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THỂ LỆ GỬI BÀI ĐĂNG TẠP CHÍ SINH LÝ HỌC VIỆT NAM

Tạp chí Sinh lý học Việt Nam là tạp chí chuyên ngành Sinh lý học. Tạp chí đăng tải các công trình nghiên cứu, các bài tổng quan, thông báo khoa học thuộc chuyên ngành Sinh lý học và các chuyên ngành có liên quan với Sinh lý học Y học, Sinh lý học Người và Động vật.

1. Quy định chung về bài đăng trên Tạp chí Sinh lý học Việt Nam

- Các thuật ngữ thống nhất theo tự điển Bách khoa Việt Nam.
- Bài gửi đăng phải đánh máy bằng tiếng Việt rõ ràng, phông chữ Unicode, kiểu chữ Arial, cỡ chữ 12, khổ giấy 19*26.5, lề trên 2cm, lề dưới 2cm, lề trái 2.5cm, lề phải 2.5cm, cách dòng 1.15 line. Các chữ viết tắt phải được chú thích các từ gốc của các chữ viết tắt đó. Thứ tự các đề mục đánh số Ả-rập, không đánh số La Mã (Thí dụ 1, 1.1, 1.1.1, 2, 2.2...).
- Bài đăng Tạp chí gửi về địa chỉ email **tapchi@sinhlyhoc.com.vn**, gửi kèm theo tên, địa chỉ liên lạc, địa chỉ email và số điện thoại của tác giả chịu trách nhiệm khoa học về bài báo (Tạp chí không nhận bản in).
- Mỗi tác giả được phép đăng nhiều bài trong 1 số nhưng chỉ được đứng tên đầu ở 1 bài. Bài không đăng được, không trả lại bản thảo.
- Tác giả chịu trách nhiệm khoa học của bài báo phải ký vào văn bản cam kết về bản quyền của mình, các số liệu nghiên cứu, nội dung được đưa ra trong bài báo, các vấn đề về đạo đức nghiên cứu và gửi về địa chỉ Ban biên tập:

Văn phòng Hội Sinh lý học Việt Nam
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2. Một số yêu cầu cụ thể về bài đăng công trình nghiên cứu khoa học

- Bài gửi đăng chưa được đăng ở bất kỳ Tạp chí quốc gia nào.
- Tổng số trang của bài đăng công trình không quá 8 trang giấy 19*26.5, không quá 10 trang với bài tổng quan.
- Tổng số các đối tượng minh họa, kết quả (gồm hình, bảng, biểu) không quá 5 (gồm bảng, biểu, hình, ảnh, biểu đồ) và/hoặc 1/4 tổng số trang của bài báo. Tên các đối tượng được ghi theo số thứ tự cho mỗi loại (ví dụ hình 1, hình 2, bảng 1, bảng 2). Tên bảng được đặt ở trên, chính giữa bảng, tên hình, biểu đồ được đặt ở dưới, chính giữa hình, biểu đồ.
- Lệ phí đăng công trình nghiên cứu là 1.000.000 đồng/bài (quy trình 1, bài báo được chấp nhận đăng theo sắp xếp của tạp chí, đã bao gồm 4 quyển tạp chí) hoặc 2.000.000 đồng/bài (quy trình 2, bài báo được chấp nhận trong số gần nhất, đã bao gồm 4 quyển tạp chí). Kinh phí được thu khi tác giả gửi yêu cầu đăng bài và không hoàn trả kinh phí khi bài báo bị từ chối đăng. Thông tin tài khoản của Hội Sinh lý học Việt Nam như sau:

Chủ tài khoản: **Hội Sinh lý học Việt Nam**
Số tài khoản: **6 4567 6668** tại **Ngân hàng Quân đội (MB), chi nhánh Thanh Xuân**

- Trình tự các mục trong bài:
  + Tên bài báo: Được viết ngắn gọn, thể hiện được nội dung chính của bài báo và bắt đầu bằng danh từ
  + Họ và tên các tác giả, địa chỉ cơ quan, nơi thực hiện công trình (không ghi học hàm, học vị, chức danh). Tác giả thực hiện chính được viết đầu tiên, tác giả chịu trách nhiệm khoa học về bài báo được viết cuối cùng nếu có (ví dụ tên thầy hướng dẫn). Cuối trang thứ nhất của bài báo cần ghi rõ tên tác giả chịu trách nhiệm khoa học về bài báo, kèm theo địa chỉ liên lạc, địa chỉ email và số điện thoại. Liệt kê đầy đủ tất cả các tác giả tham gia bài báo, đề nghị không viết "và cộng sự".
  + Tóm tắt tiếng Việt: Viết không quá 300 từ, viết dưới dạng bài văn xuôi thể hiện được mục tiêu, đối tượng nghiên cứu, phương pháp nghiên cứu, kết quả chính của nghiên cứu và kết luận. Từ khóa không quá 5 từ, cụm từ.
  + Tên bài báo và tóm tắt bằng tiếng Anh đặt ở cuối bài báo, sau tài liệu tham khảo, cần được dịch đầy đủ chính xác từ tên bài báo, tóm tắt và từ khóa bằng tiếng Việt.
  + Nội dung toàn văn gồm:

- √ Đặt vấn đề (bao gồm cả mục tiêu nghiên cứu của đề tài): Cần nêu rõ lý do hoặc giả thuyết nghiên cứu, mục tiêu nghiên cứu (không trùng lặp với tên bài báo).
- √ Đối tượng và phương pháp nghiên cứu: Viết ngắn gọn, đầy đủ thông tin bao gồm: đối tượng nghiên cứu, thiết kế nghiên cứu, công cụ nghiên cứu, phương pháp thu thập số liệu, phương pháp phân tích số liệu, đạo đức nghiên cứu.
- √ Kết quả nghiên cứu: được thể hiện bằng các bảng, biểu đồ, hình hoặc bằng lời.
- √ Bàn luận (bàn luận có thể viết chung với kết quả nghiên cứu, trong trường hợp viết chung thì đề mục cần ghi rõ "Kết quả và bàn luận"): tác giả cần so sánh kết quả nghiên cứu của mình với các tác giả khác và lý giải về kết quả thu được.
- √ Kết luận: viết ngắn gọn, trả lời đầy đủ mục tiêu đề ra.
- √ Khuyến nghị: nếu có.
- √ Lời cảm ơn: cảm ơn quỹ tài trợ, nơi thực hiện, cộng sự đóng góp cho công trình.
- √ Tài liệu tham khảo

3. Quy định về tài liệu tham khảo

- Tài liệu tham khảo (không quá 15 tài liệu) được xếp theo thứ tự vần chữ cái A, B, C..., tiếng Việt trước, tiếng nước ngoài sau.
- Nếu tài liệu là tạp chí thì ghi tên tác giả, năm xuất bản, tên bài, tên tạp chí, tập, số, trang (đầu và cuối). Ví dụ:
  + **Dean P, Michell IJ, Redgrave P (1988),** Responses resembling defensive behaviour produced by microinjection of glutamate into superior colliculus of rats. Neuroscience, 24(2):501-510.
- Trường hợp tài liệu tham khảo có từ 10 tác giả trở xuống thì ghi đầy đủ họ tên của 10 tác giả. Trong trường hợp có từ 11 tác giả trở lên thì ghi đầy đủ họ, tên của 5 tác giả đầu tiên, sau đó viết "và cs" nếu bài báo viết bằng tiếng Việt hoặc "et al" nếu bài báo viết bằng tiếng nước ngoài. Ví dụ:
  + **Dommett E, Coizet V, Blaha CD, Patricia G, Carol C et al (2005),** How visual stimuli activate dopaminergic neurons at short latency. Science, 307(5714):1476-1479.
- Nếu là sách chuyên khảo thì ghi tên tác giả, năm xuất bản, tên sách, nhà xuất bản, TP xuất bản, trang tham khảo. Ví dụ:
  + **Stein BE, Meredith MA (1993),** The merging of the senses. Cambridge, MA: MIT, pp.230-235.
- Nếu là một chương trong sách thì ghi tên tác giả của chương, năm xuất bản, tên chương, tên sách, tên người biên tập, thành phố xuất bản, nhà xuất bản, trang tham khảo. Ví dụ:
  + **Gerfen CR, Wilson CJ (1996),** The basal ganglia. In: Handbook of chemical neuroanatomy, Vol 12: Integrated systems of the CNS, Part III. (Swanson LW, Bjorklund A, Hokfelt T, eds), Amsterdam: Elservier, pp.371 - 468.
- Nếu tài liệu không thuộc hệ chữ Latinh thì phiên âm tên tác giả (theo tiếng Latinh) và dịch toàn bộ phần còn lại ra tiếng Việt, sau đó mở ngoặc ghi chú tiếng của tài liệu đó. Ví dụ: (tiếng Nga).
- Các tài liệu đưa ra phải được trích dẫn đầy đủ trong nội dung bài báo. Trong đó ít nhất 50% số tài liệu tham khảo cần xuất hiện trong phần bàn luận.

