002).

13. Schwoch, G. & Passow, H. Preparation and properties of human erythrocyte ghosts. *Mol. Cell. Biochem.* **2**, 197–218 (1973).

14. Grynkiewicz, G., Poenie, M. & Tsien, R. Y. A new generation of Ca2+ indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260, 3440–3450 (1985).

15. Paredes, R. M., Etzler, J. C., Watts, L. T., Zheng, W. & Lechleiter, J. D. Chemical calcium indicators. *Methods* 46, 143–151 (2008).

16. Kubitscheck, U., Pratsch, L., Passow, H. & Peters, R. Calcium pump kinetics determined in single erythrocyte ghosts by microphotolysis and confocal imaging. *Biophys. J.* 69, 30–41 (1995).

17. Duchen, M. R. Mitochondria and calcium: from cell signalling to cell death. *J. Physiol.* 529, 57 (2000).

18. Ryan, T. A., Millard, P. J. & Webb, W. W. Imaging [Ca2+]i dynamics during signal transduction. *Cell Calcium* 11, 145–155 (1990).

19. Tsien, R. Y., Pozzan, T. & Rink, T. J. Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with a new, intracellularly trapped fluorescent indicator. *J. Cell Biol.* 94, 325–334 (1982).

20. Takahashi, A., Camacho, P., Lechleiter, J. D. & Herman, B. Measurement of intracellular calcium. *Physiol. Rev.* 79, 1089–1125 (1999).

21. Rohrbach, P. *et al.* Quantitative Calcium Measurements in Subcellular Compartments of Plasmodium falciparum-infected Erythrocytes. *J. Biol. Chem.* 280, 27960–27969 (2005).

22. Wendt, E. R., Ferry, H., Greaves, D. R. & Keshav, S. Ratiometric Analysis of Fura Red by Flow Cytometry: A Technique for Monitoring Intracellular Calcium Flux in Primary Cell Subsets. *PLoS One* 10, (2015).

23. Brawek, B. *et al.* A new approach for ratiometric in vivo calcium imaging of microglia. *Sci. Rep.* 7, (2017).

24. Petersen, O. H., Petersen, C. C. H. & Kasai, H. Calcium and hormone action. *Annu. Rev. Physiol.* 56, 297–319 (1994).

25. Bronner, F. Extracellular and Intracellular Regulation of Calcium Homeostasis. *Sci. World J.* 1, 919 (2001).

26. Graupner, M., Erler, F. & Meyer-Hermann, M. A Theory of Plasma Membrane Calcium Pump Stimulation and Activity. *J. Biol. Phys.* 31, 183 (2005).

27. Lew, V. L. *et al.* Distribution of plasma membrane Ca2+ pump activity in normal human red blood cells. *Blood* 102, 4206–4213 (2003).

28. Lorsch, J. R. Practical steady-state enzyme kinetics. *Methods Enzymol.* 536, 3–15 (2014).

29. Cornish-Bowden, A. The origins of enzyme kinetics. *FEBS Lett.* 587, 2725–2730 (2013).

30. Delgado-Coello, B., Trejo, R. & Mas-Oliva, J. Is there a specific role for the plasma membrane Ca2+-ATPase in the hepatocyte? *Mol. Cell. Biochem.* 285, 1–15 (2006).

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LIVER FAILURE DEVELOPING SECONDARY TO OVERDOSE OF PARACETAMOL USE

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ABSTRACT

 Paracetamol (acetaminophen, N-acetyl-P-aminophenol, APAP), which is found in many preparations in our country, is the most commonly used over-the-counter analgesic in the world. The drug, which is pregnancy category B, is widely used in pregnancy. Paracetamol is the most commonly reported drug poisoning agent. Paracetamol is metabolized via the liver. Paracetamol poisoning is the most common cause of liver failure. Liver toxicity is seen in single doses of 200mg/kg or 10gr, and repeated intakes of 100mg/kg or 4gr/ day. Gastric lavage can be performed in patients who apply within the first 2 hours of oral intake, and activated charcoal should be given in the first 4 hours. N-acetyl cysteine (NAC) is given as an antidote. The mainstay of treatment in patients with hepatic failure is liver transplantation. In our case, the laboratory results of a 27-year-old patient with a 19-week pregnancy who had abdominal pain and had an oral intake of 3gr IV and 2gr in the first 48 hours, followed by an oral intake of more than 3gr per day for about 3 days after she came to the emergency room. Ph:7.32, lactate: 5.6mmol/lt (>2) in blood gas, AST:7283 U/L (0-40), ALT: 3540 U/L (0-49), ALP:162 U/L, GGT:43 U/L, total bilirubin:2.9mg/ dL, D-dimer:19800 ng/mL, INR:4.54(0, 8-1,2), APTT: 37.1 sec, creatinine 1.44 mg/dL(<1.1), hepatitis markers were negative. Considering his history, laboratory results and clinic, the patient's paracetamol toxicity was evaluated as stage 3-4, and he was referred to the center where liver transplantation was performed with a preliminary diagnosis of fulminant liver, but the patient died without transplantation in the center she went to.

INTRODUCTION

Paracetamol (acetaminophen, N-acetyl-P-aminophenol, APAP) was introduced in 1955, which is found in many preparations in our country, is the most commonly used over-the-counter analgesic and antipyretic in the world1.The drug, which is pregnancy category B, is widely used in pregnancy,and used in children. Paracetamol is used alone in preparations or combined with many drugs. As of 2015, it is included in more than 300 drug preparations in Turkey alone 2. It is the most common form of voluntary or accidental drug poisoning because it is cheap and easily accessible.

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CASE

A 27-year-old female patient was brought to the emergency department with the complaint of abdominal pain. Her medical history included diabetes mellitus, familial Mediterranean fever (FMF), and pituitary adenoma. The patient, who was 19 weeks pregnant, had a history of hospitalization 2 days ago due to the increase in FMF attacks. General condition is moderate, conscious, cooperative, blood pressure 100/60 mm/Hg, saturation 99%, heart rate 104/min, fever 36.6 C, fingertip blood glucose 72mg/dl. EKG was in sinus rhythm. There was widespread tenderness in all quadrants on abdominal examination, and other systemic examinations were normal. Ph:7.32, lactate: 5.6mmol/lt (>2), in blood gas, AST:7283 U/L (0-40), ALT: 3540 U/L (0-49), ALP:162 U/L, GGT:43 U/L, total bilirubin: 2.9mg/dL, D-dimer:19800 ng/mL, INR:4.54(0, 8-1,2), APTT:37.1s, creatinine 1.44 mg/dL(<1.1), hepatitis markers were negative. In his history, the patient who used excessive amounts of paracetamol on the 2nd day of hospitalization and after discharge (3gr IV and 2gr oral intake in the first 48 hours and more than 3gr oral intake in the following day). Hydration was provided to the patient. Upon his application to us within the first 18 hours of taking paracetamol, the patient was given NAC (150mg/kg 200 cc 5% dextrose in 15 minutes, 50mg/kg 5% 500 cc dextrose in 4 hours, 100mg/kg 5% 1000 cc dextrose in 16 hours) iv planned. Liver failure due to paracetamol toxicity was considered and urgent referral was made to the liver transplant center.

DISCUSSION

Paracetamol is used in two forms as oral and intravenous (IV). Therapeutic dose is 10-15mg/kg in children; in adults, 325-1000mg every 4-6 hours for oral use. The maximum daily dose is 75/kg in children and 4g in adults. In children over 50 kg or adults, the intravenous dose is 650 mg every 4 hours or 1 g every 6 hours. The maximum dose for intravenous administration is 4g per day. For the toxicity of paracetamol, either high-dose acute intake or repeated high-dose intake is required. In acute single dose intake, >200mg/kg or 10gr in adults and children over 6 years of age, paracetamol toxicity in children younger than 6 years of age ≥200mg/kg. In adults and children over 6 years of age, repeated doses of >200mg/

kg or more than 10g are taken, and if >100mg/kg or 4gr/day is taken for longer than 48 hours, toxicity occurs. Toxicity occurs as a result of repeated doses ≥200 mg/kg in children under 6 years of age, ≥150mg/kg in a 24-hour period, and ≥100mg/kg every 24 hours in a 48-hour period[2]. Unfortunately, no method has yet been developed for the potential toxicity that may occur after intravenous acetaminophen overdose[5].

Paracetamol is rapidly absorbed in the stomach and intestine after oral intake and is metabolized in the liver[2]. 90% of the paracetamol taken in the treatment dose range is conjugated with sulfate and glucuronide in the liver and metabolized, and the metabolites are excreted in the urine[3]. Almost 2% is excreted unchanged in the urine. The remaining about 8% is converted to a toxic and highly reactive compound known as N-acetyl p-benzokinoimine (NAPQI) by hepatic cytochrome p450 enzymes (CYP2E1, CYP1A2, CYP3A4)[3-4]. Under favorable conditions, NAPQI is rapidly converted to non-toxic metabolite by glutathione (GSH). In cases of GSH deficiency such as paracetamol poisoning, chronic alcoholism and malnutrition, NAPQI is not metabolized and causes liver damage[6,7,8]. Although it is not known exactly how NAPQI damages the liver, it is thought to cause mitochondrial damage by reacting with sulfhydryl groups, causing protein deformation, and as a result, cell death [6,7].

It was first shown to cause hepatic necrosis in a patient in Scotland in 1966[9]. The most common cause of liver failure in America and Europe is the use of paracetamol[10]. Paracetamol-related hepatic failure accounts for 42% of all hepatic failures in the United States and It causes more than 300,000 hospitalizations[6,11]. In an epidemiological study, which included 7 countries, it was seen that 20% of liver transplantations were caused by paracetamol poisoning. This rate is 52% in Ireland, 28%in the UK, 8% in the Netherlands, 1% in Italy[12].

Single-time high-dose intakes have a better prognosis than repeated intakes, and it has been shown that those who present 24 hours after intake have the worst prognosis[1, 13.]

It can be evaluated in 4 clinical stages in paracetamol-related toxicity. Stage 1 is the period in the first 24 hours after ingestion of a toxic dose. Patients may be asymptomatic at this stage or show nonspecific signs of toxicity such as nausea,

anorexia, vomiting and malaise. Stage 2; Stage 1 symptoms regress on the second-third days of toxic dose intake, but signs of hepatotoxicity such as increased serum transaminases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels and right upper quadrant pain occur. In stage 2, most of the patients recover without sequelae. Stage 3 covers 3 to 4 days, some of the patients may go to fulminant liver failure, as well as characteristic metabolic acidosis, coagulopathy, kidney failure, encephalopathy and gastrointestinal symptoms. In stage 4, after day 5, either clinical improvement or multiple organ failure and death occurs. If the patient survives complications of fulminant liver failure, it starts to improve within two weeks and after 1-3 months the liver heals without sequelae[5]. Severe liver toxicity is defined as AST or ALT above 1000IU/L.[2]

Diagnosis is mostly made by anamnesis and blood paracetamol levels. Although it is a definite diagnosis to measure paracetamol level in the blood, it cannot be applied in every hospital[5].

Gastric lavage can be performed in patients who apply within the first 2 hours of oral intake, and activated charcoal should be given in the first 4 hours. N-acetyl cysteine (NAC) is given as an antidote. The mainstay of treatment in patients with hepatic failure is liver transplantation[5].

Acetylcysteine, the essential ingredient in the prevention and treatment of paracetamol toxicity. Although the mechanism of action of acetylcysteine is not known exactly, it is thought to have two important benefits. In the first 8 hours of paracetamol poisoning, acetylcysteine prevents toxicity by preventing NAPQI from binding to hepatic macromolecules. If there is toxicity for more than 24 hours, acetylcysteine reduces hepatic necrosis by acting as an antioxidant, reducing neutrophil infiltration, increasing microcirculation or increasing tissue oxygenation[5].

In paracetomal toxicity, the treatment approach should be the same in pregnant patient, acetylcysteine has been shown to be safe and effective in pregnant patients as well[5].

Intravenous acetylcysteine is preferred because of its easy use. Standard acetylcysteine therapy; a 150mg/kg loading dose is administered over 1 hour, followed by a first maintenance dose of 50mg/kg for 4 hours and a second maintenance dose of 100mg/kg for 16 hours. It can be given with 5% dextrose or 0.45% NACl[5].

Unfortunately, fulminant hepatic failure develops in a minority of patients who take an overdose of paracetamol. Especially in patients who do not receive acetylcysteine treatment, the mortality rate is more pronounced. Deaths are usually 3-5 days after drug intake. It occurs in days and is based on complications such as cerebral edema, hemorrhage, shock, acute lung injury, sepsis, and multiple organ failure. Acetylcysteine therapy in the treatment of fulminant liver failure due to paracetamol and treatments for complications and should be referred to a center for liver transplantation without delay[5]. The most common criterion used since 1989 for patients who may benefit from transplantation with paracetamol-induced liver elevation is the King's Collage criterion[14]. Markers of king's collage criteria (Table 1); despite fluid and hemodynamic resuscitation.metabolic acidosis (arterial pH<7.30) or coagulopathy (prothrombin time>100sec), renal failure (serum creatinine >3.3mg/dl or 292micromol/L) and grade 3 or 4 hepatic encephalopathy[14]. Some studies have shown that hypoglycemia and lactic acidosis are important indicators of mortality in liver failure[14].

CONCLUSION

Since paracetamol is easily accessible and cheap, its toxicity is very common. Although it is used in children, adults and pregnant women considering how safe it is, it harms patients if it is not taken in appropriate doses. Ingestion of paracetamol in high single doses or in consecutive high doses also causes toxicity. Paracetamol toxicity can result in asymptomatic, non-specific findings, fulminant liver failure and even death. Diagnosis is made by anamnesis and blood paracetamol level, and in patients whose paracetamol level cannot be measured, but who have high-dose paracetamol intake in the anamnesis, treatment should be started quickly. Since acetylcysteine, which is our main treatment, is easily accessible and applied, treatment should be started early, thus, liver damage should be reduced in patients. It should be kept in mind that fulminant liver failure, the need for transplantation and even death will occur in patients who present late or whose treatment is delayed. In cases where liver function tests are high, it should be kept in mind to question the use of paracetomol in patients.

Our patient did not meet the King's Collage criteria because of pH: 7.32, creatinine: 1.44 mg/dl, APTT: 37.1 seconds and he was conscious. However, lactate: 5.6 mmol and high, and 72 mg/dl fingertip glucose were considered as the poor prognostic factor of the patient.

Abbreviations

ALT: Alanine aminotransferase
ALP:Alkalen fosfataz
AST: Aspartate aminotransferase
ECG: Electrocardiography
FMF:Familial mediterranean fever
GGT:Gamma glutamyl transferase
GSH:Glutathione
IV:İntravenous
NAC: N-acetyl cysteine
NAPQI: N-acetyl p-benzokinoimine
UK:United Kingdom

Conflict of interest statement

The authors declare that they have no conflicts of interests.

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REFERENCES

1. Bateman DN. Changing the management of paracetamol poisoning. Clin Ther 2015;37(9):2135-41.

2. E Mücahit. Acetaminophen (paracetamol) Turkey Clinics J Emerg Med-Special Topics, 2016 - researchgate.net

3. Manyike PT, Kharasch ED, Kalhorn TF, Slattery JT. Contribution of CYP o acetaminophen reactive metabolite forma tion. Clin Pharmacol Ther 2000;67(3):275-82

4. Bessems JG, Vermeulen NP. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues andprotective approaches. Crit Rev Toxicol2001;31(1):55-138.

5. Tintinalli Emergency Medicine comprehensive study guide, 9th Edition Judith E. Tintinalli,MD,MS 2022

6. Lancaster EM, Hiatt JR, Zarrinpar A. Acetaminophen hepatotoxicity: an updated review. Arch Toxicol 2015;89(2):193-9

7. Jaeschke H, Williams CD, Ramachandran A,Bajt ML. Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. Liver Int 2012;32

8. Bunchorntavakul C, Reddy KR. Acetaminophen-related hepatotoxicity. Clin Liver Dis 2013;17(4):587-607

9. Davidson DG, Eastham WN. Acute liver necrosis following overdose of paracetamol. Br Med J 1966;2(5512):497-9.

10. Ghallab A. Highlight report: acetami-nophen hepatotoxicity. Arch Toxicol 2015;89(12):2449-51.

11. Blieden M, Paramore LC, Shah D, Ben Joseph R. A perspective on the epidemiologyof acetaminophen exposure and toxicity in theUnited States. Expert Rev Clin Pharmacol 2014;7(3):341-8

12. Gulmez SE, Larrey D, Pageaux GP, Bernuau J, Bissoli F, Horsmans Y, et al. Liver transplant associated with paracetamol overdose: resultsfrom the seven-country SALT study. Br J Clin Pharmacol 2015;80(3):599-606

13. Pakravan N, Simpson KJ, Waring WS, BatesCM, Bateman DN. Renal injury at first pres entation as a predictor for poor outcome in se vere paracetamol poisoning referred to a liver transplant unit. Eur J Clin Pharmacol2009;65(2):163-8.

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IDENTIFICATION OF THE BELIEFS TOWARD STIGMATIZATION AND MENTAL ILLNESS AMONG RELATIVES OF SCHIZOPHRENIA PATIENTS

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ABSTRACT

Stigmatization is an important problem that causes negative results for schizophrenia patients and their relatives. This study aims to identify beliefs toward stigmatization and mental illness among relatives of schizophrenia patients followed up in community mental health centers. The target population of the study was relatives of schizophrenia patients who were registered in Hatay Community Mental Health Centers (CMHC). The sample was relatives of schizophrenia patients who attended CMHCs actively (n=170). Data were collected through the Socio-demographic Form, the Stigmatization Scale for Relatives of Schizophrenia Patients (SSRSP), and Beliefs toward Mental Illnesses Scale (BMIS) in the community mental health centers or at home visits.SSRSP total mean score of the patient relatives involved in the study was 30,64±8,35, and BMIS total mean score was 71.81±17.08. Significant differences were found in the SSRSP according to receiving help apart from the medical treatment, patient relatives' feeling differences in people's attitudes in the social activities they attended with the patient, patients' frequency of attending the CMHC, and patients' participation in social activities apart from the CMHC (p<0,05). A medium-level, positive, and significant relationship was found between SSRSP total and BMIS total scores (r=0,464) (p<0,05). The results of this study showed that patient relatives were stigmatized, and they had negative beliefs toward mental illness. Based on the results of the study, it is recommended to initiate activities to be conducted by health professionals working in CMHCs and other related institutions for improving attitudes about schizophrenia patients and their relatives and eliminating stigmatization. Through the identification of stigmatization in schizophrenia patients and their relatives and beliefs of patient relatives toward mental illness, this study contributes to patient families and society by helping the CMHC team to organize training on mental illness for these people.

INTRODUCTION

Schizophrenia is one of the most important mental health problems that cause disability, affect the individual's quality of life and social functioning, and have negative effects on patient relatives and society[1]. Care of schizophrenia patients is generally performed by family members[2]. Schizophrenia causes various difficulties not only for patients themselves but also for their families[3]. The psychological state caused by the environmental, economic, social, and emotional problems due to caring responsibilities is considered the family's burden. Stigmatization is the primary environmental problem[4].

Stigmatization is defined as referring individuals in a way to decrease their respectability as it excludes them from the criteria considered normal in

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the environments where they live. Due to stigmatization, a stigmatized individual is attributed to a characteristic that results in feeling embarrassed and disliked by others and experiencing disapproval and shyness[5,6]. Psychiatric patients are one of the groups affected by stigmatization the most[7]. As a result of stigmatization, psychiatric patients and their relatives are isolated from society and do not demonstrate health-seeking behaviors[8,9]. These individuals do not apply health institutions with the fear of being stigmatized or being excluded from society, which causes a big obstacle for maintaining their treatment. In addition to the problems with the treatment, there could also be serious problems with individuals' social relationships[10,11].

Reasons for the stigmatization of psychiatric patients by the society include seeing individuals with a psychiatric disorder different, thinking that they are violent due to this difference, blaming them, and not being able to make sense of their behaviors[12,13]. Being afraid of an individual with mental illness is the primary factor that causes stigmatization[13]. Stigmatization affects psychiatric patients both internally and externally[13]; external stigmatization includes the rejection of the individual with mental illness by their relatives, friends, and employers[15]. In such a case, not only individuals with mental illness but also their family members are stigmatized[16]. Families are considered responsible for the illness and thus blamed by society. These kinds of misbeliefs and the accompanying stigmatizing approach cause family members to feel bad, withdraw into themselves, and decrease their interactions and social activities with the environment; in fact, one of the family members may lose his/her job in some cases[7,17]. People in society treat families of psychiatric patients as if this situation was a disgraceful offense and demonstrate stigmatizing attitudes such as "being the father or mother of an individual with a psychiatric illness"; families fight with these kinds of problems. Due to these kinds of different behaviors demonstrated by society, families hide the illness from society[16]. Mental health professionals are reported to have important roles in fighting with stigmatization[17]. It is reported that societies should be informed about mental illness, individuals with mental illness should be provided with treatments and given moral support and attention in this process so that they can be reintegrated into society and negative perceptions

about mental illness can be eliminated[18]. All health professionals' attitudes towards mental illness are of importance for the prevention of stigmatization. Therefore, it is important to identify the attitudes of all health professionals and psychiatric nurses about this issue and evaluate how individuals with mental illness are affected by these attitudes[12,19]. Family education about schizophrenia and information and consultancy services to be given to patients and their relatives about mental illness and decreasing stigmatization in community mental health centers are considered to help decrease negative thoughts about mental illness and stigmatization. The results of a study show that studies to be conducted for preventing stigmatization about individuals with mental illness should focus on family psychoeducation[20].

The results of this study are considered to guide community mental health workers in providing necessary services for decreasing stigmatization of relatives of individuals with schizophrenia. The study aims to identify the beliefs of the relatives of schizophrenia patients followed up in community mental health centers toward stigmatization and mental illness.

Research Questions

1. What is the stigmatization level of relatives of schizophrenia patients?

2. What is the belief levels of relatives of schizophrenia patients toward mental illness?

3. Is there a statistically significant difference between the belief levels of patient relatives toward mental illness and their socio-demographic characteristics?

4. Is there a statistically significant difference between schizophrenia patient relatives' stigmatization levels and socio-demographic characteristics?

5. Is there a statistically significant relationship between the stigmatization levels and belief levels toward mental illness?

METHOD

This methodological and cross-sectional study aims to identify beliefs toward stigmatization and mental illness among relatives of schizophrenia

patients who were followed up in community mental health centers. The study was conducted in a community mental health center providing service affiliated to the General Secretariat of Hatay Public Hospitals Association and Iskenderun Community Mental Health Center. The target population of the study was the relatives of schizophrenia patients registered in CMHCs in Hatay. There are two CMHCs located in İskenderun and Antakya provinces. The number of patients was 439 in total, 330 in the İskenderun CMHC and 109 in the Antakya CMHC. The sample was the relatives of schizophrenia patients who were registered in the CMHC and regularly attended the CMHC at least once (n=171). In this regard, the study was planned to be conducted with 171 relatives of schizophrenia patients who attended CMHCs regularly at least once; one patient relative did not accept to participate in the study. Hence, the study was completed with 170 relatives of schizophrenia patients who accepted to participate in the study.

Ethics approval

Hatay Mustafa Kemal University Clinical Research Ethics Commitee confirmed the study with the decision number 121 and with the date 16.09.2015.

Characteristics of the Study Group

Of all the patient relatives, 64.7% were females, 27.1% were aged between 40 and 49, 66.5% received help apart from a medical treatment, 63.5% consulted a religious leader, 50.6% used non-medical methods as they thought they would be beneficial, 47.1% thought their relatives treated them differently as they had a family member with schizophrenia, 24.7% hid the individual with schizophrenia and the illness from their relatives, 11.8% did not take their schizophrenia patient to visits to relatives, 8.2% felt like a stranger when they were with relatives due to their schizophrenia patient, 54.7% felt differences in people's attitudes during the social activities they participated with the schizophrenia patient, 37,6% thought the people they met in social activities behaved them differently due to their schizophrenia patient, 28.2% hid the individual with schizophrenia and the illness from the people they met in social activities, 19.4% did not attend social activities

with the schizophrenia patient, and 7.1% felt like a stranger in the social activities they attended with their patients.

Implementation of the Study

Ethics approval was obtained from the Mustafa Kemal University Ethics committee. After the necessary permissions were obtained from the related institutions, verbal information was given to the relatives of schizophrenia patients in the community mental health centers, and their written consent was received. The study was conducted with schizophrenia patient relatives in the CMHCs between September 2015 and September 2016. After they were informed about the purpose of the study and their written and verbal consent was received, the participants were administered the socio-demographic form and the scales through face-to-face interviews in an available room in the community health centers or at home visits. The data collection procedure took about 45 minutes. Data were collected through the Socio-demographic Form, the Stigmatization Scale for Relatives of Schizophrenia Patients, and Beliefs toward Mental Illness Scale (BMIS).

The Socio-Demographic Form

The Socio-Demographic Form, prepared by the researcher in line with the related literature to form the independent variables, includes 37 questions about the socio-demographic features of the patients and patient relatives and the beliefs, attitudes, and behaviors of patient relatives toward stigmatization and mental illness[8,10,12,21,22,23].

The Stigmatization Scale for Relatives of Schizophrenia Patients (SSRSP)

The stigmatization scale developed by Kol Akıncı for measuring stigmatization among patient relatives was adapted as the Stigmatization Scale for Relatives of Schizophrenia Patients (SSRSP) after its validity and reliability were performed. SSRSP was utilized as the data collection tool. The scale was developed to identify stigmatization among relatives who have a patient with schizophrenia. The scale was composed of 17 items, and no items were eliminated from the

scale after the factor analysis. The five factors of the scale are as follows:

Social Isolation and Insufficiency Sub-scale: It includes items 1, 2, 3, 4, 9, and 15, and its Cronbach's Alpha value is 0,87.

Avoidance and Deterioration in Interpersonal Relationships Sub-scale: It includes items 5, 6, and 7, and its Cronbach's Alpha value is 0.80.

Social Negative Discrimination Sub-scale: It includes items 8, 10 and 11, and its Cronbach's Alpha value is 0.76.

Hiding and Embarrassment Sub-scale: It includes items 12, 13, and 14, and its Cronbach's Alpha value is 0,71.

Negative Internalization Sub-scale: It includes items 16 and 17, and its Cronbach's Alpha value is 0,69. SSRSP total Cronbach's Alpha value was calculated as 0.90.

The Stigmatization Scale for Relatives of Schizophrenia Patients (SSRSP) is a three-point scale responded as Yes: 3, Sometimes: 2, and No: 1. The scale is scored over both the total score and sub-scale scores; higher scores obtained from the scales and sub-scales indicate higher stigmatization. The scores to be obtained from the scale range between 17 and 51. The Cronbach's alpha value of the scale was found 0,90.

Beliefs toward Mental Illness Scale (BMIS)

Beliefs toward Mental Illness Scale utilized as the data collection tool in this study was developed by Hirai and Clum (2000) in the United States of America, and the Turkish reliability and validity of the scale were performed by Bilge and Çam (2008). The scale was developed to identify the positive and negative beliefs of individuals with different cultural characteristics toward mental illness.25 The scale is composed of three sub-scales that included Dangerousness, Poor Social and Interpersonal Skills, and Incurability.

Beliefs toward Mental Illness Scale is a 6-point scale responded as I completely disagree: 0, I largely disagree: 1, I partly disagree: 2, I partly agree: 3, I largely agree: 4, I completely agree: 5. The scale is evaluated out of both total

scores and sub-scale scores; higher scores to be obtained from the scale and sub-scales indicate negative beliefs. Cronbach's alpha value of the Beliefs toward Mental Illness Scale was found 0,82. Cronbach's Alpha values were 0,80 for the Incurability and Deterioration in Interpersonal Relationships sub-scale, 0,71 for the Dangerousness sub-scale, and 0,69 for the Embarrassment sub-scale. 25 Cronbach's Alpha value was found 0,73 in this study.

Limitations of the Study

This study is limited to the responses given by the patients and patient relatives in the data collection forms regarding personal and family characteristics. Relatives of the patients who were registered in the community mental health centers affiliated to Hatay General Secretariate of Public Hospitals Association between September 2015 and September 2016 but did not attend the centers regularly were not included in the study. The study was limited to 170 relatives of patients who attended the CMHCs regularly.

Statistical Analysis

Data were analyzed in SPSS (Statistical Package for Social Sciences) (SPSS Inc., 2012) 21.0 statistical software program. Descriptive statistics were demonstrated using numbers and percentages. Shapiro Wilk test was utilized to test whether data distributed normally; comparison of non-normally distributed data in two independent groups was done using the Mann Whitney U test and the comparison of more than two independent groups was done using Kruskal Wallis tests. The relationships between numerical variables were tested using the Spearman correlation coefficient. Descriptive statistics were used as mean±standard deviation for numerical variables and numbers and percentage values for categorical variables. Statistical significance was taken as $P<0.05$.

Findings

The participating patient relatives' Stigmatization Scale for Relatives of Schizophrenia Patients (SSRSP) mean score was found 30.64±8.35. SSRSP sub-scale mean scores were 11.29±3.85 for the social isolation and insufficiency sub-scale,

4.83±2.00 for the avoidance and deterioration in interpersonal relationships sub-scale, 4.341±1.75 for the social negative discrimination sub-scale, 5.05±1.85 for hiding and embarrassment sub-scale, and 5.12±1.13 for the negative internalization sub-scale. Patient relatives' Beliefs toward Mental Illness Scale total mean score was 71.81±17.08. Sub-scale mean scores were 26.93±8.75 for the dangerousness sub-scale, 43.18±10.48 for the incurability and deterioration in interpersonal relationships sub-scale, and 1.68±2.49 for the embarrassment sub-scale (Table 1).

Table 1. *The Stigmatization Scale for Relatives of Schizophrenia Patients and Beliefs toward Mental Illnesses Scale Mean scores*

Scales	Ort± SD	Min	Max
SSRSP			
Social Isolation and Insufficiency	11.29±3.85	6.00	18.00
Avoidance and Deterioration in Interpersonal Relationships	4.83±2.00	3.00	9.00
Social Negative Discrimination	4.34±1.75	3.00	9.00
Hiding and Embarrassment	5.05±1.85	3.00	9.00
Negative Internalization	5.12±1.13	2.00	6.00
SSRSP Total	30.64±8.35	18.00	51.00
BMIS			
Dangerousness	26.93±8.75	3.00	84.00
Helplessness and Poor Social and Interpersonal Skills	43.18±10.48	3.00	10.00
Embarrassment	1.68±2.49	0.00	10.00
BMIS Total	71.81±17.08	8.00	137.00

SSRSP and BMIS mean scores according to patient relatives' receiving help apart from the medical treatment are demonstrated. An analysis of the findings showed that those who received help apart from the medical treatment had higher and statistically significant scores in SSRSP total and sub-scales of social isolation and insufficiency, avoidance and deterioration in interpersonal relationships, and hiding and embarrassment sub-scales in comparison to those who did not (p<0,05). No significant differences were found between receiving help apart from the medical treatment and social negative discrimination sub-scale mean scores (p>0,05). Those who received help apart from the medical treatment had higher and statistically significant scores in the embarrassment sub-scale of BMIS in comparison to those who did not (p<0,05). No statistically significant differences were found between BMIS total and other sub-scales mean scores. (p>0,05, Table 2).