4. Yêu cầu đối với các bài tổng quan, thông báo khoa học và bài dịch

- Đối với các bài Tổng quan cần có đầy đủ các tài liệu tham khảo và nguồn số liệu được trích dẫn trong bài. Tác giả bài Tổng quan được ghi rõ chức danh khoa học, học vị, chuyên ngành, địa chỉ cơ quan (ghi ở cuối trang đầu của bài Tổng quan). Nếu bài tổng quan dài, Ban biên tập sẽ chia làm 2 kỳ, mỗi kỳ dài không quá 10 trang, kể cả hình ảnh, bảng, biểu và tài liệu tham khảo. Số tài liệu tham khảo không quá 20 tài liệu.
- Đối với các bài Thông tin khoa học, các bài dịch cần ghi rõ xuất xứ của nguồn dữ liệu được sử dụng để viết bài thông tin hoặc bài dịch. Đối với bài dịch cần photocopy toàn văn bản bài báo tiếng nước ngoài gửi kèm theo bản dịch.
- Đối với bài tổng quan và các bài thông tin khoa học, tác giả gửi đăng sẽ không phải nộp lệ phí khoa học.

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EVALUATION OF CONTINUOUS GLUCOSE MONITORING (isCGM) ON TYPE 2 DIABETES PATIENTS TREATED WITH BASAL – BOLUS INSULIN THERAPY

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SUMMARY

Objective: *Evaluate effectiveness of continuous glucose monitoring (isCGM) on type 2 diabetes patients treated with basal – bolus insulin therapy.* ***Method:*** *Descriptive and interventional study on 29 type 2 diabetes patients treated with basal – bolus insulin therapy at Department of Endocrinology and Diabetes, Bachmai Hospital from 1/2023 to 7/2023.* ***Results:*** *Average age is 61.4 ± 17.4. The majority oral antidiabetic drug is Metformin combined with Anti DDP4 (37%), bolus insulin is Regular (73%), basal insulin is Glargine U100 (97%). Average HbA1c is 10,8 ± 2,8 %, average serum glucose on arrival is 17.2 ± 11.6 mmol/L. Time in range on 14th day 66,2 ± 16.9% higher than the 1st day 55.1 ± 29.0% (p=0.03). CGM can detect more hypoglycemia events than point-of-care capillary blood glucose (34.2% versus 17.2%). Adverse reaction rate is 3.4% only.* ***Conclusion:*** *Continuous glucose monitoring is not only useful in controlling blood glucose level but also in detecting more hypoglycemia events than point-of-care capillary blood glucose.*

Keywords: *continuous glucose monitoring.*

1. BACKGROUND

Type 2 diabetes mellitus is characterized by increased insulin resistance and decreased beta cell function. In disease progression, insulin therapy is an essential treatment, especially in patients. When patients are treated with an intensive insulin therapy, one of complications which clinicians have to face is hypoglycemia. According to the ACCORD study (Action to Control Cardiovascular Risk in Diabetes), in the group of inpatients using intensive insulin therapy, up to 16.2% of patients with hypoglycemia needed support compared with 5.1% of the group treated without insulin. Statistics from the American Diabetes Association show that at least 50% of patients have hypoglycemia event during treatment, more than 50% them are asymptomatic. Fingertip blood sugar monitoring is the most common method for monitoring blood glucose. However, this method often causes pain and only records individual blood glucose samples, which makes it difficult for doctors to control blood glucose precisely. Meanwhile, interstitial blood glucose monitoring with a continuous blood glucose meter (isCGM) has more advantages. First, it can scan many times a day. Moreover, it is a method that can detect blood glucose level changing, especially nocturnal asymptomatic hypoglycemia event. Several studies have shown an improvement in HbA1c and reduction in hypoglycemia episodes in patients using CGMs at least 70% of the time. Because of these advantages, there are several types of CGM devices for patients in Vietnam recently, but there are a few studies related to this problem. We did research on the

results of continuous glucose monitoring (isCGM) on type 2 diabetes patients treated with basal – bolus insulin therapy.

2. METHODS

2.1. Location and time

Department of Endocrinology and Diabetes, Bachmai Hospital, from 1/2023 to 7/2023.

2.2. Participants

Inclusive criteria:

- Type 2 diabetes patients at Department of Endocrinology and Diabetes, Bachmai Hospital, diagnosed with criteria from "Guidelines for the diagnosis and treatment of type 2 diabetes" by the Ministry of Health.
- Patients received basal – bolus insulin therapy.
- Patients have indication and approve to use continuous glucose monitoring (isCGM).

Exclusive criteria:

- Pregnancy.
- Acute critical condition: sepsis, hyperosmolar hypoglycemia, diabetic ketoacidosis.
- Treated with therapy affects blood glucose: high - dose acetaminophen, vitamin C.
- Conditions affect to CGM devices and their accuracy: severe anemia, intermittent hemodialysis, magnetic resonance imaging, skin infection.

2.3. Design

- Descriptive and partly intervened study.
- Sample size: all patients meet the criteria.
- Devices: Intermittently scanned CGM (isCGM) Freestyle Libre 1 from Abbott. Patients is guided to use at least 10 times per day.

2.4. Variable and index

- Characteristics of patients in research: age, gender, occupation, geography, duration of diabetes, type of oral medicine, type of insulin therapy, some paraclinical index (Glucose at admission, HbA1c).
- Results of continuous blood glucose monitoring (CGM) in research patients (TIR, TAR, TBR, Average blood glucose, Blood glucose variation): Evaluation according to Asia-Pacific consensus recommendations for application of continuous glucose monitoring in diabetes management.

2.5. Statistical Analysis

Information and data collected is analyzed with SPSS 26.0 program.

2.6. Research ethics

This is a descriptive and partly intervened study. However, the intervention is measuring and managing patients' blood glucose more effectively, so it is no harm to patients. Information and data collected help physicians control patients' blood glucose more effective and improve the treatment results.

3. RESULTS

3.1. Characteristics of patients in research

Our study included 29 patients with a male-to-female ratio of 1.5:1. The average age of the study group was 61.4 ± 17.4; The patient is 20 years old, the oldest is 86. The average duration of diabetes is 9.8 ± 8.3 years. Among them, the group with disease duration of 5 - 14 years accounts for the majority with a rate of 38%. The average BMI recorded was 23.9 ± 3.7 kg/m^2.

On treatment characteristics, the most common therapy is using insulin combined with Metformin and DPP – IV inhibitors (37%) and insulin combined with DPP – IV inhibitors (29%). The majority of patients in the study used a bolus injection of Regular insulin and a basal injection of Glargine U100. The average total insulin dose was 0.7 ± 0.2 IU/kg. Patients in the study had poor blood glucose control with an average HbA1c of 10.87 ± 2.89 % and average blood glucose at admission of 17.24 ± 11.67 mmol/l.

Table 1. Characteristics of patients in research

Characteristics	All patients (n=29)	Male (n=17)	Female (n=12)
Age (year)	61.4 ± 17.4	56.1 ± 17.8	69.0 ± 14.3
Occupation			
Intellectual work	14 (49)	10 (59)	4 (33)
Worker	8 (27)	4 (23)	3 (25)
Farmer	7 (24)	3 (18)	5 (42)
Duration of diabetes			
< 5 years	10 (34)	8 (47)	2 (16)
5 - <15 years	11 (38)	6 (35)	5 (42)
≥ 15 years	8 (28)	3 (18)	5 (42)
Average	9.7 ± 8.3	7.5 ± 7.8	12.9 ± 8.2
Duration of insulin use (years)	2.7 ± 3.6	4.3 ± 2.7	3.3 ± 3.3
BMI (kg/m^2)	23,9 ± 3,7	24.8 ± 4.0	22.8 ± 3.0
Type of oral medicine			
Metfomin + DDP – 4 inhibitors	13 (37)		
DDP – 4 inhibitors	10 (29)		
Metformin	6 (17)		
SGLT – 2 inhibitors	4 (11)		
Metformin + SGLT – 2 inhibitors	1 (3)		
GLP-1 agonists	1 (3)		
Insulin bolus			
Regular	21 (73)		
Aspart	7 (24)		
Glulisine	1 (3)		
Insulin basal			
Glargine U100	28 (97)		
Detemir	1 (3)		
Average insulin dose (UI/kg/day)	0.7 ± 0.2		
Paraclinical index			
HbA1c (%)	10.8 ± 2.8	10.7 ± 3.2	10.9 ± 2.4
Glucose at admission (mmol/l)	17.2 ± 11.6	20.2 ± 14.2	13.0 ± 6.9

3.2. Results of continuous blood glucose monitoring (CGM) in diabetes type 2 patients received basal – bolus insulin therapy

3.2.1. Results of blood glucose control

Table 2. Results of blood glucose control at the 1st day and the 14th day

Characteristics		1st day	14th day	p
Time above range (TAR)	% of readings & time > 13.9 mmol/l ($\overline{X} \pm SD$)	16.9 ± 22,3	8.9 ± 8.5	**0.049**
	% of readings & time 10.1–13.9 mmol/L ($\overline{X} \pm SD$)	24.1 ± 14.8	20.1 ± 10.5	0.196
Time in range (TIR)	% of readings & time 3.9–10.0 mmol/L ($\overline{X} \pm SD$)	55.1 ± 29.0	66.2 ± 16.9	**0.033**
Time below range (TBR)	% of readings & time 3.0–3.8 mmol/L ($\overline{X} \pm SD$)	2.7 ± 4.3	4.0 ± 7.6	0.270
	% of readings & time < 3.0 mmol/L ($\overline{X} \pm SD$)	1.3 ± 2.8	0.6 ± 0.9	0.180
Mean glucose		9.8 ± 2.9	8.5 ± 1.7	**0.025**
Glycemic variability		30.6 ± 9.69	35.0 ± 6.4	**0.029**