Table 2. *SSRSP and BMIS mean scores according to patient relatives' receiving help apart from the medical treatment, feeling differences in people's attitudes in the social activities they participated with the schizophrenia patient, frequency of the patient's attending CMHC and participating in social activities apart from the CMHC*

	SCALE									
	SSRSP						**BMIS**			
	Social Isolation and Insufficiency $\overline{x}$±SD	Avoidance and Deterioration in Interpersonal Relationships $\overline{x}$±SD	Social Negative Discrimination $\overline{x}$±SD	Hiding and Embarrassment $\overline{x}$±SD	Negative Internalization $\overline{x}$±SD	SSRSP Total	Dangerousness $\overline{x}$±SD	Helplessness and Poor Social and Interpersonal Skills $\overline{x}$±SD	Embarrassment $\overline{x}$±SD	BMIS Total
Receiving help apart from the medical treatment										
Yes (n=113)	11,78±3,86	5,10±1,99	4,53±1,92	5,31±1,95	5,22±1,06	31,97±8,41	27,55±8,82	43,65±9,41	1,93±2,63	73,15±16,26
No (n=57)	10,31±3,68	4,29±1,92	3,94±1,28	4,52±1,50	4,92±1,25	28,01±7,62	25,70±8,55	42,26±12,38	1,19±2,11	69,15±18,46
Z*	-2,450	-2,856	-1,570	-2,410	-1,390	-3,091	-1,451	-1,447	-1,981	-1,852
p	**0,014**	**0,004**	0,116	**0,016**	0,165	**0,002**	0,147	0,148	**0,048**	0,064
Feeling differences in people's attitudes in the social activities they participated with the schizophrenia patient										
Yes (n=93)	12,41±3,89	5,58±2,13	4,93±2,05	5,59±1,95	5,44±0,94	33,96±8,52	27,73±10,10	43,41±9,61	1,73±2,51	72,88±18,13
No (n=77)	9,93±3,36	3,93±1,38	3,62±0,87	4,40±1,48	4,74±1,22	26,63±6,11	25,97±6,71	42,90±11,50	1,63±2,47	70,51±15,74
Z*	-4,157	-5,388	-4,171	-4,169	-4,073	-5,608	-1,345	-1,217	-0,379	-1,452
P	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	0,179	0,224	0,705	0,147
Frequency of the patient's attending CMHC										
1-2 days a week (n=56)	11,14±3,75	4,78±2,02	4,21±1,71	5,46±1,79	5,16±1,04	30,76±8,29	26,80±8,42	44,51±13,02	1,05±2,05	72,37±18,53
3-5 days a week (n=52)	10,40±3,96	4,50±1,92	4,00±1,49	4,59±1,70	4,84±1,30	28,34±8,10	25,65±7,19	42,17±9,28	1,25±2,06	69,07±14,92
1-3 days a month (n=57)	12,01±3,75	5,05±1,99	4,73±1,98	5,03±1,97	5,29±1,05	32,14±8,23	27,80±10,30	42,56±8,85	2,45±2,77	72,82±17,69
1 day in two months (n=5)	14,00±3,16	6,40±2,30	4,80±1,30	5,40±1,81	5,60±0,89	36,20±8,46	31,80±7,39	46,00±7,68	4,60±3,84	82,40±11,37
χ^2*	7,388	6,009	6,598	**7,824**	5,131	**8,413**	4,656	2,152	**14,852**	4,295
P	0,061	0,111	0,086	**0,050**	0,162	**0,038**	0,199	0,541	**0,002**	0,231
Participating in social activities apart from the CMHC										
Never (n=45)	13,46±3,40	5,44±2,26	5,13±2,09	5,53±1,98	5,46±0,86	35,04±7,85	27,71±8,44	43,93±11,33	2,26±2,80	73,91±19,12
Sometimes (n=92)	10,80±3,60	4,57±1,70	3,95±1,33	4,96±1,79	5,11±1,16	29,42±7,50	27,20±9,24	43,33±8,66	1,63±2,41	72,17±15,18
Frequently (n=33)	9,69±3,97	4,72±2,28	4,33±1,96	4,63±1,72	4,66±1,24	28,06±9,22	25,12±7,70	41,75±13,68	1,06±2,10	67,93±19,00
χ^2*	**21,487**	4,988	**11,331**	4,583	**9,574**	**19,557**	3,892	5,804	4,473	**6,850**
P	**<0,001**	0,083	**0,003**	0,101	**0,008**	**<0,001**	0,143	0,055	0,107	**0,033**

Z* Mann Whitney U Test
χ^2* Kruskal Wallis test

SSRSP and BMIS mean scores are demonstrated according to feeling differences in people's attitudes in the social activities they participated with the schizophrenia patient. SSRSP total and all sub-scale mean scores were higher and statistically significant in those who felt differences in people's attitudes in the social activities they participated with the schizophrenia patient in comparison to those who did not feel so (p<0,05). No significant differences were found between BMIS and all sub-scale mean scores according to feeling differences in people's attitudes in the social activities they participated with the schizophrenia patient (p>0,05, Table 2).

Patient relatives' SSRSP and BMIS mean scores according to the frequency of the patient's attending CMHC are shown. An analysis of the findings indicates that there is a statistically significant difference between the patient's frequency of attending the CMHC and the scores of SSRSP total and sub-scales of hiding and embarrassment mean scores (p<0,05). Further analysis of all pairwise test was performed to find out which group caused this difference. The group that caused a statistical difference was composed of patients who went to the CMHC 1 to 3 days monthly (p<0,05). No statistically significant differences were found between the frequency of attending the CMHC and SSRSP and other

sub-scale mean scores (p>0,05). No statistically significant difference was found between patients' frequency of attending the CMHC and embarrassment sub-scale mean score of BMIS (p<0,05). Further analysis of all pairwise test was performed to find out which group caused this difference. The group that caused the statistical difference in the embarrassment sub-scale was composed of the patients who went to the CMHC once in two months. No statistically significant differences were found between the frequency of the patient's going to the CMHC and BMIS total and other sub-scale mean scores (p>0,05, Table 2).

SSRSP and BMIS mean scores of the patient relatives according to the patient's participation in social activities apart from the CMHC are demonstrated. An analysis of the findings indicated a statistically significant difference between the patient's participating in social activities apart from the CMHC and their SSRSP total and sub-scales of social isolation and insufficiency, social negative discrimination, and negative internalization sub-scale mean scores (p<0,05). Further analysis of all pairwise test was utilized to find out which groups caused this difference. The group that caused a statistical difference in

SSRSP total, social isolation and insufficiency, social negative discrimination and negative internalization sub-scale was the group that was composed of patients who never participated in social activities apart from the CMHC (p<0,05). No statistically significant differences were found between participation in social activities and SSRSP other sub-scales mean scores (p>0.05). No significant differences were found between patients' participation in social activities apart from the CMHC and BMIS total mean scores (p<0,05). Further analysis of all pairwise test was utilized to find out which groups caused this difference. The group that caused a statistically significant difference in BMIS total score was found to be composed of patients who never participated in the social activities apart from the CMHC (p<0,05). No significant differences were found between patients' participation in social activities apart from the CMHC and BMIS all sub-scale mean scores (p>0,05, Table 2).

A positive, medium-level relationship was found between SSRSP total and BMIS total scores (r=0,464) (p<0,001, Table 3).

Table 3. *The relationship between the Stigmatization Scale for Relatives of Schizophrenia Patients and Beliefs toward Mental Illnesses Scale*

SSRSP		BMIS			
		Dangerousness	Poor Social and Interpersonal Skills	Embarrassment	BMIS Total
Social Isolation and Insufficiency	r	0,380*	0,294*	0,365*	0,435*
	p	0,001	0,001	0,001	0,001
Avoidance and Deterioration in Interpersonal Relationships	r	0,317*	0,194*	0,340*	0,334*
	p	0,001	0,011	0,001	0,001
Social Negative Discrimination	r	0,286*	0,108	0,337*	0,280*
	p	0,001	0,162	0,001	0,001
Hiding and Embarrassment	r	0,316*	0,291*	0,198*	0,350*
	p	0,001	0,001	0,010	0,001
Negative Internalization	r	0,389*	0,336*	0,174*	0,422*
	p	0,001	0,001	0,023	0,001
SSRSP Total	r	0,422*	0,311**	0,385**	0,464*
	p	<0,001	<0,001	<0,001	0,001

DISCUSSION

The total scores to be obtained from the SSRSP range between 17 and 51. Hence, the scores obtained from the SSRSP total and sub-scales indicate a high level of stigmatization. Studies show that families of individuals with mental illness experience high levels of stigmatization,[26,27] family members who spend the highest amount of time with the individual who has mental illness feel like they do not belong to the external world,[28] patient relatives want to hide the illness, and they are exposed to stigmatization due to the presence of mental illness in family,[29] and patients and patient relatives do not consult a psychiatrist with the fear of stigmatization due to the psychiatric illnesses although they are referred to a psychiatrist.[30,31] Patient relatives who take the responsibility of the care for the schizophrenia patient could be affected physically, psychologically, economically and emotionally. In addition, they worry about the patient's future and about who will take care of him/her when they get older or are not alive anymore, which might lead to blaming themselves and the patient and being ashamed of the patient. All these factors could cause stress.[32]

BMIS total and sub-scales mean scores of the participating patient relatives were found to be high. Büyüksandıç Özşen similarly reported the BMIS total and sub-scale mean scores to be high. Higher scores obtained from the scale and sub-scales indicate negative beliefs. Based on the BMIS total and sub-scales mean scores, Büyüksandıç Özşen reported that patient relatives had above-average negative beliefs.[33] The results of the present study and the ones reported by Büyüksandıç Özşen are similar. It could be concluded that the BMIS total and sub-scale levels of the participating patient relatives were high, and thus patient relatives had negative beliefs toward mental illness.

SSRSP total and sub-scales of social isolation and insufficiency, avoidance and deterioration in interpersonal relationships, hiding and embarrassment sub-scales mean scores were high and statistically significant according to patient relatives' receiving help apart from the medical treatment. Patient relatives' receiving help apart from the medical treatment and BMIS sub-scales showed that the embarrassment sub-scale mean score was high and statistically significant. Several studies reported that

misinformation about psychiatric illnesses could direct patient relatives to inaccurate solution-seeking behaviors[34,35]. Some studies report that the treatment of psychiatric illnesses is performed by people who do not have any relation and authority about psychiatric illnesses. Wizards, hodjas, priests, people who previously had the same illness, relatives, neighbors, and friends who have hearsay information about the illness are reported to have more place than health personnel and doctors about this issue[36,37,38,39]. People in our country and other countries reported that these illnesses are caused by the factors such as hostile spirits, devilish spirits, thoughts infused in the individual's head by force, evil eye, spell, and magic[36].It is also reported that lack of rapid recovery causes families to feel helpless, and these feelings of helplessness could lead them to seek different treatment options[39].This case could result from patient relatives' lack of knowledge or misinformation about mental illness.

SSRSP total and all sub-scale scores were significantly higher in the patient relatives who reportedly felt differences in people's attitudes in the social activities they attended together with the schizophrenia patient. The study conducted by Oban and Küçük (2011) on the identification of the factors indicating stigmatization in psychiatric illnesses in high school students reported that the participants tended to have prejudices, negative attitudes, and desires to put a social distance about these illnesses[40].A study conducted by Struening et al. (2001) in New York reported that family members who had a psychiatric patient in family thought that majority of people in society looked down on families who lived with an individual who had a mental disorder[41].This situation could be caused by the fact that patient relatives thought that they were looked down on and excluded due to the schizophrenia patient they have.

According to the CMHC registration durations, social negative discrimination, negative internalization sub-scale mean scores of SSRSP sub-scale mean scores of patient relatives were statistically significant. BMIS total and embarrassment sub-scale mean scores were statistically significant according to the duration of registration. The study conducted by Avcil in 2014 with 82 psychiatric patient relatives showed that in time patient relatives saw the patients who received psychiatric treatment less as a patient, but they began to feel more embarrassed[42].This

finding could result from the high number of patients who were enrolled in the CMHC for 12 months or more.

Patient relatives' SSRSP total and sub-scales scores according to patients' frequency of going to the CMHC showed that hiding and embarrassment sub-scale mean scores were statistically significant. There was an increase in the stigmatization level with a decrease in the patients' frequency of going to the CMHC. The majority of individuals with mental illness were reported to have difficulties in continuing the treatment regularly[43]. Studies show that stigmatization is a factor that affects compliance to treatment[43] and that individuals who have mental illness and their relatives accept to be isolated at home by refusing the treatment due to stigmatization[10]. This situation could be considered to result from some factors; for instance, patients do not go to the CMHC as they feel embarrassed, they generally go to the CMHC just to get the prescription of their medicine and thus do not benefit from the services provided by the center.

According to the participation of patients to social activities apart from the CMHC, patient relatives' social isolation and insufficiency, social negative discrimination, negative internalization sub-scale mean scores of the SSRSP total scores were statistically significant. According to the findings of the study conducted by Arkar in 1991, people believed that there should be more distance when there is a need to have personal interactions with patients who have mental illness, they tend to stop interaction with individuals who are defined as "mentally ill", and the social acceptance of individuals with mental illness is high in general settings[44]. This case could result from the fact that the patients and patient relatives do not gain acceptance in social environments due to negative attitudes of society towards these illnesses in general settings.

An analysis of the relationship of patient relatives' SSRSP total and sub-scales and BMIS total and sub-scales indicated a positive, medium-level relationship between SSRSP total and BMIS total scores (r=0,464).

The more the mental disorder is recognized, the higher the stigmatization will be[45]. A study on the factors that identify stigmatization about psychiatric disorders reported that the participants tended to have prejudices, desires to put social distance, and negative attitudes toward these illnesses[40]. Another study also reported that families that have a member with mental illness experienced problems as they believed that people thought badly about them, they also reportedly felt hate, fear, and shame in society, thus going out with the individual with mental illness could be a source of fear for families[46]. It is also reported that patient relatives are ashamed of this condition, thinking that they played a role in the psychiatric illness to develop[47,48,49]. Some studies indicate that individuals with a psychiatric illness generally have to live alone and live their life as a person who is misunderstood due to stigmatization; families kept the individual with mental illness at home and isolated him/her from society; patient relatives similarly isolated themselves from society, experienced fear of being stigmatized, and felt helpless; and they also feared that the individual with mental illness would do harm[50,51,52,53,54,55]. BMIS mean score was found to increase with the increase in the SSRSP mean score. Hence, the negative beliefs of patient relatives toward mental illness might have increased stigmatization in a parallel way.

CONCLUSION

In conclusion, belief levels of patient relatives toward stigmatization and mental illness were found to be at a moderate level. It was found that patient relatives felt ashamed of the presence of the individual with mental illness in the social activities they attended together and thus felt differences in people's attitudes, and their interpersonal relationships were affected negatively due to the illness.

Going to the CMHC every day regularly was found to lead to less stigmatization of patients and their families and have positive effects on their beliefs toward mental illness.

Therefore, it is recommended to prepare special programs that include accurate information about schizophrenia through both audio and written media, initiate activities in CMHCs and other related institutions to help society to improve their attitudes towards schizophrenia patients and their relatives and eliminate isolation and prejudices, and to provide patient relatives with consultancy services by identifying the factors indicating

stigmatization so that more understanding behaviors and attitudes could be developed towards psychiatric patients.

It is recommended that all health professionals should communicate with patients and patient relatives for whom they are in charge in the region they work, inform them about the purposes and functions of the center, invite patients and patient relatives to the center, help patients and patient relatives to realize the importance of the centers, emphasize the importance of CMHC nurses and other health professionals, and conduct more comprehensive studies about stigmatization in schizophrenia patients that include the effects of CMHCs and CMHC health team.

Conflict of interest statement

The authors declare that they have no conflicts of interests.

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REFERENCES

1. Aziz, KA., Mohammed, H., Elamin, MH., El-Saadouni, NM., El-Gabry, DA., Mahmoud Barakat, M., Alhayyas, F., Moselhy, HF. Schizophrenia: Impact of psychopathology, faith healers and psycho-education on adherence to medications. *Int J of Soc Psych*, 2016; 62:719–725.

2. Duman, ZC., Bademli, K. Families of chronic psychhiatric patients: a systematic review. *Current Approaches in Psychiatry,* 2013;5(1):78-94

3. Koroglu, A., Hocaoglu, C. The impact of schiophrenia on the family. *Düzce University Health Sciences Institute Journal,* 2017;7(3):170-175

4. Ozlu, A. Yildiz, M. Aker, T. Validity and reliability study of zarit caregiver burden scale in relatives of schiophrenia patients. *Archives of Neuropsychiatry,* 2009;46.

5. Cam, MO., Bilge, A., Engin, E., Akmese, ZB., Turgut, EO., Cakir, N. ınvestigation of the effectiveness of anti-stigma training given to headmen regarding mental illness. *Journal of Psychiatric Nursing*, 2014; 5:129-136.

6. Bilge, A., Cam, O. Fighting the stigma of mental illness. *TAF Preventive Medicine Bulletin,* 2010; 9:71-78.

7. Sarikoc, G., Demiralp, M., Ozsahin, A., Acikel, C. Help seeking in mental illness: A research on patients' relatives attitudes. *Balikesir Journal of Health Sciences*, 2015;4 (1)

8. Cam, O., Bilge, A. Beliefs, attitudess and stigma process toward mental illness/patient in turkey: systematic review. *Journal of Psychiatric Nursing,* 2013; 4(2):91-101

9. Taskin, EO. Stigma process and stigma towards schzophrenia. *Anatolian Journal of Psychiatry*, 2009; 10 (2): 58-9

10. Kus Saillard, E. Psychiatrist views on stigmatization toward people with mental ıllness and recommendations. *Turkish Journal of Psychiatry*, 2010;21:14-24.

11. Halter, MJ. Stigma in Psychiatric Nursing. *Perspectives in Psychiatric Care,* 2002;38(1):23

12. Ergun, G., Isik, I., Dikec, G. Roles of psychiatry nurses within a therapeutic environment of psychiatry clinics in Turkey. *Archives of psychiatric Nursing*, 2017;*31*(3), 248-255.

13. Taskin, EO. Stigma: attitudes and stigma toward mental illnesses. Taskin EO., Editor. Izmir: Meta Basim Matbaacilik Hizmetleri, s.3-7; 2007.

14. Cobanoglu, ZSÜ., Aker, T., Cobanoglu, N. Treatment compliance problems in patients with schzophrenia and orher psychotic disorders. *Dusunen Adam,* 2003;*16*(4): 211-218.

15. Kivircik Akdede, BB., Alptekin, K., Topkaya, SO., Belkiz, B., Nazli, E., Özsin, E. and et. all. Level of stigmatization of schzophrenia in youn people. *New Symposium. 2004,42:113-117.*

16. Baysan Arabacı, L., Başogul, C., Buyukbayram, A. Internalized stigma and social functioning levels of forensic psychiatric patients. Anatolian Journal of Psychiatry, 2015; 16(2):113-121

17. Ozkan, S. "Kronik Psikoz", İ.Ü. İstanbul Tip Fakültesi Hasta Okulu.[http://www.frmtr.com/saglik_makaleleri/1209527_sizofrenide_damgalamayi azaltmakpdt.html 2009. 07.06.2017].

18. Soygür, H. Society's Rejection: Discrimination Against People with Mental Illness. *Federation of schizophrenia associations,* Ankara, 2014.

19. Gulseren, L., Cam, B., Karakoc, B., Yigit, T., Danaci, AE. and et al. Factors Affecting the Family Burden in Schizophrenia. *Turkish Journal of Psychiatry,* 2010, 21:203-212 .

20. Shibre T, Negash A, Kullgren G, Kebede D, Alem A, Fckadu A, et al. Perception of Stigma Among Family Members of İndividuals with Schizophrenia and Major Affective Disorders in Rural Ethiopia. *Soc Psychiatry Epidemiol,* 2001, 36(6):299-303

21. Ben-Noun, L. Characterization of Patients Refusing Professional by Relatives of Psychotic Patients. *J Psychiatr Assoc Thailand,* 1996, 42(4):226-33

22. Nicholas, R. et al., "Stigma, discrimination, social exclusion, andmental health – A Public health perspective, In: Richard P., Marni S., Routledgehandbook in global public health, 1st publish, RoutledgeTaylor & Francis Group, 2011, 394-9.

23. Kaya, Y., & ÖZ, F. Global social functioning of patients with schizophrenia and care burden of caregiving relatives. *Journal of Psychiatric Nursing*, 2019; *10*(1)

24. Buyuksandic Ozsen, P. Knowledge, Beliefs and Help-Seeking Behaviors of Relatives of Schizophrenic Patients Regarding Schizophrenia. Master's Thesis, Gaziantep University Health Sciences Institute, Department of Nursing, Gaziantep, 2013.

25. Ozbas, D., Kucuk, L., & Buzlu, S. Attitudes of Families with Individuals Mental Disorders Towards the Disease. *Dusunen Adam: Journal of Psychiatry and Neurological Sciences,* 2008;*21*(1-4).

26. Angermeyer, MC., Matschinger, H. Relatives' Beliefs About The Causes of Schizophrenia. *Acta Psychiatr Scand*, 1996, 93:194-199

27. Tuna, A. Investigation of the effects social rejection of mentally patients on the patient's family. Master's Thesis, Istanbul University Health Sciences Institute, Istanbul, 1997.

28. Oksuz, E., Vural, H., Oflaz, F. Attitudes of Family Members towards Psychiatric Patients. *Zonguldak School of Health Education Research Journal*, 2005;*1*(3):11-20.

29. Akbulut, Ş. Attitudes of relatives of schizophrenic patients towards and the difficulties they experience in care of the patient. Master's Thesis, Harran University Health Sciences Institute, Departman of Psychiatric Nuring, Sanliurfa, 2009.

30. McCann G, McKeown M, Porter I. Understanding The Needs of Relatives Of Patients Within A Special Hospital For Mentally Disordered Offenders. A Basis For İmproved Services. *Journal of Advanced Nursing,* 1996, 23:346-352.

31. Yildiz, M., Yazici, A., Cetinkaya, O., Bilici, R. and Elcim, R. Information and Opinions of Relatives of Schizophrenia Patients About the Disease. *Turkish Journal of Psychiatry,* 2010; 21(2):105-113.

32. Ziyalar, A. The effect of Culture on Psychiatric Approach. *Yeni Symposium,* 1995; 33(2-3):59-63.

33. Ozkorumak, E., Gulec, H., Kose, S., Borckardt, J., Sayar, K. Nonimedical Help Seeking Behavior in Depression Patients: Could Alexithymia Be A Factor? Journal of *Clinical Psychiatry,* 2006; 9:161-169.

34. Bahar, A., Savaş, H. A., & Bahar, G. Evaluation of Non-Medical Help Seeking Behavior in Psychiatry Patients. *In New Symposium,* 2010; 48(3): 216-222.

35. Yildiz, M. Psychological Social Skills Training for Schizophrenia Patients. Ankara, Türkish Social Psychiatry Association Publications, 2011.

36. Wahl, O. F., & Harman, C. R. Family views of stigma. *Schizophrenia Bulletin*, 1989; *15*(1):131-139.

37. Oban, G., & Küçük, L. Factors affecting Stigma Towards Mental Illnesses in Adolescents. Journal of *Psychiatry Nursing,* 2011;*2*(1), 31-39.

38. Struening, EL., Perlick, DA., Link, BG., Hellman, F., Herman, D., Sirey, JA. Stigma as A Barrier to Recovery: The Extent to Which Caregivers Believe Most People Devalue Consumers and Their Families. *Psychiatr Serv,* 2001, 51(12):1633-1638.

39. Cansayar, S. Why How Schizoprenia. Ankara: Sen Matbaa, 2005.

40. Arkar, H. The Social Refusing of Mental Patients. *Journal of Psychiatry Neurologic Science*, 1991;4:6–9.

41. Soygür, H., Alptekin, K., Atbasoglu, E. C., Herken, H. Schizophrenia and Other Psychotic Disorders. *Türkish Psychiatric Association, s.500;* 2005.

42. Durmaz, H. Examining the Effect of the Self-Efficacy Levels of Family Members Caregiving to a Schizophrenia Patient on the Caregiving Burden. Master's Thesis, Ataturk University Health Sciences Institute, Departman of Psychiatric Nuring, Erzurum, 2011.

43. Tel, H., Terakye, G. A Psychoeducational Approach Application Trial for Families of Schizophrenic Patients. *Anatolian Journal of Psychiatry,* 2000; 1:133-142.

44. Tanriverdi, D. The Effect of psyceducation Given to Caregivers of Schizophrenia Patients on Their Care Burden. Unpublished Doctoral Thesis, Ataturk University Health Sciences Institute, Erzurum, 2008.

45. Caqueo-Urizar, A., Gutierrez-Moldonado, J., Miran-da-Castillo, C. Quality of Life in Caregivers of Patients with Schizophrenia: A Literature Review. *Health Qual Life Outcomes*, 2009;7:84.

46. Gurhan, N. Support for The Social Support Needs of Families with Psychiatric Patients. *Nursing Buletini,*1991;5(22):67-70

47. Yildiz, M. Understanding Schizophrenia and Learning to Live with It. Ankara: Hekimler Yayin Birligi, 1999.

48. Angermeyer, MC. "Public İmage of Psychiatry. Results of A Representative Poll in The New Federal States of Germany". *Psychiatrische Praxis,* 2000;27:327-329.

49. Dogan, O. Home Care of Schizophrenia Patients. *Anatolian Journal of Psychiatry*, 2001; 2(1):41-46

50. Dogan, O., Dogan, S., Tel, H., Coker, F., Polatoz, O., Basegmez, FD. Psychosocial Approaches to Schizophrenia: Families. *Anatolian Journal of Psychiatry,* 2002; 3:133-139.

51. Gulseren, L. Schizophrenia and The Family: Difficulties, Burdens, Emotions, Needs. *Turkish Journal of Psychiatry,* 2002; 13(2):143-151

52. Kayahan, M. Nursing Students' Attitudes Towards Schizophrenia ant the Effect of Psychiatry Education. *Harran University Faculty of Medicine Journal,,* 2009; 6(1):27-34

53. Ozer, U., Varlık, C., Ceri, V., Ince, B., Delice, MA. Change Starts With Us: Stigmatizing Attitudes Towards Mental Illnesses and Use of Stigmatizing Language Among Mental Health Professionals. *Dusunen Adam*, 2017; *30*(3), 224-232.

54. Kapikiran Acun, N., Kapikiran, S. Self Stigma Scale For Seeking Psychological Help: Validity And Reliability. *Turkish Psychological Counseling and Guidance Journal,* 2013, 5 (40), 131-141

55. Koksal, H. Evaluation of Society's Perpective on Mental Illneses. Master's Thesis, Marmara University Institute of Health Sciences, Department of Public Health, Istanbul, 2012.

56. Avcil, C. Psikiyatrik Hasta Yakınlarında Damgalama ve İlişkili Faktörler. Master's Thesis,, Üsküdar Üniversity Institute of Social Sciences, Department of Clinical Psychology, Istanbul, 2014.

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INVESTIGATION OF VIBURNUM OPULUS L. EXTRACT'S ANTIOXIDANT EFFECTS IN DIFFERENT SOLVENTS AND CYTOTOXIC EFFECTS ON COLON CANCER CELL LINES

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ABSTRACT

Cancer is a disease that develops as a result of the uncontrolled proliferation of cells and can be fatal if left untreated. Side effects of cancer treatment reduce the cure rate of this disease. For this reason, studies in the field of health have shifted to new treatment methods in cancer. As a result of these studies, natural products have become important targets for the development of new anticancer agents. There are many plants belonging to Viburnum opulus L. species. These plant species have economic importance in many sectors due to essential and aromatic oils and secondary metabolites. These plant species are commonly used for the treatment of coughs, stomach aches, and infectious diseases. In this study, the antioxidant and antiproliferative properties of the endemic species Viburnum opulus L. extract were investigated in colon cancer cell lines. In this way, the aim is to develop an effective therapeutic agent for the treatment of cancer.

INTRODUCTION

Colorectal cancer (CRC) is a serious cancer with high incidence and mortality rates in developed countries. Colorectal cancer (CRC) is the third most common cancer diagnosed in both men and women in the United States. Colon cancer and rectal cancer are often grouped together because they have many common features. The precursors of colon cancer are polyps that develop into cancerous cells over time. Colonoscopy is the most widely accepted standard for the detection of these polyps and colon cancer screening[1].

The treatment method in colon cancer varies from person to person depending on the size, distribution and stage of the tumour. The main treatment modalities are surgery, chemotherapy and radiotherapy[2].

Natural therapeutics are very important because they support treatment in all stages of cancer, are less toxic or non-toxic compared to chemotherapeutic agents, are easily accessible, easy to use and generally show synergistic effects with drugs[3].

Recent research has shown that many compounds derived from seeds, fruits, bark, roots and leaves of plants have anti-carcinogenic properties. They can regress colon cancer growth in many ways, such as increasing the level of

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superoxide dismutase; reducing DNA damage mediated by oxidative stress; inducing cell cycle checkpoint arrest in G1 phase, G1/S phase, S phase and G2/M phase to increase apoptosis; reducing anti-apoptotic protein levels such as BCL2 (B-cell lymphoma 2) and BCL-XL (B-cell lymphoma-extra large); reducing the expression of PI3K (phosphoinositide 3-kinase), AKT (Ak strain transformation) and MMP (matrix metalloproteinase) levels; induces the expression of various cell cycle inhibitors such as p53, p21 and p27 and apoptotic markers such as BCL2-related cell death agonist, BCL2-related X protein (BAX), Caspase3, Caspase7, Caspase8 and Caspase9 protein[4].

The use of natural sources such as strawberries, grapes, plums, pomegranates, green tea, cruciferous vegetables, vegetables, soya beans, tomatoes, garlic, turmeric, ginger, olives, whole grains and mushrooms, garlic and pomegranate can inhibit development and colon carcinogenesis by promoting apoptosis and cell cycle arrest. About 35,000 herbal bioactive compounds are extracted from plants, seas and other sources, which minimise the negative effects of using modern technology to treat cancer, such as chemotherapy and radiological therapy. Medicinal plants are the most reliable source of bioactive compounds for natural medicines that improve medicines to alternative systems as a green approach in CRC treatment. Terpenoids, saponins, essential oils, flavonoids, phenolics, quinones and alkaloids have a strong cytotoxic effect against CRC cells with lower risk and fewer side effects[4].

Viburnum opulus L. (VO) is a plant belonging to the genus Viburnum L. of the family Adoxaceae, sometimes included in the monotypic family Viburnaceae, formerly in the family Caprifoliaceae. In Turkey it is known as viburnum rose, European viburnum, cranberry bush, water elder, rose elder, rose marbling, cherry tree, krampbark, viburnum tree and gilaburu. VO is a valuable decorative, medicinal and food plant. In the countries of Russia and Ukraine, red VO berries, despite their astringent-bitter-sour taste, are used in traditional cuisine, for example, as a component of marmalades, jams, liqueurs and liqueurs and "Kalinnikov" pies, as well as herbal teas. VO is widely used for medicinal purposes. Gilaburu juice is traditionally used to treat coughs, colds, tuberculosis, rheumatic pains, ulcers, liver disease, diabetes and hypertension, as well as to prevent certain stomach and kidney problems. VO bark (Cortex Viburni) is used in the treatment of gastric or uterine bleeding and haemorrhoids. The results of published in vitro studies show that it has antimicrobial, antidiabetic, anti-obesity,

anti-inflammatory and anti-cancer effect. The properties of different morphological parts of VO have been shown in animal studies to have a beneficial effect on the urinary system, anti-inflammatory and vasorelaxant activities of VO. The health benefits of VO are due to the presence of bioactive components such as phenolic compounds, vitamin C, carotenoids, iridoids and essential oils, among others[5]. In this study, we aimed to investigate the antioxidant and anti-cancer activity of Viburnum opulus L. in colon cancer cell line.

MATERIALS AND METHODS

Preparation of Extracts

The collected Viburnum opulus L. were washed with tap water, then distilled water and dried in the laboratory for 2 weeks. The dried samples were ground and 100 gram samples were extracted twice in 1000 mL of different solvents (water, ethanol, n-hexane and ethyl acetate solvents) at 45°C for 45 min in an ultrasonic bath. The extracts were filtered through Whatman No.1 filter paper and concentrated in vacuo to obtain the extracts. The extracts obtained were stored at -20°C for use in experimental procedures.

Determination of Antioxidant Properties

Determination of Radical Scavenging Activity (DPPH)

DPPH determination was performed according to the method developed by Cuendet et al. DPPH- radical (2,2-diphenyl-1-picrylhydrazyl) is a commercially available radical and was dissolved in 100 μM ethanolic solution of this radical. According to this method, the sample was treated with DPPH- radical and kept in the dark for 50 min and then absorbance was measured at 517 nm in a spectrophotometer. (Ascorbic acid was used as standard).

Determination of Iron (Fe+3) Reducing Power in Extracts

The presence of reducing agents such as antioxidants causes the reduction of the Fe+3-ferricyanide complex to Fe+2. In this method, the colour of the test solution changes from yellow to green depending on the reducing power of the sample under test. The resulting green colour gives maximum absorbance at 700 nm and increasing absorbance indicates increasing reducing power [100]. According to this method, trolox was used as standard antioxidant compound.

The experiment was modified and instead of spectrophotometric cuvettes, the first six steps of pipetting in Table 1 were performed in 1.5 ml ependorfs and the subsequent steps were performed in 96-well microplane [101].