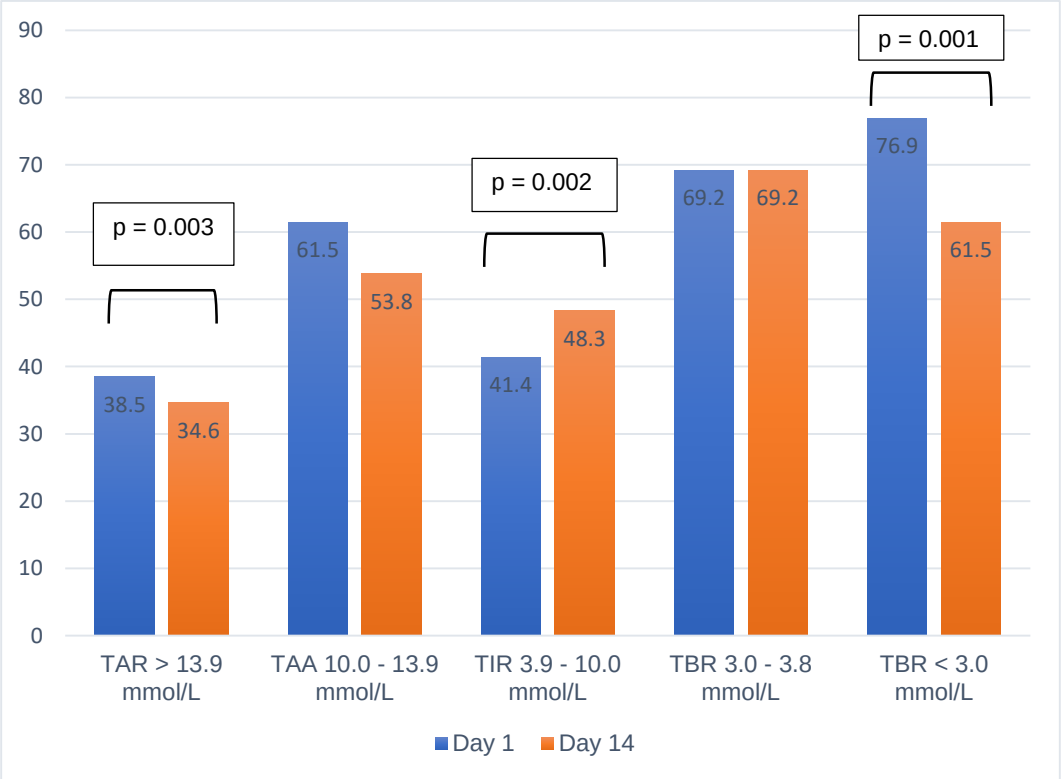

Figure 1. TIR, TAR, TBR reached target after 1 and 14 days of treatment

Our study found an improvement in blood sugar control of patients after 14 days of using CGM. The time above range (TAR) on day 14 was 8.9 ± 8.5 (%) which saw statistically significant drop compared to the first day of treatment (p = 0.049). Similarly, the time in range (TIR) ratio of the 14th day was 66.2 ± 16.9 (%), improved compared to day 1 of 55.1 ± 29.0 (%) with statistical significance (p = 0.033). The mean blood sugar after 14 days of treatment also saw statistically significant increase with p = 0.025. We divided the pass and fail rates of the blood glucose monitoring criteria by CGM. Figure 3.1 shows that the pass rates of TAR, TIR and TBR all improved between day 1 and day 14.

3.2.2. Detecting hypoglycemia by CGM and point-of-care capillary blood glucose

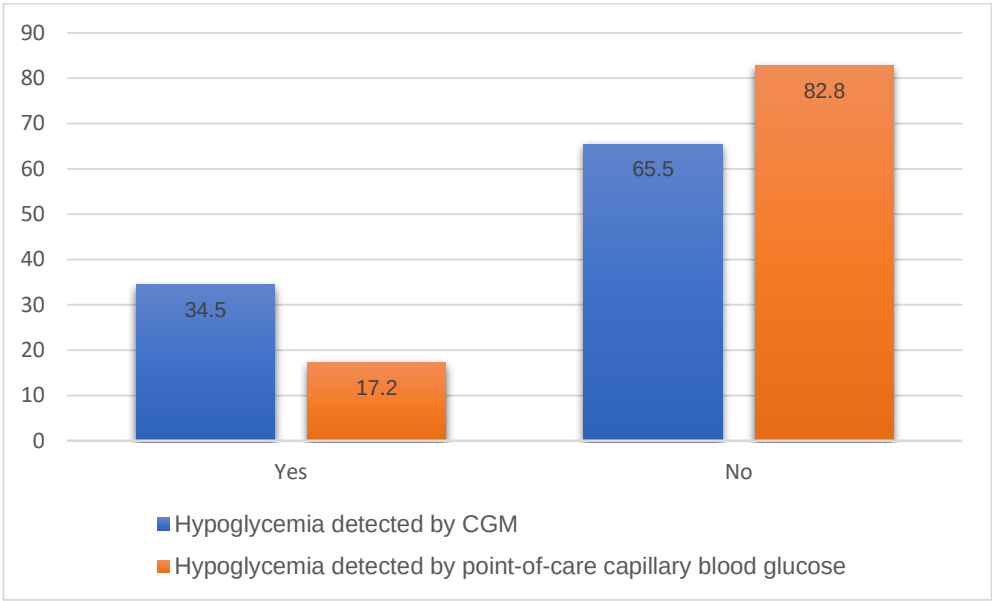

Figure 2. Rate of hypoglycemia detected by CGM and point-of-care capillary blood glucose

CGM detected more hypoglycemic episodes (34.5%) than the point-of-care capillary blood glucose. (17.2%). Among them, CGM detected 38.1% of hypoglycemic events which capillary blood glucose could not detect.

3.2.3. Adverse events

The proportion of patients in our study experiencing side effects when using CGM was relatively low. We only recorded 1 patient with muscle soreness and pain at the point of attachment. No side effects such as skin irritation or infection were noted.

4. DISCUSSION

The mean age of patients in the study was 61.4 ± 17.4 (years). This age is lower than that of western studies such as Gomez's (2016) which was 66.1 ± 8.6 (years) or Morton's (2013) which was 70 ± 15 (years). The ratio of male to female in the study is 1.5:1, some studies on CGM also have a higher male-to-female ratio, for example Gomez's study (2016) was 1.4:1.

The patients in the study suffered diabetes for an average of 9.7 ± 8.3 years, significantly lower than in Gomez's study (2016) of 14.8 ± 9 years.

The study showed that most of the bolus insulin in treatment is Regular insulin, accounting for 73%, while basal insulin, most of the patients treated with Glargine U100 accounted for 97%. The reason why these insulins account for such

high percentages was because this is a type of insulin that was widely available in Bach Mai hospital drugstore.

The average HbA1c index of patients was 10.87 ± 2.89%, higher than that of foreign studies such as Gomez's (2016) which was 9.26 ± 2.62% or Morton's (2013) which was 8.3 ± 1.7%. The patients' blood sugar at hospital admission was 17.24 ± 11.67 mmol/L which was also higher than that of Gomez's study (2016) which was 13.9 ± 0.5. Therefore, patients need more aggressive blood sugar control and CGM will be a safe and effective support solution.

The time above range (TAR) on day 14 of 8.9 ± 8.5 was much lower than on the first day of treatment (p=0.049). Similarly, the time in range (TIR) of the 14th day was 66.2 ± 16.9, which improved compared to the first day of 55.1 ± 29.0 (p = 0.033). The reason for such a difference might be because a part of patients received dose adjustment based on their daily blood sugar measured by CGM.

The rate of hypoglycemia detection by CGM in our study was 34.5%, higher than that of point-of-care capillary blood glucose of 17.2%. In addition, more than 1/3 of cases could only be detected by CGM but not by point-of-care capillary blood glucose. This is similar to most other studies. In Gomez's study (2016), CGM detected 55 hypoglycemia events, while point-of-care capillary blood glucose detected only 12 times. Moreover, asymptomatic hypoglycemia 86.7% could only be detected by CGM, especially nocturnal hypoglycemia. In the research of Morton (2013), only 10% of patients with hypoglycemia from 0h – 7h could be detected by point-of-care capillary blood glucose, especially no patients with blood sugar less than 3.0 mmol/L were detected by this method. hypoglycemia has been identified as a factor that can affect the adherence to treatment and the effectiveness of blood sugar control in patients with diabetes. The detection and prevention of hypoglycemia, especially asymptomatic cases, will help patients achieve the target of blood sugar control and ensure patients' safety during treatment.