Table 1. *Determination of iron (Fe+3) reducing power of Viburnum opulus L. extract*

	Blank	Sample	Standart
Solvent	40 µl	-	-
Extract	-	40 µl	-
Standart	-	-	40 µl
0.2 M pH:6.Phosphate Buffer	100 µl	100 µl	100 µl
1%-K₃Fe (CN)₆	100 µl	100 µl	100 µl
Incubated at 50°C for 20 minutes and cooled.			
10% TCA	100 µl	100 µl	100 µl
Centrifuged at 3000 g for 10 minutes.			
100 µl of the upper phases were taken and transferred to a 96-well micropleyt.			
Distilled water	100 µl	100 µl	100 µl
0.1% FeCl₃	20 µl	20 µl	20 µl
Incubated at room temperature for 5 minutes in the dark.			
At 700 nm, absorbance was measured on a micropleyt reader.			

Total Flavonoid Content Determination

Total flavonoid content of the extracts was determined by aluminium chloride colorimetric method. The principle of the method is based on the fact that $AlCl_3$ forms acid stable complexes with C-4 keto group and C-3 or C-5 hydroxyl groups of flavones and flavonols. In addition, $AlCl_3$ forms complexes with ortho-dihydroxyl groups of A- or B- rings of flavonoids [98]. According to this method, quercetin was used as a standard [99].

Table 2. *Determination of filavonoid content of Viburnum opulus L. extract*

	Blank	Sample	Standart
Solvent	20 µl	-	-
Extract	-	20 µl	-
Standart	-	-	20 µl
80% Ethanol	172 µl	172 µl	172 µl
10% Al (NO₃)₃	4 µl	4 µl	4 µl
1 M KCH₃COO	4 µl	4 µl	4 µl
Incubated at room temperature for 40 minutes in the dark.			
At 415 nm, absorbance was measured on a micropleyt reader.			

Cell Culture

Cells

In this study, colon cancer (HT-29-HTB-38™) cell lines were grown in DMEM medium containing 10% (v/v) fetalbovine serum (FBS), 1% penicillin-stretomycin and healthy mouse fibroblast cells (L-929) were grown in RPMI-1640 medium containing 10% (v/v) fetalbovine serum (FBS), 1% penicillin-streptomycin in an incubator at 5% CO2, 95% humidity and 37°C. Mouse fibroblast cell line L929 was used as a healthy cell line.

Cytotoxicity Tests

The cytotoxic effects of Viburnum opulus L. extract, which was found to be the most effective antioxidant, on colon and healthy cells were performed using MTT method. Firstly, cells were seeded in plates at 5 × 104 cells/well, respectively. All cells were treated with different concentrations of the extracts and incubated for 24 hours. After incubation, MTT solution was added and incubated for 4 hours. After the plate was poured, DMSO was added and formazone crystals were formed. Absorbance values were obtained by reading on an ELISA reader at 545 nm.

FINDINGS

Antioxidant Parameters

DPPH is a dark purple radical and in the presence of antioxidant, it takes a proton and turns into a colourless compound DPPH reduced molecule. Measurement of the absorbance at 517 nm of the reaction of DPPH with antioxidant is one of the most widely used decolarisation analysis methods [103].DPPH (1,1-Diphenyl-2-picrylhy-drazyl) is used for the determination of free radical scavenging activity. DPPH radical was determined in the extracts of Viburnum opulus L. obtained from ethanol, water, ethyl acetate and n-hexane. DPPH is purple in colour and as the plant shows antioxidant effect, the DPPH radical is scavenged and the purple colour lightens. The colour change is determined by absorbance change with UV-Vis spectrophotometer. Ascorbic acid was used as standard. DPPH scavenging activity of Viburnum opulus L. extract in the study is given in the table below.

DPPH radical scavenging activity was determined for different concentrations of Viburnum opulus L. extract and ascorbic acid (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562 µg/mL). For this purpose, % inhibition values at each concentration were calculated from the absorption values obtained and plotted against the concentration. The following formula was used for calculation [104].

Figure 1. *Ascorbic acid standard graph*

$$I\,(\%) = \left[\frac{A_{blank} - A_{sample}}{A_{blank}}\right] \times 100$$
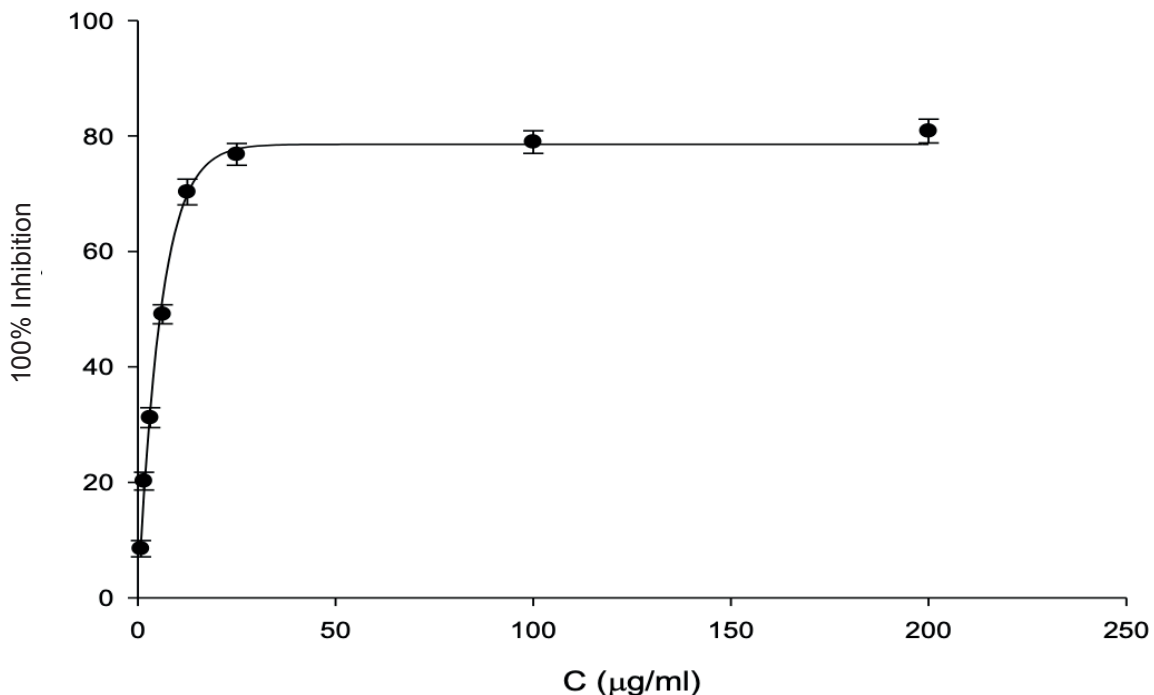
IC50 value of Viburnum opulus L. extract was 62.5±1.23 µg/mL water, 53±2.76 µg/mL ethanol, 65±2.34 µg/mL ethyl acetate, 44±1.98 µg/mL n-hexane. The IC50 value of ascorbic acid was found to be 4.67±0.09 µg/mL (Table 3).

Table 3. *DPPH values*

	Water	**Ethanol**	**Ethyl acetate**	**n-hexane**	**Ascorbic Acid**
µg/mL	62.5±1.23	53±2h.76	65±2.34	44±1.98	4.67±0.09

Total Flavonoid Content Determination

Viburnum opulus L. extract was analysed according to the method of Chang et al. The amount of total phenolic matter was calculated as quercetin equivalent. The concentrations of total flavanoid compounds in Viburnum opulus L. extracts (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562 µg/mL) were calculated as quercetin equivalent from the equation obtained from the quercetin standard graph calibration curve given in Figure 2. The obtained graph equation was found as y = 0.0032x+0.0768 (R2=0.9997). The results of the total flavonoid content of Viburnum opulus L. extracts of water, ethanol, ethyl acetate and n-hexane solutions are given in Table 4.

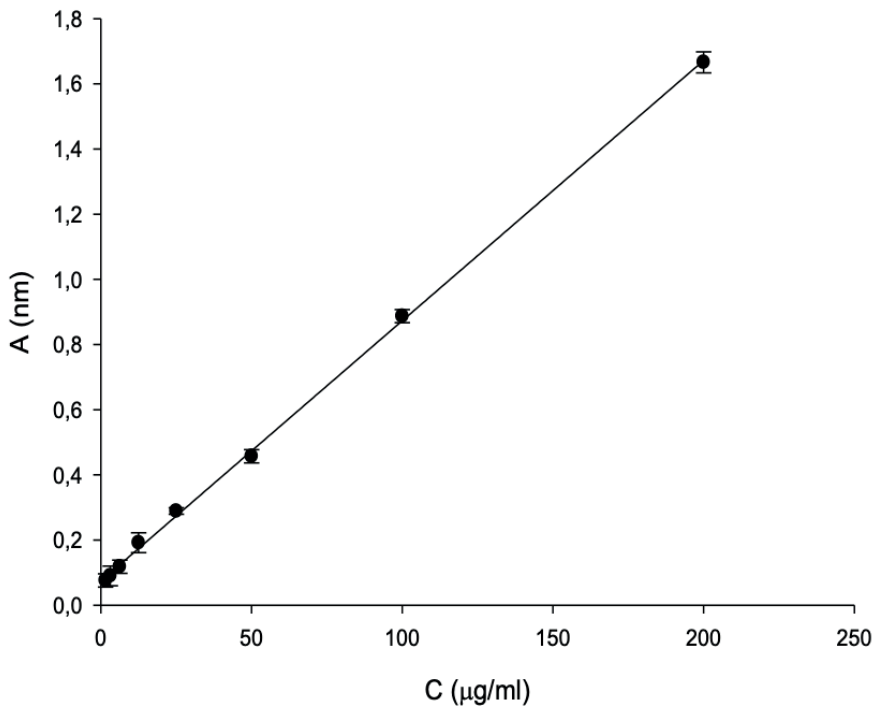**Figure 2.** *Standard graph of quercetin*

Table 4. *Flavonoid values*

	Water	Ethanol	Ethyl acetate	n-hexane
µg/mL	28.67	29.87	25.98	27.75

FRAP Antioxidant Power Determination Results

In the analysis of Viburnum opulus L. extracts according to Benzie and Strain method, the amounts of FRAP antioxidant power were calculated as trolox equivalents. The concentrations of iron reducing compounds (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562 µg/mL) in *Viburnum opulus L.* extract were calculated as equivalent to trolox from the equation obtained from the calibration curve of the trolox standard graph given in Figure 3. The obtained graph equation was found as $y = 0.0031x-0.0012$ ($R^2=0.9999$).

Figure 3. *Trolox standard graph*
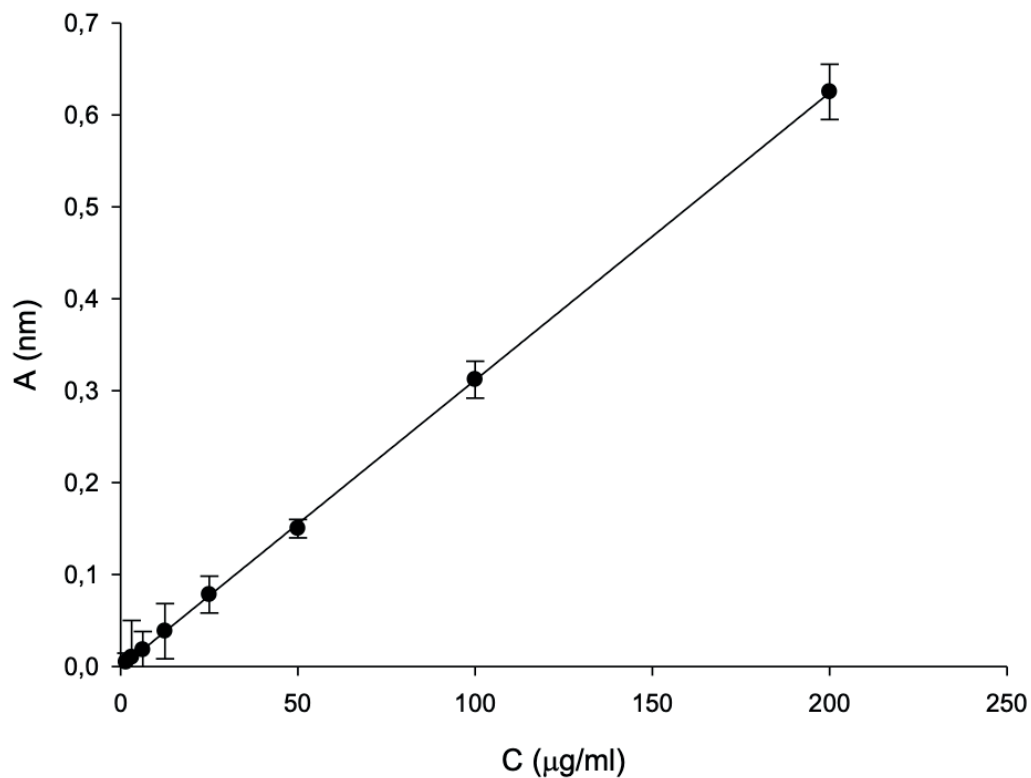
The results of FRAP antioxidant power of Viburnum opulus L. extracts obtained from ethanol, water, ethyl acetate and n-hexane solvents are given in Table 5.

Table 5. *FRAP values*

	Water	Ethanol	Ethyl acetate	n-hexane
µg/mL	189.86	178.08	215.98	165.87

Cell Culture

In the study, Viburnum opulus L. extracts prepared in water, ethanol, ethyl acetate and n-hexane solvents were applied to colon cancer in cell culture with 24 hours incubation. IC50 values were calculated and given below (Table 6 and Table 7).

Table 6. *IC$_{50}$ values of Viburnum opulus L. calculated in different solvents in colon cancer*

	Water	Ethanol	Ethyl acetate	n-hexane
µg/mL	189.73±2.65	108.98±1.93	135.87±2.07	152.98±1.04

Table 7. *IC50 values of Viburnum opulus L. calculated in different solvents in healthy cell linecancer*

	Water	Ethanol	Ethyl acetate	n-hexane
µg/mL	276.56±1.96	250.72±1.90	300.72±2.73	350.82±3.98

DISCUSSION

Various parts of different species of the genus Viburnum opulus L. are widely used in Chinese medicine due to numerous pharmacological properties such as antimicrobial, anti-inflammatory, antiallergic and antioxidant activities. In the literature, the antimicrobial effect of leaf and flower essential oil of Viburnum opulus L. is observed[6]. Antioxidants can protect human cells from reactive oxygen species (ROS). Antioxidants can convert free radicals into non-radical compounds by methods such as chain reaction of lipid oxidation, inhibition of pro-oxidative enzymes and chelation of metal ions. Thus, antioxidants present in the diet may have a significant impact on disease prevention and progression. Water-soluble vitamin C is found in cellular fluids such as cytosol or cytoplasmic matrix, whereas lipid-soluble vitamin E and carotenoids are predominantly found in cell membranes[7]. Vitamin C may act directly by reacting with aqueous peroxyl radicals and indirectly by restoring the antioxidant properties of fat-soluble vitamin E. Vitamin C content in VO fruit ranged from 12.4 to 164 mg/100 g fresh weight, depending on growing location and genotypes. The vitamin C content of VO fruits grown in Turkey ranged from 25.0 to 59.5 mg per 100 g of fresh fruit [8, 9, 10, 11, 12, 13].

In a study, the essential oils of the leaves and flowers of Viburnum opulus L. were extracted and analysed. Of the 16, 53 and 35 compounds identified in the stem, leaf and flower of Viburnum opulus L., only 5 were found to be present in all three segments of the plant. The essential oil in the plant stem was found to consist mainly of spathulenol, carvacrol, santolina alcohol and trans-caryophyllene oxide. The main constituents of the leaf oil were 1,8-cineole, camphor, ascaridol, trans-isoacaridole and piperitone oxide, while the main constituents of the flower oil were ascaridol, trans-isoacaridol, 1,8-cineole, p-cymene and camphor. These factors provide strong anticancer and antiproliferative properties of Viburnum opulus L.

In our study, when the DPPH free radical scavenging activity of Viburnum opulus L. extract was studied, ascorbic acid, which is a strong antioxidant, was 4.67±0.09, while 44±1.98 n-hexane solvent was the highest free radical scavenging. Viburnum opulus L. ethanol solvent 53±2.76, water solvent 62.5±1.23, ethyl acetate solvent 65±2.34. In our study, the antioxidant power of the extract of n-hexane solvent was found to be high. The highest total flavonoid content was 30.75±0.12 µg/g in n-hexane solvent. In water solution 28.67±1.56 µg/g, in ethanol solution 29.87±2.97 µg/g and in ethyl acetate 25.98±2.34 µg/g. It was observed that total polyphenol content was low. Iron reducing power was found as 215.98±2.78 mg/g in n-hexane solvent. Iron reducing power of Viburnum opulus L. Extract was found to be at medium level in our study.

Colorectal cancer is one of the most common malignancies. Colorectal cancer is the 4th most common cancer and the 2nd most common cause of death in the United States of America (USA). The lifetime probability of a person developing colorectal cancer is 6%. From this point of view,

it is a serious public health problem. More than 90% of the patients are over the age of 50 years and 75% have no other known risk factors other than age[14,15].Although screening programmes in colorectal cancer are widely used in western societies, this point has not been given the necessary importance in our country. In a study, it was confirmed that commercially prepared gilaburu juice (80 µl/mL) showed a cytotoxic effect against HeLa and Caco-2 cells, while no inhibition of metabolic activity was observed in normal HUVEC cells[16]. In another study, the preparation obtained from defatted VO fruit pulp after extraction with pressurised ethanol reduced the proliferation of human colon adenocarcinoma HT-29 cells (IC50=0.39 mg/mL) without toxic effect on Caco-2 cells[17].

Viburnum opulus L. After the extract was obtained in different solvents, it was applied to colon cancer cell line and the proliferation effect of colon cancer was examined. In our cell culture study, the IC50 value of ethanol extract was found to be the most effective. The IC50 values of Viburnum opulus L. water extract were 189.73±2.65, ethanol 108.98±1.93, ethyl acetate 135.87±2.07, n-hexane 152.98±1.04 ug/mL. While an extract kills cancer cells, it should not damage healthy cells. For this reason, in our study, healthy cells were applied as a positive control and it was observed that the doses affecting cancer had a low effect on healthy cells. Our study seems to be supportive of the literature.

CONCLUSION

In this study, Viburnum opulus L. can be considered as one of the promising aromatic plants on colon cancer in the future. Considering the results of this study; the effect of Viburnum opulus L. on colon cancer should be investigated at different incubation times and new studies should be included to elucidate apoptotic and cancer mechanisms.

Conflict of interest statement

The authors declare that they have no conflicts of interests.

Acknowledgements

REFERENCES

1. Pacal, I., Karaboga, D., Basturk, A., Akay, B., & Nalbantoglu, U. (2020). A comprehensive review of deep learning in colon cancer. Computers in Biology and Medicine, 126, 104003.

2. Ahmed, M. (2020). Colon cancer: a clinician's perspective in 2019. Gastroenterology research, 13.

3. Zhang, M., Chen, X., & Radacsi, N. (2021). New tricks of old drugs: Repurposing non-chemo drugs and dietary phytochemicals as adjuvants in anti-tumor therapies. Journal of Controlled Release, 329, 96-120.

4. Esmeeta, A., Adhikary, S., Dharshnaa, V., Swarnamughi, P., Maqsummiya, Z. U., Banerjee, A., ... & Duttaroy, A. K. (2022). Plant-derived bioactive compounds in colon cancer treatment: An updated review. Biomedicine & Pharmacotherapy, 153, 113384.

5. Kajszczak, D., Zakłos-Szyda, M., & Podsędek, A. (2020). Viburnum opulus L.—A review of phytochemistry and biological effects. Nutrients, 12(11), 3398.

6. Yilmaz, N., Yayli, N., Misir, G., Karaoglu, S., & Yayli, N. (2008). Chemical composition and antimicrobial activities of the essential oils of Viburnum opulus, Viburnum lantana and Viburnum orientala. Asian Journal of Chemistry, 20(5), 3324.

7. Nimse, S.B.; Pal, D. Free radicals, natural antioxidants, and their reaction mechanisms. RSC Adv. 2015, 5, 27986–28006.

8. Özrenk, M.; Gündogdu, N.; Kenskin, N.; Kaya, T. Some physical and chemical characteristics of gilaburu ˇ (Viburnum opulus L.) fruits in Erzincan region. I ˇgdır Univ. J. Inst. Sci. Technol. 2011, 1, 9–14.

9. Akbulut, M.; Calsir, S.; Marakoglu, T.; Coklar, H. Chemical and technological properties of European cranberrybush (Viburnum opulus L.) fruits. Asian J. Chem. 2008, 20, 1875–1885.

10. Ersoy, N.; Ercisli, S.; Gundogdu, M. Evaluation of European cranberrybush (Viburnum opulus L.) genotypes for.

11. Ozkan, G.; Ercisli, S.; Ibrahim, H.; Gulce, S. Diversity on fruits of wild grown European cranberrybush from.

12. Cam, M.; Hisil, Y.; Kuscu, A. Organic acid, phenolic content and antioxidant capacity of fruit flesh and seedof.

13. Çam, M.; Hişil, Y. Comparison of chemical characteristics of fresh and pasteurised juice of gilaburu (Viburnum.

14. Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. CA Cancer J Clin 2001;51(1):15-36.

15. Winawer SJ, Fletcher RH, Miller L, Godlee F, Stolar MH, Mulrow CD, et al. Colorectal cancer screening: clinical guidelines and rationale. Gastroenterology 1997;112(2):594-642.

16. Kopral, A.T. In vitro evaluation of gilaburu (Viburnum opulus L.) juice on different cell lines. Anadolu J. Educ. Sci. Int. 2019, 9, 549–571. .

17. Dienaite, L.; Pukalskiene, M.; Pereira, C.V.; Matias, A.A.; Venskutonis, P.R. Valorization of European cranberry bush (Viburnum opulus L.) berry pomace extracts isolated with pressurized ethanol and water by assessing their phytochemical composition.

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THE SCABIES CASE WITH RE-INFECTION OBSERVED IN HEALTHCARE PERSONNEL LIVING IN THE TENT CITY AFTER THE EARTHQUAKE IN HATAY

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ABSTRACT

Scabies is a disease caused by the parasite called Sarcoptes scabiei var. hominis, which is invisible to the human eye, is seen mostly in public areas, and is characterized by itchy rashes. It is thought that there are approximately 300 million cases per year in the world. A 23-year-old male patient, working as a healthcare professional, applied to the Dermatology clinic with complaints of itching and skin rash, which continued day and night for approximately three months and became more severe at night. The patient's history was taken, a physical examination was performed, and the patient was diagnosed with scabies. A 5% permethrin solution applied to the whole body every three days, a 1% permethrin shampoo to be applied to the scalp during each bath, a 5 mg antihistamine tablet before sleeping at night, and two doses of 18 mg ivermectin tablets seven days apart were prescribed. The patient, who improved clinically, applied to the clinic a week later due to recurrent complaints and re-infection was observed. The treatment was repeated a second time and a check-up was recommended after two weeks. The patient recovered at the end of the process. In this case report, it is aimed to draw attention to scabies and re-infection, which is a common disease in people living in tent cities after the earthquake.

INTRODUCTION

Scabies is a disease characterized by itching and skin rashes caused by a parasitic mite called *Sarcoptes scabiei var. hominis*, which is not easily noticeable to the human eye. It is particularly common during the winter months and is often observed in communal living spaces such as schools, dormitories, nursing homes, and prisons. The causative agent of scabies was first discovered by Bonomo in the year 1687. Sarcoptes scabiei is derived from the Greek words sarcoptes and scabie, originating from sarx (flesh), coptein (to cut), and scabere (to scratch). Scabies is commonly observed worldwide, irrespective of age, gender, race, or socioeconomic level[1,2].

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The World Health Organization added scabies to the "neglected tropical diseases and other neglected official diseases" list in 2013 to draw attention and create awareness[3]. It is estimated that there are approximately around 300 million cases of scabies worldwide annually[1,2]. According to research, the prevalence of scabies has been found to vary in the range of 0.2% to 71%. Children constitute 5-10% of the reported cases[4].

Scabies cases are more commonly observed during the winter season due to the increased prevalence of communal living spaces and the ability of the scabies mite to survive longer in cold environments[5,6]. Particularly worsening during the night intense itching, is the most significant symptom of scabies[7,8]. The formation of wounds on the body due to itching leads to infection. The presence of pearl-like lesions, the size of a pinhead, containing fluid on the surface of the skin and within the genital area, where female Sarcoptes scabiei is also found, is another significant clinical manifestation of scabies. Especially observed between the fingers, raised, line-shaped tunnels (sillons) ranging from 1 to 10 mm in length and grayish-white in color, caused by the scabies mite, are seen on the skin surface. Rashes especially occur in the folded areas of the skin[9,10].

In this case presentation, the occurrence of scabies in healthcare personnel staying in a tent camp for three months following an earthquake, the applied two-week treatment process (application of 5% permethrin solution every three days, use of 1% permethrin shampoo for the head during each bath, antihistamine tablet before bedtime, and 6 tablets of 3 mg ivermectin once a week) resulting in the patient's recovery is emphasized. However, the intention is to draw attention to the reoccurrence of infection in the patient one week later.

CASE REPORT

S.A, a 23-year-old male patient, resides in the Hatay/Antakya region. He is 176 cm tall and weighs 75 kg. For the past 4 months, S.A has been working as a healthcare personnel in a hospital in Hatay. According to the obtained medical history, after the earthquake, S.A stayed with his family in the Hatay/Antakya Expo tent camp center for three months. It was noted that shared toilets and bathrooms were used in the tents.

The patient has been experiencing persistent itching and skin rash complaints for approximately three months. The patient sought treatment at the MKU Faculty of Medicine Dermatology clinic and has been referred to the Parasitology Department laboratory for mite examination from the lesions. During the physical examination, it was observed that the inner side of the wrist (Image 1), inner side of the ankle (Image 2), genital area (Image 3), and chest region had itchy, red, raised, line-shaped whitish lesions, and occasionally crusted lesions.

Image 1. *Whitish lesions and rashes observed on the inner side of the wrist*

The systemic examinations of the patient were observed to be normal. Particularly, with the history of itching intensifying at night, the physical examination of the patient revealed pathognomonic lesions, including erythematous rash, redness due to itching, and the presence of tunnels (sillons) on the skin, confirming the diagnosis of scabies for the patient.

Image 2. *Crusted lesions and redness observed on the inner side of the ankle*

The patient has been prescribed topical 5% permethrin solution, 1% permethrin shampoo, oral antihistamines, and 3 mg tablets of ivermectin. Additionally, information has been provided to the patient regarding scabies, instructions on how to use the medications and lotions have been explained.

Before applying Permethrin 5% lotion, it is recommended to wash the body, then apply the lotion to dry skin, and take a shower again 24-48 hours later. The treatment should be repeated one week later, and improvement is expected within 15 days after the treatment. Family members have been informed to initiate treatment as well.

It has been explained that all clothing and bed linens should be washed at 60 degrees Celsius, dried, and ironed. If items cannot be washed, it is recommended to place them in a plastic bag without air for one week.

The patient has applied the medication as instructed and expressed that they have diligently adhered to the precautions during the treatment process. A few days after completing the treatment, the patient reported a decrease in the intensity of itching that worsened at night. Additionally, there has been a noticeable reduction and improvement in the presence of burrows (sillons).

Image 3. *Sillons (tunnels), redness, and crusted lesions observed in the genital area*

Image 4. *Redness and lesions observed in the groin area*

Image 5. *Redness and lesions observed in the leg area*

Patient S. A. has completely recovered upon completing the two-week treatment process. There has been no itching or lesions for one week; however, at the end of the first week, mild itching started in the middle of the chest.

Two days later, itching started in the groin area (Image 4), genital region, and on the fourth day, it began on the legs (Image 5), intensifying during the nights. Patient S.A, who returned to the dermatology clinic, narrated the history of the disease. Based on the physical examination conducted at the dermatology clinic and the observed symptoms, a re-infection was determined. The patient was again prescribed the previously used 5% permethrin solution, 1% permethrin shampoo, antihistamine tablets, and 3mg ivermectin tablets. The crucial points to be considered were explained to the patient. After the

two-week treatment, the patient has completely recovered.

DISCUSSION

During the physical examination of the patient, burrows (sillons) were observed. The presence of burrows (tunnels) is pathognomonic for scabies. Diagnosis is typically made based on the severe itching, redness, and characteristic rash experienced especially at night. The presence of itching and similar rash in other family members also supports the diagnosis. In the presented case study, the patient exhibits severe itching at night, along with a typical rash and redness; however, there is no history of scabies in the patient's family.

In scabies, itching, redness, and lesions are more commonly observed in folded areas such as the wrists, spaces between fingers, ankles, and the areas between the genitals and groin[11]. In the presented case study, similarly, itching, redness, and lesions have been observed in the folded areas, including the wrists and ankles, genital region, and chest region. In a study conducted in Turkey in 2018 and 2019, a significant increase was observed in the number of diagnosed scabies cases and cases of topical resistant scabies. There has been an increase in the number of patients with scabies in our country since 2018. In 2018, 186 patients and in 2019, 805 patients were diagnosed with scabies. Until the year 2018, cases of topical resistant scabies had not been observed in our country; however, the first cases were seen in 2018. In 2018, resistant scabies was observed in 20 patients (13.3%), and in 2019, it was observed in 87 patients (13.14%). The percentage increase in these cases nationwide was 81% in 2018 and 138% in 2019 in Turkey.

The study included a total of 17,803 patients diagnosed with scabies from ten different provinces. Antakya district in Hatay province was one of the areas included in the study, with 2029 patients (11.4%) diagnosed with scabies[12]. Particularly after the earthquake centered in Kahramanmaraş, referred to as the Disaster of February 6th, there was a significant increase in scabies cases in Hatay province due to the rise in communal living spaces like tent camps and difficulties in meeting hygiene needs.

Current treatment for scabies involves the use of 5% permethrin, 3 mg ivermectin, and antihistamine drugs. 5% Permethrin induces neurotoxicity in the sodium channels it inhibits, leading to paralysis and subsequently, the death of scabies mites. Permethrin is applied to the entire body, ensuring that no part below the neck is overlooked. It is crucial to repeat the treatment within 7-10 days for a more effective outcome. The initial treatment may not always be lethal to all mite eggs, and the administration of a second treatment will kill mites that have hatched from new eggs.

Ivermectin has been used in the institutional treatment of scabies in France since 2001. The normal dosage range for adults is 9-15 mg, with 1 tablet per 15 kg. A second dose is administered 2 weeks after the initial dose[13]. In our case, patient S.A, weighing 70 kg, took a single dose of 6 tablets and repeated the same dosage the following week.

In Hatay province, after the earthquake on February 6th, many tent cities were established, and people had to live in crowded spaces. Due to the lack of hygiene and infrastructure, and the inability to fully meet basic needs such as bathing and laundry, this situation created a conducive environment for the spread of certain parasitic diseases, especially scabies. In communal living areas like tent cities, attention has been drawn to considering scabies when diagnosing patients with similar clinical complaints and the risk of re-infection after treatment. It has been emphasized that a single-dose treatment may not be sufficient.

Conflict of interest statement

The authors declare that they have no conflicts of interests.

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REFERENCES

1. Romani, L., Steer, A. C., Whitfeld, M. J. & Kaldor, J. M. Prevalence of scabies and impetigo worldwide: a systematic review. *The Lancet infectious diseases* 15, 960-967 (2015).

2. Hengge, U. R., Currie, B. J., Jäger, G., Lupi, O. & Schwartz, R. A. Scabies: a ubiquitous neglected skin disease. *The Lancet infectious diseases* 6, 769-779 (2006).

3. Thomas, J. et al. Scabies: an ancient global disease with a need for new therapies. *BMC infectious diseases* 15, 1-6 (2015).

4. Akgöl, J. & Köroğlu, A. Scabies Disease (Scabies), Treatment, and Plants Used in the Treatment of Scabies Disease. *Journal of Faculty of Pharmacy of Ankara University* 46, 600-618 (2022).

5. GÜREL, G. & GÜREL, A. Multidisciplinary Approach to Sexually Transmitted Diseases. *Academician Publishing House* (2023).

6. Tüzün, Y. et al. The epidemiology of scabies in Turkey. *International Journal of Dermatology* 19, 41-44 (1980).

7. Arlian, L., Bruner, R., Stuhlman, R., Ahmed, M. & Vyszenski-Moher, D. Histopathology in hosts parasitized by Sarcoptes scabiei. *The Journal of Parasitology*, 889-894 (1990).

8. Ünver, A. Y. and Turgay, N. Approach to Scabies Patient. *Turkish Journal of Parasitology* 30, 78-83 (2006).