The rate of side effects of CGM in our study was very low, only 1 patient, accounting for 3.4%. Moreover, this was only a mild side effect, not dangerous for the patient. This shows that the use of CGM is very safe and ensures patient satisfaction.

5. CONCLUSION

Continuous blood glucose monitoring (CGM) is a new, effective, and safe method for assessing glycemic control in patients on intensive insulin therapy. There is a significant improvement in blood sugar control of diabetic patients when using CGM. Moreover, the ability to detect hypoglycemia is also better than that of fingertip capillary blood glucose.

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ASSOCIATION BETWEEN RISK OF FALL AND RELATED FACTORS IN SARCOPENIA PATIENTS

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ABSTRACT

Objectives: *This study aims to evaluate related factors affecting the risk of fall in older sarcopenic patients.* ***Methods:*** *A cross-sectional study was conducted in 294 participants aged ≥ 60 years old at National Geriatric Hospital in Hanoi, Vietnam, from July to October 2022. Sarcopenia was defined by the criteria proposed by Asian Working Group for Sarcopenia (AWGS 2019). Risk of fall was assessed through 21-item index. The T-test and Chi-square were used to compare groups. The relationship between sarcopenia and risk of fall was estimated by deriving odd ratios from multiple logistic regression models.* ***Results:*** *Mean age 75.68±8.20, 78.2% female. In multivariate logistic regression, age (adjusted OR=2.907, 95%CI:1.777-4.754), marital status (adjusted OR=2.352, 95%CI:1.390-3979), diabetes (adjusted OR=1994, 95%CI:1225-3246), heart failure (adjusted OR=1.947, 95%CI:1.086-3.493), depression (adjusted OR=2.298, 95%CI:1.005-5.253), hypertension (adjusted OR=1.885, 95%CI:1.166-3.046), ADL (adjusted OR=4.724, 95%CI: 2.867-7.783), IADL (adjusted OR= 8.403, 95%CI:4.689-15.061), sleep quality (adjusted OR=3.027, 95%CI: 1.837-4.987) were significantly associated with high risk of fall in sarcopenic patients defined by AWGS 2019 criteria.* ***Conclusion:*** *Patients with sarcopenia have high prevalence of fall and high risk of fall. Fall risk assessment and implementation of fall prevention should be carried out in everyday practice.*

Keywords*: sarcopenia, risk of fall, sarcopenic patient, AWGS 2019*

1. INTRODUCTION

Sarcopenia is classified as a musculoskeletal disorder that characterized by low muscle mass, low muscle strength and low physical performance. In recent years, more and more studies have shown that sarcopenia is associated with multiple adverse health outcomes, including hospitalization multiple trauma, functional decline, poor quality of life [1] particularly higher risk of falls [2]. Falling is the second leading cause of unintentional injury death, after road traffic injuries. The majority of fall-related deaths occur in low- and middle-income countries in the Western Pacific and Southeast Asia regions. The highest mortality rate in adults over 60 years old [3]. Some studies have been conducted the increased risk of clinical relevant outcomes among sarcopenic older adults compared to non-sarcopenic population such as the recurrent rate of fall, the incidence of falls [4], previous falls, fall-related injuries and also fall related fractures. Sarcopenia is predicting factor for falls in community-dwelling older adults [4]. A study on the prevalence of fall-related injury (2021) show higher among those with sarcopenia than in those without this condition (7.9% vs. 4.3%). In Vietnam, the rate of falls in outpatients is relatively

high, almost 1 out of every 4 outpatients experienced fall and highly concentrated in group ≥ 60 years old typically sarrcopenia older patients. In Vietnam, there have not been many research papers on sarcopenia. In 2018, cross-sectional studies was performed on patients aged ≥ 60 years visiting outpatient geriatric clinics in Vietnam, sarcopenia the prevalence of sarcopenia was quite high and varied from 48.3% - 61.1% according to the criteria used [5]. Therefore, this study was conducted on sarcopenia and patterns in older adults to evaluate related factors affecting the risk of falls in older sarcopenic patients.

2. METHOD

2.1. Participants

Patients 60 years old and older were being examined and treated at National Geriatric Hospital.

Recruited criteria

- Patients who were diagnosed with sarcopenia by specialist doctor according to the AWGS criteria [6].
- Patients who had already been diagnosed with and received treatment for sarcopenia before.
- Patients and patient's family agreed to participate in the study (agree to answer questions regarding general health and disease status according to the outline proposed by the research).
- The patients are fully conscious and have the physical and cognitive abilities to do a face-to-face interview.

Exclude criteria

- Patients or families refused to participate in the study.
- Patients who do not have the physical and cognitive abilities to do a face-to-face interview.
- Patients with advanced dementia and terminal illness, acute and malignant diseases (advanced cancers, end-stage chronic diseases, acute myocardial infarction, acute stroke, symptomatic cardiovascular disease).

2.2. Study design

The study is cross-sectional descriptive study.

Sample size and sampling

The sample size is calculated using the formula: n = $(Z_{1-\frac{\alpha}{2}})^2 \frac{p(1-p)}{d^2}$

n: study sample size;

α: statistical significance level, with α = 0,05; (Z1- α/2) = 1,96

p = 0.25 (Sex differences in impact of sarcopenia on falls in community-dwelling Korean older adults in 2021) [7]

d = expected error (d = 0.05).

From the formula, the estimated sample size was 288 sarcopenic patients. The number of sarcopenic patients in our study was 294 patients.

Sampling: convenience sampling

Location and time

Inpatients and outpatients at National Geriatric Hospital excepted for Emergency and Stroke departments, Intensive care unit from July to October 2022.

2.3. Tools and data collection method

Data were collected by using designed tools included: General information, history of fall, Mini Nutrition Assessment (MNA-SF), Instrumental Activities of Daily Livings (IADLs), Activities of Daily Livings (ADLs), 21-item fall risk index. Data were collected by using research questions through interview, diagnosis test, laboratory test and medical record at National Geriatric Hospital.

2.4. Sarcopenia criteria to diagnose

Sarcopenia diagnosis by the standard of the Asian Working Group for Sarcopenia (AWGS) [6].

- Criterion 1: Low muscle mass (kg): Each patient was assessed whole-body skeletal muscle mass by using a Bioelectrical Impedance Analysis (BIA) (InBody 770, Biospace Co., Ltd.). The results show the muscle mass of each body part (kg) standardized to height2: SMI = ALM (kg)/height2 (m).

- Criterion 2: Low grip strength: The low grip strength is evaluated using Jamar TM (Hydraulic Hand Dynamometer J1, USA), 2 trials were performed on both hands, the better of the two trials was used for scoring purposes.

- Criterion 3: Low physical performance is evaluated by 6m walk test, calculated by the patient's time to cover the 6m distance outline.

Based on the AWGS, diagnosis of sarcopenia required the present of low muscle mass plus low muscle strength or/and low physical performance. Sarcopenia can be separated into 2 types:

Mild sarcopenia: decreasing in muscle mass, muscle strength or physical performance.

Severe sarcopenia: decreasing in muscle mass, muscle strength and physical performance.

2.5. Falls

Include 6 questions to identify prevalence of falls in the elderly in the community.

Risk of fall

❖ 21 item fall risk index

Include 21 questions to identify possible risks of falls in the elderly in the community. The answer is one in 2 options: Yes = 1; No = 0;

- Evaluation: < 10 points: Normal

≥ 10 points: Risk of fall [46]

2.6. Data analysis

- The process of data coding, entry and analysis would be done using SPSS software version 22.0
- Descriptive statistics were adopted to examine data characteristic: number, %, mean, median, and minimum, maximum, range. Inferential statistics would be done to compare between groups, using $\chi 2$, ANOVA (if compare between more than 2 mean groups, use ANOVA and ≥ 2 groups, use $\chi 2$).
- The correlation between lower body strength and its related factors was determined by using crosstabs method for classified variables
- The correlation between handgrip strength and factors was also analyzed by using simple binary regression and multiple binary regressions to see the relative.
- Statistical significance was defined as any p-value is less than 0.05

2.7. Ethical consideration

- The participants were explained clearly about the purposes of the study and they consented to take part in the study.

3. RESULTS

The study was conducted on patients aged 60 years and older who came to the National Geriatric Hospital for examination and treatment from July 2022 to October 2022. A total number of 294 older sarcopenic patients were selected for this study. After completing the data analysis, the demographic and baseline characteristics of the participants were shown below:

Table 1. Patient's demographic (n=294)

Characteristics		Frequency (n)	Percent (%)
Aged group	60 – 69	73	24.8
	70-79	115	39.1
	≥80	106	36.1
	Mean age ±SD	75.68±8.20	
Gender	Male	64	21.8
	Female	230	78.2
Educational level	Below high school	140	47.6
	High school	55	18.7
	Above high school	99	33.7
Occupation	Retirement	160	54.4
	Others	134	45.6
Marital status	Married	215	73.1
	Others	79	26.9
Living with	Family	273	92.9
	Alone	1	0.3
	Caregiver	20	6.8

Demographic details of patients in this study are shown in the table 1. In the total 294 participants, the age of sample ranged from 60 to 97 with the mean age was 75.68 ± 8.20 years old. The greatest distribution was generated by people aged from 70-79 years old, with percentage of 39.1%. In which, female subjects accounted for 78.2%, higher than male subjects accounted for 21.8%. The highest percentage of sarcopenic patients was below high school group, with 47.6% and the lowest was high school group with 18.7%. About half of the participants were retired (54.4%), the percentage of married participants was 73.1% and nearly three times the total percentage of the remaining groups.