9. Özcel, M. Increasing Importance in Immunodeficiency Parasitic Diseases. Ok Üz, Üner A, Korkmaz M, Blastocystosis. *Türkiye Parasitology Association Publishing*, 1 (1995).

10. Burns, D. The treatment of human ectoparasite infection. *British Journal of Dermatology* 125, 89-93 (1991).

11. Şimşek, E., Keskin, A. & Dağcıoğlu, B. F. Sik Scabies, a common and often overlooked disease: a case presentation. *Ankara Medical Journal* 19, 205-209 (2019).

12. Moscarella, E. et al. A survey on teledermatology use and doctors' perception in times of COVID-19. *Journal of the European Academy of Dermatology and Venereology* 34, e772 (2020).

13. Richards, R. N. Scabies: diagnostic and therapeutic update. *Journal of Cutaneous Medicine and Surgery* 25, 95-101 (2021).

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A CASE OF NECROTIZING FASCIITIS DEVELOPED AFTER TENOXICAM INJECTION[a]

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ABSTRACT

Necrotizing fasciitis is a rapid and mortal subcutaneous infection that is observed in the superficial fascia and settles in the tissues between the skin and deep muscle layers. Although its incidence is 0.4 per 100 thousand cases, its overall mortality is as high as 52%. It should be considered when clinically severe pain and systemic toxicity are encountered. It was observed that our case was in septic shock, and the patients clinical condition progressed aggressively in the emergency department follow-up. In this article, a 55-year-old patient who developed necrotizing fasciitis as a result of tenoxicam injection and underwent debridement is presented.

INTRODUCTION

Necrotizing soft tissue infections are a group of diseases with sudden onset and rapidly worsening, widespread soft tissue necrosis and systemic toxicity with high mortality[1]. These Infections may initially appear benign.Risk factors for necrotizing soft tissue infections; advanced age, diabetes mellitus, alcoholism, peripheral vascular disease, heart disease, kidney failure, human immunodeficiency virus (HIV), cancer, NSAID use, decubitus ulcers, chronic skin infections, IV drug use and immune system disorder[2].

CASE

A 55-year-old male patient applied with the complaints of swelling, pain and redness in the right hip after tenoxicam injection, which was administered at home one week ago. He had no known chronic disease. Examination findings include painful joint movements in the right hip and knee, ecchymotic appearance in the right hip, swelling, redness. There was widespread tenderness and crepitation in the lateral thigh and hip. The general condition of the patient was moderate when he came

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to the emergency department; blood pressure: 100/60 mmHg, pulse rate: 98/min and rhythmic, fever: 38.2C°, respiratory rate: 22/min. Laboratory findings; leukocytes: 26.5x103/L(4000-10000), hemoglobin: 6.5 g/dL(11-15), CRP: 279 mg/L(0-5). In the emergency superficial US examination, fluid reaching 2 cm in width in its widest part was observed in the right gluteal region. On this background, the skin had a subcutaneous volumnous, edematous appearance. Computed tomography imaging of the pelvis and lumbosacral region with the preliminary diagnosis of necrotizing fasciitis showed a collection containing free air near the right gluteal region, heterogeneity and dirty appearance in the subcutaneous tissue, free air values between the muscle planes of the right thigh. The patient was given meropenem 2 gr, vancomycin 1 gr, clindamycin. 500 mg was started. The patient with low hemoglobin was given 2 units of erythrocyte suspension. Plastic surgery was consulted. The patient diagnosed with necrotizing fasciitis was taken to emergency surgery for debridement. The patient was followed up by plastic surgery for about 50 days and was discharged with full recovery.

DISCUSSION

Necrotizing soft tissue infections are mostly polymicrobial. These microorganisms are gram positive cocci, gram negative bacilli and anaerobes. Monomicrobial infections are caused by group A streptecocci. It is mostly seen in people who have a history of trauma or who have had a recent operation from the infection site. Community-acquired MRSA is particularly common in IV drug users, athletes, and hospitalized patients.The rapid necrotizing process typically results from an external trauma (IV injection, surgical incision, abscess, insect bite, ulcer) or begins with direct invasion of subcutaneous tissue, spreading through hollow organ perforation[1,3]. Skin involvement develops secondary to vasculitis and thrombosis of blood vessels. There is little skin change in the early stage to show the extent of the infection, as multiple capillary beds must be thrombosed before skin manifestations develop.

As the disease progresses, diffuse gangrene occurs in the skin,subcutaneous fatty tissue, fascia and skeletal muscle.Clinical symptoms are severe pain, anxiety and sweating. Pain is usually hypersensitive in the area of erythema and is disproportionate to physical examination findings[4,5]. Therefore, it is the most important feature in early diagnosis. Therefore, emergency surgical consultation and surgical intervention are the main treatment. The patient was followed up by plastic surgery for about 50 days and was discharged with full recovery.

On examination, subcutaneous edema that does not easily collapse with pressure in the painful area can be seen and there may be crepitation caused by bacterial gas production.The main symptoms of necrotizing fasciitis are crepitation, skin necrosis, bullae, hypotension and gas on the radiograph.

Gas may not always be seen on the radiograph. Contrast-enhanced CT is the most sensitive method in necrotizing fasciitis. The most reliable indicator of necrosis in CT is the deep tissues that are not enhanced. Due to tissue ischemia in necrotizing skin infections, the immune system cannot destroy bacteria and prevents antibiotic activity[6].

CONCLUSION

Necrotizing fasciitis is a rare but progressive and fatal soft tissue infection. Despite all the interventions, effective treatment protocols and patient care, it is a disease that is difficult to manage. It should never be forgotten that it progresses with high mortality despite early diagnosis and treatment.

Image 1. *Case of necrotizing fasciitis developed after tenoxicam injection*

Image 2. *Abdominal CT scan detecting intramuscular free air.Subcutaneous emphysema of the right thigh root.*
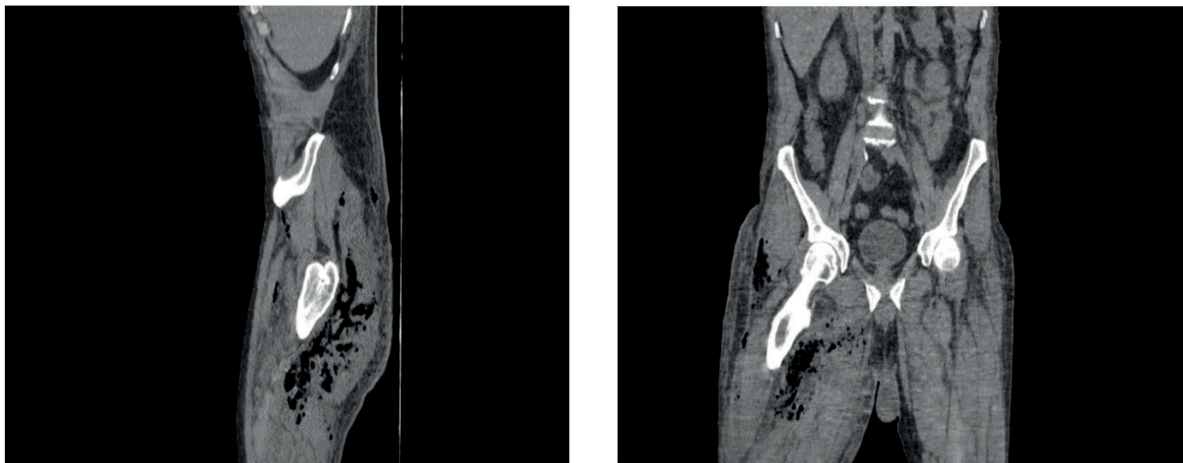

Image 3. *First day after surgical debridement*

Image 4. *Granulation tissue after debridement postoperatively*

Conflict of interest statement

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REFERENCES

1. Wallace HA, Perera TB. Necrotizing Fasciitis. [Updated 2023 Feb 21]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430756/

2. Edlich RF, Cross CL, Dahlstrom JJ, Long WB 3rd. Modern concepts of the diagnosis and treatment of necrotizing fasciitis. J Emerg Med. 2010 Aug;39(2):261-5. doi: 10.1016/j.jemermed.2008.06.024. Epub 2008 Dec 11. PMID: 19081698.

3. Abbate A, Almasio PL, Mongitore M, Di Vita G, Patti R. Necrotizing Soft Tissue Fasciitis after Intramuscular Injection. Case Rep Surg. 2018 May 8;2018:3945497. doi: 10.1155/2018/3945497. PMID: 29854541; PMCID: PMC5964413.

4. von Glinski M, Dadras M, Wallner C, Wagner JM, Behr B, Lehnhardt M. Die nekrotisierende Fasziitis [Necrotizing Fasciitis]. Handchir Mikrochir Plast Chir. 2021 Jun;53(3):312-319. German. doi: 10.1055/a-1381-8077. Epub 2021 Jun 16. PMID: 34134163.

5. Hsiao GH, Chang CH, Hsiao CW, Fanchiang JH, Jee SH. Necrotizing soft tissue infections. Surgical or conservative treatment? Dermatol Surg. 1998 Feb;24(2):243-7; discussion 247-8. PMID: 9491119.

6. Young MH, Aronoff DM, Engleberg NC. Necrotizing fasciitis: pathogenesis and treatment. Expert Rev Anti Infect Ther. 2005 Apr;3(2):279-94. doi: 10.1586/14787210.3.2.279. PMID: 15918785.

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COMPARISON OF TURKEY AND LUXEMBOURG IN TERMS OF HEALTH SYSTEMS

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ABSTRACT

Comparative health systems approach refers to a research methodology that aims to reach useful conclusions and analyses by comparing certain facets of the health systems of two or more countries[1]. As in other areas, the exchange of information on health systems between countries has been very intensive. In the late 1980s and early 1990s, under the influence of globalization dynamics and the liberal economic and political policies that emerged as a result of these dynamics, there has been a significant impact on various systems around the world and especially on national health systems, policies and services[2]. This study was conducted with the aim of providing general information about the health systems of Luxembourg and Turkey, comparing the health systems of these countries and determining the differences between their health data.

INTRODUCTION

The structures of healthcare systems of countries, such as how healthcare services are delivered and how healthcare expenditures are financed, are critical factors directly affecting various outcomes in the field of health. In our era, different countries adopt various models of healthcare systems and implement them within their socio-economic and political frameworks. The provision of healthcare services, methods of financing expenditures, and whether they are managed through the public or private sector are examined as fundamental dynamics of each healthcare system. As a result of these factors, significant differences are observed among countries in parameters such as per capita healthcare expenditures, the share of healthcare expenditures in Gross Domestic Product (GDP), the distribution of expenditures between public and private sectors, and the proportion of out-of-pocket healthcare expenditures by citizens. These differences are directly reflected in the health indicators of countrie[3].

Each country's choice regarding healthcare financing is shaped according to its economic situation, social structures, and political preferences. How the burden of financing will be distributed within society varies from country to country. Especially in developed countries, it has been stated that the increase in healthcare expenditures is related more to the quality of demand for healthcare

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services rather than demographic changes such as the increase in the elderly population and the age structure of the society. Economic growth, technological advancements in medicine, and the increasing demand for healthcare services, as well as innovations in healthcare policies (such as changes seen in healthcare systems in the USA and Turkey), can lead to an increase in healthcare expenditures and changes in expenditure dynamic[4].

Controlling the increasing healthcare expenditures is of great importance to ensure fair access to healthcare services for the population, to enhance the quality of healthcare services, and to achieve improvements in health indicators. Therefore, countries continuously review their healthcare systems and strive to manage the delivery and financing of healthcare services more effectively. This process indicates the need for the continuous evaluation and improvement of healthcare policies and practices[5].

This research aims to provide a comprehensive overview of the basic features of the healthcare systems in Turkey and Luxembourg, and how these systems differ from each other. The structural elements of the healthcare model adopted by the examined countries, healthcare expenditures, financing mechanisms, and critical factors such as equity in access to healthcare services are thoroughly examined. The potential effects of these various healthcare systems, in terms of their impact on the delivery and financing of healthcare services, as well as on overall health indicators and citizens' access to healthcare services, are also analyzed from a comparative perspective. Thus, the aim is to develop a deeper understanding of the effectiveness of different healthcare systems in practice and their potential effects on healthcare services.

LUXEMBOURG

General Information

Located in the heart of Western Europe and without a coastline, Luxembourg holds a significant place among Europe's smallest countries. Situated west and north of Belgium, east of Germany, and south of France, this small country, with a population of approximately 600,000, stands out due to its strategic location. Despite its small size, Luxembourg is ethnically diverse and economically highly developed; according to IMF and World Bank statistics, it ranks second in the world in terms of per capita income, following only Qatar.

Luxembourg, with its capital bearing the same name and the only country to have been awarded the title of European Capital of Culture in 1995 and 2007, respectively. Officially known as the Grand Duchy of Luxembourg, this country is the only grand duchy in the world and is governed by a constitutional monarchy. Divided into three by its administrative structure – Diekirch, Grevenmacher, and Luxembourg – Luxembourg, although shaped under the influence of French and German cultures due to its historical and geographical location, has preserved its unique culture and identity. With more than 160 nationalities living in this country, it is known for its multiculturalism, tolerance, and openness. Although Luxembourgish is mostly spoken, French and German are also widely used in state institutions[6].

Luxembourg's population structure is dominated by people of French and German descent, and the low population growth rate has led to a contraction in the labor market. This contraction is compensated for by immigrants and foreign workers from Portugal, Italy, and other Southern European countries. Many immigrants and foreign workers work in mining, steel industry, finance, and international companies in the country. While the majority of the population is Catholic, there are also religious minorities such as Protestants and Jews. A large part of the population lives in the capital Luxembourg and the small towns in the southwest, which is the industrial center of the Grand Duchy.

The village of Schengen, renowned worldwide as the place of signing the Schengen Agreement, is also located in Luxembourg. This small winemaking village opened the doors to unlimited and passport-free travel in Europe on June 14, 1985. Luxembourg is a founding member of important international organizations such as the European Union, NATO, the United Nations, the Benelux Union, and the Western European Union. The historic neighborhoods and walls in the city, protected by being included in the UNESCO World Heritage List in 1994, are preserved. In Luxembourg, especially in the capital, many museums showcase the country's cultural and artistic richness. The National History and Art

Museum, Luxembourg City History Museum, Grand Duke Jean Museum, and Military History Museum in Diekirch are among the country's most important museums. The Luxembourg Ardennes, or the Eisleck region, with its high plateaus and rivers, is one of the country's remarkable natural beauties.

A General Overview of the Healthcare System of Luxembourg

Healthcare services in Luxembourg operate within a framework of a "Welfare-Oriented/Insurance-Based" system. In this system, patients have the freedom to choose their healthcare providers, and there is an obligation for private healthcare workers to adhere to agreements between professional associations and insurance companies. Public health and healthcare services in Luxembourg are regulated by the Ministries of Health and Social Security. The Ministry of Health is responsible for the provision of treatment and prevention-focused healthcare services to the public, while the Ministry of Social Security regulates the basic components of the social security system, such as health insurance and dependency insurance[1].

In 1901, following a similar model implemented by Bismarck in Germany, Luxembourg established a mandatory health insurance system targeting workers in the industry and production sectors. In 1925, a law combining sickness, accident, old age, and disability insurances came into effect as the insurance sector expanded and became more complex[7].

Until the early 20th century, most hospitals in the country were administered by religious organizations. However, increasing costs over time necessitated state intervention in this area. In 1976, a law on hospital planning was enacted to support the state's planning efforts in the hospital sector.

By the 2000s, eight out of ten deaths in Luxembourg were due to non-communicable diseases. Cardiovascular diseases were the leading cause of these deaths, accounting for one-third of all deaths. However, mortality rates due to these diseases have shown a regular decline in recent years and have fallen below the European average[8].

Provision of Healthcare Services

In Luxembourg, healthcare services operate under a system where individuals covered by insurance can freely choose any healthcare provider or institution (such as hospitals or clinics) according to their preferences. This flexibility facilitates access to healthcare services for patients and creates a competitive environment among healthcare providers[1].

Primary healthcare services are generally provided by independent and private general practitioners. These practitioners play a significant role in referring patients to secondary healthcare services, leading to direct competition with specialist physicians. The organization and provision of preventive healthcare services are the responsibility of the Ministry of Health. In addition to services provided by the state, healthcare services offered by private and non-profit organizations are also financed through the ministry's budget. As of 2017, Luxembourg had 10 acute care hospitals with a bed ratio of 4.7 beds per 1,000 people (OECD; 2021).