Table 2: Geriatric characteristics (n=294)

Characteristics	Classification	Frequency (n)	Percentage (%)
Nutritional status	Malnourished	33	11.2
	Risk of malnutrition	138	46.9
	Normal nutritional status	123	41.8
ADL	Independent	173	58.8
	Dependent	121	41.2
IADL	Independent	207	70.4
	Dependent	87	29.6
Sleep quality	Good sleep	120	40.8
	Poor sleep	174	59.2
Charlson index score (Mean ± SD)		1.80±1.59	

In nutritional status, average core of MNASF was 10.73±2.49. 46.9% participants were identified as "at risk of malnutrition" and only 11.2% were malnourished. The proportion of normal nutritional status closely followed the proportion of malnourished patients with 41.8%.

In this study, 41.2% of study subjects were dependent on functional

activity and 58.8% of subjects were completely independent. Dependent in daily activities were also reported in 87 subjects (29.6%) of participants. There was 174 patients (59.2%) suffering from poor sleep. The mean value of sleep quality was 7.25±5.42. The total number of comorbidities was calculated according to the Charlson scale with mean value of 1.80±1.59.

Table 3. Association between demographic and risk of fall (n=294)

		Low risk of fall		High risk of fall		p
		n	%	n	%	
Gender	Male	38	22.6	26	20.6	0.68
	Female	130	77.4	100	79.4	
Age	60-69	57	33.9	16	12.7	<0.001
	70-79	68	40.5	47	37.3	
	≥ 80	43	25.6	63	50.0	
Living area	Urban	95	56.5	74	58.7	0.71
	Rural	73	43.5	52	41.3	
Marital status	Married	135	80.4	80	63.5	0.001
	The other	32	19.6	46	36.5	
Living status	Family	154	91.7	119	94.4	0.36
	The other	14	8.3	7	5.6	
Occupation	Retired	93	55.4	67	53.3	0.71
	The other	75	44.6	59	46.8	
Education	Below high school	77	45.8	63	45.0	0.38
	High school	36	21.4	19	15.1	
	Above high school	55	32.7	44	34.9	
BMI (kg/m2)	Underweight	18	10.7	25	19.8	0.09
	Normal	94	56.0	62	49.2	
	Overweight + Obesity	56	33.3	39	31.0	
		Mean ± SD		Mean ± SD		
Mean age		73.61±7.7		78.44± 8.08		0.04
Mean BMI		21.75± 2.68		21.08± 2.92		<0.001

The table 3 showed association between demographic and fall risk in elderly. The mean age and mean BMI in the group of patients at high risk of falling was higher than the group with low risk of falling with p <0.05. Age group and marital status were associated with risk of fall in older sarcopenic patients. The risk of falls increased with age group (p< 0.05).

The differences were not statistical significance between gender, living status, living area, education level and body mass index and risk of falls in the older sarcopenic patients (p > 0.05).

Table 4: Association between of falls and geriatric characteristics

Factor		Low risk of fall		High risk of fall		p
	Classification	n	%	n	%	
Nutritional status (MNA-	Normal	86	51.2	37	29.4	0.001
	Risk of	65	38.7	73	57.9	

SF)	malnutrition					
	Malnourished	17	10.1	16	12.7	
ADL	Independent	125	74.4	48	38.1	<0.001
	Dependent	43	25.6	78	61.9	
IADL	Normal	148	88.1	59	46.8	<0.001
	Impaired	20	11.9	67	53.2	
Quality of sleep (PSQI)	Good sleep	87	51.8	33	26.2	<0.001
	Poor sleep	81	48.2	93	73.8	

The results showed that patients with impairment in instrumental activities daily living and daily activities had higher of falls than those without impairment in instrumental activities daily living and activities daily living. The differences were statistically significant (p<0.05).

The MNA-SF scale and PSQI showed that there was a significant association between level of malnutrition, sleep quality and risk of fall (p<0.05).

Table 5: Multivariable regression models on some factors related to risk of fall in older sarcopenic patients.

Factors	High risk of fall			p
	OR	95% confident interval		
		Lower	Upper	
Age (≥80 years old)	2.907	1.777	4.754	0.001
Marital status (Other)	2.352	1.390	3.979	0.001
Diabetes (Yes)	1.994	1.225	3.246	0.005
Heart failure (Yes)	1.947	1.086	3.493	0.024
Depression (Yes)	2.298	1.005	5.253	0.044
Hypertension (Yes)	1.885	1.166	3.046	0.009
Nutrition (Malnourished)	1.292	0.625	2.669	0.488
ADL (Dependent)	4.724	2.867	7.783	<0.0010
IADL (Dependent)	8.403	4.689	15.061	<0.001
Quality of sleep (Poor sleep)	3.027	1.837	4.987	<0.001

The table 5 showed the factors associated with risk of fall in older sarcopenic patients were age increasing (OR=2.907, 95%CI: 1.777-4.754), patients with dependent in activities daily living (OR=4.724, CI95%: 2.867-7.783), dependent in instrumental activities daily living (OR=8.403, CI95%: 4.689-15.061), decreasing in sleep quality (OR =3.027, CI95%:1.837-4.987). Risk of fall also increased in patients suffering from depression, diabetes, heart failure, depression, hypertension. But risk of fall in lower in married group (OR=2.352, CI95%:1.390-3.979).

4. DISCUSSION

The average age of participants in this study was 75.68 years old (SD=8.20). According to Nguyen et al., average age of sarcopenic participants was 72.2±8. However in the research conducted by Landi et al in 2012 evaluated sarcopenia as a risk of fall in older population, the average age of participants was 86.7 years old (SD=5.4) and the same as sarcopenic group in this study [8]. Another research was conducted in Chinese participants with the age range was 80–99 years in which mean age of sarcopenic group was 87.9±3.7 years old. The reasons can be

explained by the research area was different research subjects or sample size.

The majority of patients in the study were married with rate of 73.1%. This large disparity in percentage was explained by the elderly population and research location of our study. The similar result was found in the research of Nguyen et al. with 75% sarcopenic participants reported married [9]. However, in the research of Landi et al., the group of married participants was diagnosed with sarcopenia only 25% [8]. This difference may be explained by different research location.

The Mini Nutritional Assessment Short Form (MNA-SF) scale was used assess the nutritional status of the patients and normal nutritional status in our study accounted for a high rate. The group of normal nutritional status had 123 people, accounting for 41.8%. The group of risk of malnutrition had 138 people, accounting for 46.9%. Meanwhile, the malnourished group with 33 people, accounting for 11.3%. In Nguyen et al.'s study, the group of malnourished rate was 13.7% [9]. The different sample sizes and time of study were also the reasons for the difference.

With ADL, mainly patients in the dependent group had 121 people, accounting for 41.2%, meanwhile were 173 independent people (58.8%). According to Xu et al.'s research in 2020, the percentage of patients with daily independent activities based on ADLs was 34.5% and dependent was 65.5% [10].

Based on IADLs scale, mainly patients in the normal group had 207 people, accounting for 70.4%, meanwhile there was 29.6% of the people experienced impairment in daily functional activities with one or more items in the instrument activities of daily living. According to research of Tanimoto et al. in 2012, the percentage of disability for IADL was 33.3% with sarcopenia and normal group accounted for 66.7% [11]. Because sarcopenia is a common condition in older adults that contributes to functional decline, disability, frailty, and falls. It affects to declines in activity and make patients dependent and impaired daily activity.

The significantly association between age and risk of fall in the research of Nguyen Trung Anh in hypertension patients. Aged ≥ 80 was accouted for the highest proportion in high risk of fall group in the research of Nguyen Trung Anh [12]. However, in research of Smith in 2017, there was no association between age increasing and risk of fall in elderly living at home. The difference in result could be from difference of sample size between study and difference subject study.

With ADL of independent group high risk of fall sleep is 38.1%, independent of low risk of fall is 74.4%. With IADLs of normal group having high risk of fall is 46.8%, normal group of low risk of fall is 88.1%. Thus, with $p < 0.001$, we found a correlation between activity of daily living and high risk of fall. In Yamazaki et al. research in 2017 in older adults showed reduced in functional status was associated with a greater risk of falls ($p < 0.01$) [13].

Our results show that the group of older sarcopenic patients with comorbidity including diabetes, heart failure, depression and hypertension increased the risk of falling, 1.994 times with diabetes (95% CI:1.225-3.2466), 1.947 times with heart failure (95% CI:1.086-3.493), 2.298 times with depression (95% CI: 1.005-5.253) and 1.885 times with hypertension (95% CI:1.166-3.046). Therefore, assessing the comorbidities in sarcopenic patients

is necessary. It helps us to come with more effective fall prevention measures. Our results also show that patients with poor sleep quality and dependent in activities of daily living and instrumental activities of daily living increased the risk of falling in sarcopenic older patients. Sarcopenic patients with poor sleep increased 3.027 times higher risk of fall than those with good sleep quality with 95%CI: 1.837-4.987. The risk of falls in patients with dependent in dependent in activities of daily living increased 4.724 times higher risk of fall with 95%CI: 2.867-7.783 and patients with dependent in instrumental activities of daily living increased 8.403 times risk of fall with 95%CI: 4.689-15.061 the risk of fall. Therefore, we should further assess the level of disability in older sarcopenic patients.

5. CONLCUSION

Patients with sarcopenia have high prevalence of fall and high risk of fall. Fall risk assessment and implementation of fall prevention should be carried out in everyday practice.

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HANDGRIP STRENGTH AND SOME RELATED FACTORS AMONG OLDER PATIENTS WITH OSTEOPOROSIS

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SUMMARY

Objectives: *To assess handgrip strength (HGS), lower limb strength, and some related factors among older people with osteoporosis.* ***Subjects and Methods****: A cross-sectional study on 141 older patients with osteoporosis examined and treated at National Geriatric Hospital from June to October 2022. HGS was measured using the handgrip strength dynamometer, and lower limb strength was assessed by the 5-time chair stand test. General information, characteristics of osteoporosis, and geriatric syndromes were collected.* ***Results:*** *56% of individuals had impaired HGS, and 24.8% of participants had impaired lower limb strength. The older age group was significantly associated with lower handgrip strength (p<0.05). Those with HGS impairment had lower mean BMI (p<0.05). The co-incidence of lumbar spondylosis and diabetes significantly differed between the two groups (p< 0.05). Participants who experienced pain and lumbar spine malformation had a higher rate of impaired HGS (p<0.05). Declining cognitive function increases 5.7 times the risk of HGS decline. The risk of deterioration in HGS was increased in participants with low physical activity levels (OR=3.9), sleep disturbance (OR=3.8), depression (OR=3.7), frailty syndrome (OR=3.5), risk of fall (OR=3.4) and malnutrition (OR=2.2).* ***Conclusion:*** *Half of the subjects had reduced HGS and a quarter of the subjects had reduced lower limb muscle strength. Age, BMI, diabetes, lumbar spondylosis or other joints, living status, and geriatric syndromes were significantly associated with impaired HGS.*

Keywords*: handgrip strength, lower limb strength, elderly, osteoporosis.*

1. INTRODUCTION

Osteoporosis is a “silent disease"- with no typical symptoms, so you may not realize you have it until a low-trauma fracture occurs. Although osteoporosis affects people of all ages and genders, it is more prevalent in Caucasians, women, and the older. These effects create a cycle of impairment, with fractures leading to deterioration in physical function and reduced physical capacity, putting them at a greater risk for further fractures and the possibility of more physical restrictions [1].

The contribution of associated factors in physical function varies between studies. A previous study showed that significant associations include advanced age, females, and pain density [2]. Handgrip dynamometers are typically used to assess maximum isometric handgrip strength (HGS) with excellent inter-tester and test-retest reliability. Low HGS is frequently a sign of poor upper extremity strength and lower extremity function. Weak muscle strength is typically seen in older persons as reduced mobility and an increase in dependency on their activities of daily living and it is a predictor of mortality and body function. In addition, HGS is regarded as a reliable measure of

physical decline and potential outcomes among the older adult population [3].

Exploring the HGS and its associated factors among older patients with osteoporosis can develop interventions to promote activities as much as possible. However, data about HGS are lacking regarding the older population with osteoporosis.

Thus, the aims of this study were to assess HGS and some related factors among older people with osteoporosis examined and treated at the National Geriatric Hospital.

2. SUBJECTS AND METHODS

2.1. Subjects

Patients aged 60 years old and older were diagnosed with osteoporosis according to WHO criteria, were examined and treated at the National Geriatric Hospital from June to October 2022, and had the physical and cognitive abilities to do a face-to-face interview.

Exclusion criteria: patients with severe acute conditions such as ketoacidosis coma, hyperosmolar coma, coma due to cerebrovascular accident, exacerbation of heart failure decompensation, liver failure, exacerbation of chronic obstructive pulmonary disease.

2.2. Study design

- A cross-sectional descriptive study

- The sample was selected according to the convenience sampling method

- According to the formula, the smallest sample size is n = 135 patients. In fact, 141 patients participated in this study.

2.3. Variables

- General information: age, gender, educational level, weight, height, body mass index (BMI).
- Comorbidities characteristics
- Osteoporosis characteristics: T-score, diagnosed duration, symptoms of osteoporosis, treatment
- **Handgrip strength (HGS)**: The HSG of both hands was measured using the handgrip strength dynamometer (Jamar TM Hidraulic Hand Dynamometer 5030 J1, USA). Participants sat comfortably with their hands at their sides in a neutral position. The inner lever of the dynamometer was adjusted to fit the hand, and the respondents were instructed to squeeze the dynamometer as hard as possible for a few seconds. Test each hand twice and record the best effort rating of each on the participant's handout and on the aggregate form.

  Low HGS was defined as < 28 kg for males and < 18 kg for females [4].
- **Lower limb strength:** The 5-time chair stand test measures how quickly a patient transfers five times from sitting to standing and back to a sitting position. A stopwatch and a standard-height chair with a straight back are among the tools required for the test. The test-taker is instructed to sit on the chair with their backrest. The test-taker is also instructed to cross their arms over their chest. The test-taker should then be encouraged to perform five sit-to-stands as quickly as they can at the count of go,

without putting their back or leg on the chair in between repetitions. Individuals with times for 5 repetitions of this test ≥ 12 seconds following can be considered to have performed poorer than average [4].

- **Characteristics of some geriatric syndromes:** Physical activity level was assessed according to the International Physical Activity Questionnaire-Short Form (IPAQ-SF). Polypharmacy was assessed by asking patients and families/ caregivers, viewing prescriptions, and referring to medical records. Nutrition status was evaluated using the Mini Nutritional Assessment - Short Form (MNA-SF). Depression was performed using the Geriatric Depression Scale (GDS-15). Sleep disturbance was screened by the Pittsburgh Sleep Quality Index (PSQI). Cognitive impairment was assessed by the Mini-Mental State Examination (MMSE). The risk of falls was assessed by asking the 21-item fall risk index. Frailty syndromes were assessed by the Clinical Frailty Scale (CFS).

2.4. Tools and data collection method

Data were collected by using a research questionnaire through interviews, diagnosis tests, and medical records at the National Geriatric Hospital.

2.5. Data processing and data analysis

Data coding, entry into REDCap, and analysis were done using Statistical Package for Social Science (SPSS) software (version 22.0). Descriptive statistics were adopted to examine characteristic data: frequency, percentage, and mean with standard deviation. T-test, Chi-square, and Binary logistic regression were performed to evaluate the factors affecting physical function in osteoporosis patients. Statistical significance was accepted at the 95% confidence level ($p < 0.05$).

3. RESULTS

3.1. General characteristics

In the total 141 older patients with osteoporosis, the mean age of the patient was 73.1 ± 8.6 years with a minimum of 60 and a maximum of 97. Participants were divided into 3 groups: 60 – 69 years old (35.5%), 70 – 79 years old (39.9%), and 80 years old and over (25.5%). The majority of study participants were females, accounting for 94.3%. The mean BMI was 21.9±3.0 kg/m^2. The percentage of underweight and overweight were 13.5% and 37.6%, respectively.

3.2. Handgrip and lower limb strength in older patients with osteoporosis

Table 1. HGS and lower limb strength measurements in older osteoporosis patients

Test		Normal *n (%)*	Impaired *n (%)*	Mean ± SD
Handgrip strength (HGS)	Male	4 (50.0)	4 (50.0)	27.9 ± 4.7 (kg)
	Female	58 (43.6)	75 (56.4)	16.1 ± 5.1 (kg)
	Total	62 (44.0)	79 (56.0)	16.7 ± 5.7 (kg)
Lower limb strength (5XSTS)		106 (75.2)	35 (24.8)	16.4 ± 6.8 (s)

Male patients with low HGS accounted for 50% of the total number of males. Females having impaired upper limb strength had a higher rate compared to those having normal upper limb strength (56.4% and 43.6%, respectively). In total, 56% of individuals had impaired HGS. The mean time to accomplish the five-time sit-to-stand test was 16.4 ± 6.8 seconds. Over three-four participants got normal, while 24.8% of the total sample was estimated as impaired lower limb strength.