Except for medical expenses not covered by insurance, hospital visits, inpatient services provided in second-class rooms, and outpatient services are covered by insurance. Patients contribute a co-payment of 8.86% for the services received. While the management structure of hospitals varies by institution, generally, each hospital is governed by a board of directors, and annual budgets are determined through negotiations with the Association of Sickness Funds[8].

The financing of healthcare services in Luxembourg is based on a model that combines mandatory social insurance with complementary and voluntary health insurance. This system relies on mandatory social insurance, which forms the legal framework of the insurance system, aiming to ensure fair and comprehensive access to healthcare services[8].

Health Financing and Expenditures

Similar to its neighbors Belgium, France, and Germany, Luxembourg provides access to healthcare services through a social health insurance system. In this system, approximately half of the costs of long-term care services

are covered by the state, while the remaining portion is supported by taxes paid by workers and contributions from electricity-generating companies. The majority of insurance revenues are collected through contributions from insured individuals, while the remaining portion is supplemented by the state budget[9].

In Luxembourg, healthcare services are financed through a triple insurance system consisting of mandatory, complementary, and voluntary insurance. According to this system, all regular workers, regardless of income or profession, are covered, including those paying for unemployment insurance and even students not covered by insurance. Additionally, minors and disabled individuals can benefit from this insurance, and the state assumes insurance payments for those in need[8].

Residents of Luxembourg who are not covered by insurance can obtain access to healthcare services by purchasing voluntary insurance after a certain period. Individuals over the age of 18 and no longer dependent on their families can apply within six months to continue benefiting from the healthcare services offered by insurance. Voluntary insurance covers services not covered by public insurance, but it offers lower reimbursement rates and provides fewer services. Nevertheless, a large portion of workers take advantage of the benefits of voluntary insurance[1].

Health Reforms

Health reforms carried out in Luxembourg during the 1980s and 1990s primarily focused on ensuring the financial stability of sickness funds. Significant developments during this period included increasing contributions to healthcare services, establishing the Union of Sickness Funds to balance budgets, and transferring the responsibilities of various sickness funds to this union.

In 1995, significant changes were made to the payment structure due to rising hospital costs, initiating a process where hospital budgets were determined through negotiations between the Union of Sickness Funds and hospitals[1].

In 1998, a law was enacted formally recognizing patients' basic rights. These rights include:

- Information: Patients have the right to be informed about their health conditions and appropriate treatment methods, although they also have the right to refuse to be informed.

- Informed Consent: Any diagnostic or treatment procedure cannot be performed without the informed consent of the patient. Patients have the right to refuse proposed diagnoses or treatments.

- Examination of Medical Records: Patients have the right to access their medical records, although they do not have access to personal notes kept by healthcare professionals.

- Confidentiality: Healthcare professionals are obligated to maintain the confidentiality of patient files and should not share this information with third parties.

These regulations were enacted to safeguard patients' rights and improve the quality of healthcare services.

TURKEY

General Information

The Republic of Turkey, the country that bears its name, is located on the Anatolian Peninsula for the majority of its territory, with a small part in Thrace, an extension of the Balkan Peninsula. Turkey's population is around 82 million and its total area is 814,600 square kilometers, ranking 36th among the world's countries. Surrounded by seas on three sides, this unique geography consists of two important peninsulas divided into two by the Bosporus and Dardanelles straits. These two strategic waterways physically separate the continents of Asia and Europe, separating Anatolia from Thrace. This geographical location makes Turkey a natural bridge and an important geostrategic power, as well as a country noted for its rich historical and cultural heritage.

Turkey is a constitutional republic based on democratic principles, with a secular structure and a unitary state organization, and its administration is based on a presidential system. Its official language is Turkish, the mother tongue of the majority of the population. The Turkish ethnic group constitutes 70-80% of the population,

while minorities recognized under the Treaty of Lausanne and other unrecognized ethnic groups enrich the country's cultural diversity. In terms of religious belief, the majority follow Sunni Islam[10].

Turkey is a member of many important international organizations, including the Council of Europe, NATO, OECD, OSCE and G-20. It became an associate member of the European Economic Community in 1963, joined the EU Customs Union in 1995 and started negotiations for full membership with the European Union in 2005. It also actively participates in regional and cultural organizations such as the Organization of Turkic States, the International Organization of Turkic Culture, the Organization of Islamic Cooperation and the Organization for Economic Cooperation. Turkey is recognized as a regional power with its military capacity and diplomatic activities, and is a geopolitical actor of global importance with its strategic location at the crossroads of Europe and Asia.

A General Overview of the Healthcare System of Turkey

The Turkish Health System is divided into three main periods.

- **Post-Republican Period (1920-1960):**

The foundations of the Ministry of Health of the Republic of Turkey were laid in 1920. In the first years of its establishment, the Ministry focused on rebuilding the health infrastructure of the country emerging from the war and developing the basic laws that would form the health system. As Turkey's first Minister of Health, Dr. Refik Saydam took important steps to strengthen the infrastructure of health services for 14 years. He opened public hospitals, maternity and child care centers in Ankara, Erzurum, Diyarbakır, Sivas and many other cities. Attaching great importance to the training of health personnel, Saydam established health education courses and dormitories for medical students, launched the Institute and School of Public Health in 1928, and opened dispensaries in Istanbul and Ankara to combat tuberculosis[11].

Between 1923 and 1946, the cornerstones of Turkey's current public health system were laid. During this period, many laws were passed that clearly defined the responsibilities and duties of the Ministry of Health. Preventive health programs, especially for the control of infectious diseases such as tuberculosis, malaria and leprosy, came to the fore. The organization of health services showed a vertical structuring in this period[11].

Between 1946 and 1960, Turkey entered a period in which health centers were established to provide integrated health services. At the same time, all hospitals were taken from local governments and placed under the control of the Ministry of Health. In 1946, the Social Insurance Institution (SSK) was established to provide health insurance services for workers in the private sector and blue-collar workers in the public sector. This period was an important turning point in strengthening Turkey's healthcare infrastructure and improving public health[12].

- **Socialization Period (1961-1980)**

In 1961, Law No. 224, which would fundamentally transform Turkey's healthcare infrastructure, was enacted. This legal regulation laid the groundwork for an inclusive and national healthcare system in the country. The law emphasized the importance of providing healthcare services impartially, continuously, and in response to the needs of the people, thereby giving momentum to the establishment of an Integrated Healthcare Services System. The main goal was to provide either completely free or partially free healthcare services to all citizens. The financing of these services was provided through insurance payments and allocations from the state budget. Especially, the development of the necessary infrastructure for preventive healthcare and environmental health services, as well as the spread of health education throughout the country, was targeted. However, the investments required for this expansion could not be fully realized. Due to the significant portion of resources allocated to personnel expenses, the necessary infrastructure, medical equipment, and other needs could not be adequately met[13].

In 1963, for the first time in Turkey, the five-year development plan included the health sector. The aim of this plan was to prioritize preventive healthcare services, provide general healthcare services through the Ministry of Health, distribute healthcare personnel fairly across the country, improve public health services, promote the national pharmaceutical industry, support the

opening of private hospitals, implement General Health Insurance, and establish the revolving capital system in state hospitals. This plan is recorded in history as one of Turkey's significant steps in the healthcare sector[11].

- **Neoliberal Reforms Period (1980+)**

Between 1980 and 2002, significant constitutional rights regarding access to social insurance and healthcare services were granted in Turkey. The 1982 Constitution recognized social security as a right for every citizen and stated that the state must provide social insurance opportunities to its citizens. Additionally, provisions regarding the organization of healthcare services and the implementation of General Health Insurance (GSS) were included in this constitution. Between 1986 and 1989, the government passed laws, including the Basic Healthcare Services Law and the initiation of health insurance through Bağ-Kur. These laws aimed to address the shortcomings in the Integrated Healthcare Services System established in 1960. However, the Basic Law could not create a comprehensive healthcare policy or introduce legal regulations to support systemic reforms[14].

As of 2003, public healthcare financing in Turkey was based on a social security system established in 1946, which showed significant developments in the following decades. In 1992, the government initiated the Green Card program, aiming to provide healthcare services to financially disadvantaged citizens. This program was considered a temporary solution until the full implementation of GSS. Applications for the Green Card were evaluated by commissions at the district level.

By 2003, approximately 85% of the population had some form of health insurance coverage, while the remaining 15% lacked access to health insurance. However, preventive and primary healthcare services provided through the Ministry of Health network were indirectly financed. SSK (Social Insurance Institution), Bağ-Kur (Social Security Organization for Artisans and the Self-Employed), and Emekli Sandığı (Retirement Fund) were the institutions with the largest share in insurance coverage, while the coverage of private insurances was quite limited. However, there were issues with insurance coverage data for this period, such as multiple insurances by different social security institutions, deficiencies in the registration system, and uncertainties regarding the number of individuals responsible for insuring others. There are significant differences in health insurance coverage rates between SPO data and surveys conducted by the Ministry of Health and TurkStat, revealing uncertainties and gaps in official data[15].

Recent Health Reforms in Turkey

In Turkey, the provision of health services by the state became a constitutional obligation with the Law No. 224 adopted in 1961, which stipulated the socialization of health services. However, with the 1982 Constitution, health services were no longer directly provided by the state, but were limited to the state's duty to plan and regulate health institutions. Throughout the 1980s and 1990s, reforms were introduced to promote liberalization in the financing and delivery of health services. These reforms included measures such as financing health services through insurance premiums and contributions instead of taxes, privatization of health institutions and the introduction of performance-based payment systems[11].

Launched in 2003, the Health Transformation Program (HTP) introduced a comprehensive approach to the health system. The SDP includes important changes such as centralization of health financing, implementation of the family medicine system, semi-autonomous structure of public hospitals and performance-based remuneration of health workers. In addition, the number of subcontracted workers in the health sector was increased and the focus was on issues such as quality and accreditation to improve the quality of health services[16].

Among the main objectives of the SDP were the role of the Ministry of Health as a planner and regulator, the inclusion of all citizens under the General Health Insurance (GSS), the provision of accessible and effective healthcare services, and the enhancement of quality and efficiency in healthcare services. This transformation process included significant steps such as transitioning from health centers to the family medicine system, establishing healthcare enterprises, and consolidating social security institutions under a single roof. With the implementation of the Family Medicine Pilot Application Law and other legal regulations in 2004, the healthcare system in Turkey aims to be more efficient, accessible, and sustainable[1].

Financing Health Expenditures in Turkey

While the delivery of health services in Turkey is carried out by various organizations in both the private and public sectors, access to health services is differentiated between individuals with and without insurance coverage. Insured individuals receive services under different health regimes. The country's health services are financed by the central government's general budget revenues, i.e. taxes, compulsory insurance premiums and direct payments by individuals for services. Some insured individuals receive health care through systems such as SSK, Bağ-Kur or private insurance, with premiums paid by employees and employers, while others, such as pensioners and active public servants, are covered by the state. Individuals who are not covered by insurance pay directly for health services, but those who cannot afford to pay are covered by various state funds.

Insured people who are linked to these funds have to pay a certain portion of the health services they consume, such as medicines, eyeglasses, dental services, etc., as a consumer contribution. This diversity makes Turkey's health financing system quite complex[11].

Health expenditures are financed by various institutions and organizations and a unified organizational structure has not been established throughout the country (Tokat 1993:12). In the current system, the Ministry of Health, the Ministry of National Defense, universities, autonomous and special budget institutions, municipalities and social security institutions such as SSK, Bağ-Kur and Emekli Sandığı provide health services. In addition to these, foundations, foreign and minority communities and the private sector are also part of health services (Yıldırım, 2000).

Table 1. *Comparison of Health Data between Luxembourg and Turkey*

Indicator	Luxembourg	Turkey
Total Population	600,000	82,000,000
Population Growth Rate	Decreased by 3%	Increased by 1.5%
Population Over 65 Years Old (%)	14.08	8.5
Number of Hospitals	10	1510
Hospital Beds per Capita	4.7	2.5
Total Fertility Rate (%)	1.47	2.07
Life Expectancy at Birth	82.29 (Male=80.1, Female=84.6)	78 (Male=75.3, Female=80.8)
Maternal Mortality Rate (per 100,000)	10	16 (2006: 28.5)
Under-5 Mortality Rate (per 1,000)	10	16
Vaccination (%)	99	98
DTaP Vaccination (%)	99	97
DTaP 3 and BCG Vaccination		DTaP3=96, BCG=93
HBV 3 Vaccination (%)		96
Measles Vaccination (%)	99	98
Hib 3 Vaccination (%)	38	7
GDP (Billion $)	62.404	851.102
GDP per Capita ($)	111,710	27,000
Physicians per 1,000 People	2.92	1.8
Nurses per 1,000 People	11.7	1.9
Healthcare Expenditure	Total: $6,475, State: $5,286, Private: $1,188	Total: $1,194, State: $938, Private: $260
Smoking Prevalence (%)	14.9	27.3
Tuberculosis Incidence (per 100,000)	5.8	18
Adolescent Fertility Rate (2016)	5.44	26.93 (1986: 74.65)

Source: https://data.oecd.org/luxembourg.htm (Accessed on 16.04.2021)

Conflict of interest statement

The authors declare that they have no conflicts of interests.

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REFERENCES

1. Sargutan, E. "Comparative Health Systems - Concept, Method, and Applications, Country Examples with General System and Financial Structures of Countries, Types of Health Systems and Prominent Features with Examples of Turkey's Health System." Hacettepe University Publication, Ankara, 2006.

2. Yıldırım, H. H., Tarcan, M. "Comparative Health Systems: Lessons to be Learned for Turkey." In: Proceedings of the 1st National Health Administration Congress. Editors: Tatar M, Erigüç G, Sahin. Ankara, May 20-21, 2000. pp. 577-592.

3. Çelik, Y. "Analysis of Health Expenditures in Turkey and Evaluation of the Appropriateness of Health Expenditure Levels." Social Security Journal, 1(1): 62-81, 2011.

4. Çakır, Ö. D. "Evaluation of Health Financing Policies Implemented in Turkey and the European Union." Unpublished Master's Thesis, Department of Business Administration, Faculty of Economics and Administrative Sciences, Marmara University, 2016.

5. Grabowski, H. "The Evolution of the Pharmaceutical Industry Over the Past 50 Years: A Personal Reflection." International Journal of the Economics of Business, 18(2): 161-176, 2011.

6. https://en.wikipedia.org/wiki/Luxembourg. Access date: 30.06.2022

7. WHO. "Health Care Systems in Transition." Luxembourg, European Observatory on Health Care Systems, World Health Organization, 1999. [Accessed on May 7, 2019]. Available at: http://www.euro.who.int/document/e67498.pdf.

8. WHO. "Health Care Systems in Transition." Luxembourg, European Observatory on Health Care Systems, 2015.

9. Karagan, E. "Comparison of Some European Union Country Health Systems with the Turkish Health System." Marmara University Institute of Health Sciences, 2008.

10. https://en.wikipedia.org/wiki/Turkey. Access date:14.02.2021.

11. Yüksel, O. "Health Services and Health Expenditures in the Process of Health Reforms: Examples of Selected Countries and Turkey." Master's Thesis, Institute of Social Sciences, Namık Kemal University, 2017.

12. Demirci, B. "Transformation in Health Services: What Kind of System Does the Health Transformation Program (HTP) Bring?" Mediterranean Journal of Economics, Business and Finance, 15(30): 122-135, 2015.

13. Yurdadoğ, V. "Financing and Analysis of Health Expenditures in Turkey." Journal of Çukurova University Institute of Social Sciences, 16(1): 591-610, 2007.

14. TÜSİAD. "A Healthy Future: Feasible Solution Proposals on the Path to Health Reform." Publication No. TÜSİAD-T/2002-09/380, Istanbul, 2004.

15. Cevahir, Egemen. "Transformation of the Health System in Turkey: Examples of Social Reflection." Kibele Publications, Istanbul, 2016.

16. Daştan, I., Çetinkaya, V. "Comparison of OECD Countries and Turkey's Health Systems, Health Expenditures, and Health Indicators." Social Security Journal, 5: 104-134, 2015.

17. Yıldırım, H. H. "Why Can't Turkish Health Reforms Be Implemented? An Evaluation in the Light of European Health Reforms." Proceedings of the 1st National Health Administration Congress: Management of Health Services and Institutions in the 2000s, Ankara, May 20-21, 2000.