3.3. Association between HGS and general characteristics

Table 2. Association between HGS and general characteristics (n=141)

Characteristics		Normal HGS		Impaired HGS		p-value
		n	%	n	%	
Age	60 – 69	30	48.4	20	35.3	**< 0.05**
	70 – 79	23	37.1	32	40.5	
	≥ 80	9	14.5	27	34.2	
Gender	Male	4	6.5	4	5.1	> 0.05
	Female	58	93.5	75	94.9	
BMI	< 18.5	4	6.5	15	19.0	> 0.05
	18.5 – 22.9	30	48.4	39	49.4	
	≥ 23	28	45.2	25	31.6	
Lumbar spondylosis or other joints	Yes	7	11.3	20	25.3	**< 0.05**
	No	55	88.7	59	74.7	
Diabetes	Yes	6	9.7	19	24.1	**< 0.05**
	No	56	90.3	60	75.9	
Living status	Family	62	100.0	70	88.6	**< 0.05**
	Alone	0	0.0	9	11.4	
		Mean ± SD				
Age (year)		70.1 ± 7.1		75.5 ± 9.1		**< 0.05**
BMI (kg/m^2)		22.6 ± 2.7		21.4 ± 3.1		**< 0.05**

The overall older age group was significantly associated with lower handgrip strength ($p<0.05$). The mean age in those with HGS decline was higher, and statistically significant ($p<0.05$). The percentage of people living alone with impairment of upper extremity strength was higher (11.4% vs 0.0%) and people living with family had less strength impairment (100.0% vs 86.4%) were statistically significant ($p< 0.05$). Those with HGS impairment had lower mean BMI ($p< 0.05$). The co-incidence of lumbar spondylosis and

diabetes was significantly different between the two groups (p< 0.05).

3.4. Association between HGS and characteristics of osteoporosis

Table 2. The association between HGS and characteristics of osteoporosis

Characteristics		Normal HGS		Impaired HGS		p-value
		n	%	n	%	
Diagnosed duration (year)	Newly	34	54.8	36	45.6	> 0.05
	<5	17	27.4	32	40.6	
	5 – 10	11	17.7	11	13.9	
Symptoms	Pain (back, …)	52	83.9	76	96.2	**< 0.05**
	Loss of height	27	43.5	35	44.3	> 0.05
	Spine malformations	8	12.9	22	27.8	**< 0.05**
	Bone fractures after minor injuries	3	4.8	3	3.8	> 0.05
	No symptoms	4	6.5	2	2.5	> 0.05
Treatment	Yes	35	56.5	37	46.8	> 0.05
	No	27	43.5	42	53.2	
Mean ± SD	T-score of hip	-1.4 ± 1.3		-1.6 ± 0.9		> 0.05
	T-score of spine	-3.3 ± 0.7		-3.4 ± 0.8		> 0.05

Participants who experienced pain and lumbar spine malformation had a higher rate of loss of upper limb strength (83.9% vs. 96.2% and 12.9% vs. 27.8%, respectively) (p<0.05). There is no statistically significant relationship between HGS and diagnosed duration, treatment or not, and BMD in osteoporosis patients (p>0.05).

3.5. Association between HGS and geriatric syndromes

Table 3. The association between HGS and geriatric syndromes (n=141)

Geriatric syndromes	OR	95%CI
Cognitive impairment	**5.7**	2.2 – 14.9
Low physical activity level	**3.9**	1.9 – 8.1
Sleep disturbance	**3.8**	1.7 – 8.4
Depression	**3.7**	1.7 – 7.8
Frailty syndrome	**3.5**	1.5 – 8.4
Risk of fall	**3.4**	1.7 – 6.8
Malnutrition	**2.2**	1.1 – 4.3
Polypharmacy	1.6	0.8 – 3.2

Cognition was an important factor in HGS impairment. Declining cognitive function increased 5.7 times the risk of HGS decline, 95%CI 2.2 – 14.9 (p< 0.05). The risk of deterioration in HGS was increased in participants with low physical activity levels (OR=3.9), participants with sleep disturbance (OR=3.8), participants with depression (OR=3.7), participants with frailty syndrome (OR=3.5), risk of fall (OR=3.4) and malnutrition participants (OR=2.2).

4. DISCUSSION

Our results highlighted characteristics of HGS, lower limb strength, and some related factors with impaired HGS among older patients with osteoporosis at the National Geriatric Hospital. The mean handgrip strength in osteoporosis was 16.7 ± 5.7 kg. The

prevalence of impaired HGS was 56.0%. The mean grip in our study was significantly lower than the study by Yixuan, with 24.17 ± 9.20 kg [5]. This difference is due to the women/men ratio disparity of this study compared to our study, with the female/male ratio being 1.7 compared to 16.6, respectively. Many authors identified that muscle strength was negatively associated with osteoporosis, and reduction in grip strength was associated with an increased risk of osteoporosis [5].

The mean time to accomplish the five-time sit-to-stand test was 16.4 ± 6.8 seconds. Over three-four participants were normal, while 24.8% of the total sample was estimated as impaired lower limb strength. The studies by Khazzani et al., and Dai et al. showed that low 5XTST performance was associated with low BMD, and a high risk of falls and fractures [6].[7].

Many studies among older adults identified that HGS declined gradually with age [4],[8]. A study showed that individuals aged >75 years increased the risk of having low handgrip strength by 2.3-fold [9]. It was well understood that the aging process leads to degenerative changes, and reduction in muscle mass makes HGS decline. Our study found out that the mean age in those with HGS decline was higher and for people in the age group of 80 or older, the risk of HGS decline can increase 3.1 times. These results were consistent with the study among women with low bone mass, with the tendency of handgrip strength decreased with age [10].

Our results demonstrated an association of decreased grip strength with diabetes and lumbar spondylosis but not with comorbidities. Our finding was consistent with the study by Uman et al. conducted in older people aged ≥ 60 years, there was a significant relationship between type II diabetes and low handgrip strength (OR, 2.3; 95% 1.2 – 4.7) [11]. On the other hand, the study by Lino et al. found no association between low grip strength and diabetes [12]. Maybe the differences in subject, sample size, and study method can explain this occurrence

Participants who experienced pain and lumbar spine malformation had a higher rate of loss of upper limb strength (83.9% vs. 96.2% and 12.9% vs. 27.8%, respectively) ($p< 0.05$). Severe vertebral deformities were associated with substantial pain and disability [13]. However, to our knowledge, no studies have investigated the relationship between lumbar spine deformity and hand muscle strength. Therefore, in the future, more research is needed to evaluate this issue.

Cognition impairment was a risk factor for HGS impairment. Declining cognitive function increased 5.7 times the risk of HGS decline, 95% CI 2.2 – 14.9 ($p< 0.05$). The result was consistent with the population-based study in Korean adults that decreasing handgrip strength was associated with a decline in cognitive function [14]. Physical activity directly stimulates skeletal muscle and subsequently leads to improved muscle mass and higher HGS. Higher physical activity level was associated with greater HGS [15]. We found that individuals with low levels of physical activity can have a 3.9 times higher risk of deteriorated HGS ($p<0.05$). The risk of fall was assessed using the 21-item risk of fall, which was associated with impaired handgrip strength. Similar to our finding, the study

by Nga Thi Thuy Nguyen et al. using the TUG test to assess the risk of falls identified that low HGS was associated with a higher risk of falls [8].

As demonstrated by other authors [12],[16], we also identified participants with depression who had higher odds of having poor HGS (OR 3.7). The assessment of nutritional status had shown a significant association with hand grip strength. Older adults with osteoporosis had malnutrition that increased the odds of poor upper extremity strength 2.2 times ($p < 0.05$). The results were in line with the study by Riviati et al., which showed the association between MNA score and handgrip strength ($p<0.001$), and malnutrition increased the risk of having low handgrip strength by 1.9-fold [9]. Impaired muscle strength in malnourished conditions is due to decreasing muscle protein supply which is a useful alternative energy source [17].

5. CONCLUSIONS

Half of the study subjects had reduced HGS and a quarter of the study subjects had reduced lower limb muscle strength. Age, BMI, diabetes, lumbar spondylosis or other joints, living status and geriatric syndromes were significantly associated with impaired HGS in older patients with osteoporosis.

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HEALTH-RELATED QUALITY OF LIFE AND SOME RELATED FACTORS AMONG OLDER PATIENTS WITH KNEE OSTEOARTHRITIS

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SUMMARY

Objectives: *To assess health-related quality of life (HRQoL) and associations with some characteristics among older people with knee osteoarthritis (OA).* ***Subjects and Methods****: A cross-sectional study on 184 older patients with knee OA treated at National Geriatric Hospital from May to August 2022. HRQoL was assessed using the EQ–5D–5L questionnaire. Knee OA characteristics were also collected.* ***Results:*** *The proportion of patients with difficulties in aspects such as mobility was 88%; self-care was 68.9%; usual activities was 66.1%; pain/discomfort was 90.8% and anxiety/depression was 66.1%. The results showed that HRQoL in older age was significantly lower than in the younger in terms of mobilities and usual activities ($p<0.05$). Patients who had the lower KOA stage had better HRQoL, especially in mobility and pain dimensions. The smaller the VAS score, the better the HRQoL. About the subclinical tests of KOA patients, the thickening synovial membrane had a significant difference with HRQoL in mobility, usual activities, and pain/discomfort dimensions. Patients with bone spurs had significant differences with HRQoL, only except mobility dimension. All negative correlation coefficients showed that this is the opposite correlation, the higher WOMAC pain, stiffness, and function patients got, the worse QoL patients experienced.* ***Conclusion:*** *More than two-thirds of the participants self-reported as having problems with one of the aspects of HRQoL among older patients with knee OA. Our results highlighted that aspects of the EQ-5D-5L are related to age, severe knee OA stages, severe VAS pain, and WOMAC scores.*

Keywords*: Health-related quality of life, elderly, knee osteoarthritis*

1. INTRODUCTION

Osteoarthritis (OA), also known as degenerative joint disease, is a leading cause of disability in worldwide [1]. It is typically a progressive disease that may eventually lead to increased physical limitations, pain, and functionality restriction. The intensity of the clinical symptoms may vary for each individual. However, they typically become more severe, more frequent, and more debilitating over time.

Knee osteoarthritis (KOA), also known as degenerative joint disease of the knee, is typically the result of wear and tear and progressive loss of articular cartilage. It is most common in the elderly [2]. In Vietnam, roughly 8.5% of people between age 40 and 49 had knee osteoarthritis, compared with 30% between age 50 and 59, and 61% over the age of 60 [3].

Health-related quality of life (HRQoL) is defined by the World Health Organization as "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns". These individuals with knee OA suffer from

progressive increased impact on their activities of daily living, which leads to losses in labor relations, leisure, social life, and sleeping quality, leading also to an important decrease in their quality of life [4].

Exploring the HRQoL and its associated factors among older patients with knee OA can develop interventions to promote the HRQoL as much as possible. However, data about HRQoL are lacking regarding the older population with knee OA in Vietnam. Thus, the aims of this study were to assess HRQoL and some related factors among older people with knee OA examined and treated at the National Geriatric Hospital.

2. SUBJECTS AND METHODS

2.1. Subjects

Osteoarthritis of knee patients aged 60 years old and older was being treated at the National Geriatric Hospital from May to October 2022.

- Inclusion criteria: Patients aged 60 years old and older were diagnosed by doctors with ACR 1991 (American College of Rheumatology); and had the physical and cognitive abilities to do a face-to-face interview.

- Exclusion criteria: Patients with an inability to communicate or subjects who have severe or acute diseases or are unable to answer questions.

2.2. Study design

- A cross-sectional descriptive study

- The sample was selected according to the convenience sampling method

- The sample size is calculated using the formula: $n = (Z_{1-\frac{\alpha}{2}})^2 \frac{p(1-p)}{d2}$

p = 0.13 (Health-related quality of life in patients with knee osteoarthritis attending two primary care clinics in Malaysia) [5].

From the formula, the estimated sample size was 174 KOA patients

The number of KOA patients in our study was 184 patients

2.3. Variables

- General information: age, gender, weight, height, body mass index (BMI).
- Knee OA characteristics: stage (according to knee X-ray Kellgren and Lawrence classification), knee ultrasound features, pain intensity (VAS score), and WOMAC score.
- **Health-related quality of life (HRQoL)**: The European Quality of Life-5 Dimendions-5 Level scale (EQ-5D-5L) Questionnaire has two components: EQ-5D-5L descriptive system and the EQ visual analog scale (EQ-VAS) [6].

  The descriptive system comprises 5 dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety/depression), and each dimension has 5 levels: “1 = extreme problems”, “2 = severe problems”, “3 = moderate problems”, “4 = mild problems” and “5 = no problems”. Evaluation: The levels of problems in each dimension were classified into 2 groups: 1-4: Problems and 5: No problems.

  The EQ visual analog scale (EQ-VAS) records the patient’s self-rated health status of the interviewed day on a 20 cm vertical analog scale with 2 endpoints “0 = The worst health

you can imagine" and "100 = The best health you can imagine".

- **Visual Analog Scale (VAS)** was used to measure knee pain intensity [7]. The corresponding 0 line is no pain = 0 points, the maximum 10 line is the most intense pain = 10 points. The range of VAS scores is divided into 3 levels: 0-3.4 (mild); 3.5-7.4 (moderate); ≥ 7.5 (severe).
- **Western Ontario and McMaster Universities Arthritis Index (WOMAC)** [8] is a self-administered questionnaire consisting of 24 items divided into 3 subscales:

- Pain (5 items): during walking, using stairs, in bed, sitting or lying, and standing upright.

- Stiffness (2 items): after first waking and later in the day.

- Physical Function (17 items): using stairs, rising from sitting, standing, bending, walking, getting in/out of a car, shopping, putting on taking off socks, rising from bed, lying in bed, getting in/out of bath, sitting, getting on/off toilet, heavy domestic duties, light domestic duties.

Evaluation: Calculating the average of WOMAC scores with each knee.

2.4. Tools and data collection method

Data were collected by using a research questionnaire through interviews, diagnosis tests, and medical records at the National Geriatric Hospital.

2.5. Data processing and data analysis

Data coding, entry into REDCap, and analysis were done using Statistical Package for Social Science (SPSS) software (version 22.0). Descriptive statistics were adopted to examine characteristic data: frequency, percentage, mean. Inferential statistics was done to perform comparisons between groups, using χ^2 and ANOVA. Statistical significance was accepted at the 95% confidence level ($p<0.05$).

3. RESULTS

3.1. General characteristics

In the total 184 older patients with knee OA, the mean age of the patient was 73.6 ± 8.3 years with a minimum of 60 and a maximum of 94. They are evenly distributed into 3 age groups, 60-69 patients (35.9%), 70-79 patients (38%), and patients over 80 years old (26.1%). The majority of study participants were females, accounting for 83.7%. 42.9% of people surveyed had normal BMI. The mean BMI is 23.1±3.1 (kg/m^2).

3.2. Health-related Quality of Life of older knee osteoarthritis patients

Table 1. EQ-5D-5L characteristics of older KOA patients (n=184)

	Mobility		**Self-care**		**Usual Activities**		**Pain/Discomfort**		**Anxiety/Depression**	
	n	%	n	%	n	%	n	%	n	%
No problems	22	12	57	31	44	23.9	17	9.2	44	23.9
Mild problems	77	41.8	76	41.3	80	43.5	81	44	104	56.5
Moderate problems	66	35.9	40	21.7	52	28.3	72	39.1	26	14.1
Severe problems	17	9.2	8	4.3	5	2.7	12	5.6	10	5.4
Extreme problems	2	1.1	3	1.6	3	1.6	2	1.1	0	0
EQ-VAS	63.2±15.8									

The proportion of participants who reported moderate or severe problems in the dimension of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression were 45.1%; 26.0%; 31.0%; 44.7%, and 19.5%, respectively. Besides that, there are a few patients who reported that they have extreme problems in the dimension of mobility (1.1%), self-care (1.6%), usual activities (1.6%), and pain/discomfort (1.1%). The Mean±SD EQ-VAS score was 63.2±15.8.

3.3. Association between HRQoL and age, KOA characteristics

Table 2. Association between HRQoL and age groups (n=184)

EQ-5D DIMENSION		**60-69 years**		**70-79 years**		**≥80 years**		**p-value**
		n	%	n	%	n	%	
Mobility	No problems	10	15.2	7	10	5	10.4	**<0.01**
	Problems	56	84.8	63	90	43	89.6	
Self-care	No problems	25	37.9	24	34.3	8	16.7	>0.05
	Problems	41	62.1	46	65.7	40	83.3	
Usual activities	No problems	21	31.8	16	22.9	7	14.6	**<0.05**
	Problems	45	68.2	54	77.1	41	85.4	
Pain/Discomfort	No problems	8	12.1	6	8.6	3	6.2	>0.05

	Problems	58	87.9	64	91.4	45	93.8	
Anxiety/Depression	No problems	20	30.3	15	21.4	9	18.8	>0.05
	Problems	46	69.7	55	78.6	36	81.2	
EQ-VAS Score		64.2±16.2		63.6±14.6		61.1±17.0		>0.05

The result of data analysis revealed statistically significant differences in the perception of HRQoL between age groups. Increasing age is related to a high frequency of reporting difficulty in daily life, such as mobility and usual activities.

Table 3. Associated to EQ-5D by clinical characteristics of KOA patients (n=184**)**

	n	% responding with a problem (moderate or extreme)					EQ-VAS Score Mean ± SD
		Mobility	**Self-care**	**UA**	**Pain**	**Anxiety**	
KOA Stages							
1	36	**3.8**	4.1	4.1	**3.8**	3.9	65.1±15.8
2	82	**3.6**	3.9	3.9	**3.6**	4.0	63.7±16.1
3	52	**3.2**	3.8	3.6	**3.3**	3.8	60.3±15.0
4	14	**3.0**[b]	3.7	3.5	**3.0**[b]	3.9	65.7±16.9
VAS							
Mild	48	**4.0**	**4.3**	**4.3**	**3.9**	**4.2**	**68.2±17.6**
Moderate	117	**3.4**	**3.9**	**3.7**	**3.5**	**4.0**	**63.7±13.0**
Severe	19	**3.1**[a]	**3.6**[b]	**3.6**[a]	**2.8**[b]	**3.8**[b]	**47.9±18.4**[c]
Ultrasound							
Thickening synovial membrane							
No	126	**3.6**	4.0	**3.9**	**3.6**	4.0	64.5±14.8
Yes	58	**3.2**[b]	3.7	**3.6**[a]	**3.2**[b]	3.9	60.4±17.6
Bone spurs							
No	60	3.5	**4.1**	**4.1**	**3.7**	**4.2**	66.4 ±12.8
Yes	124	3.5	**3.8**[a]	**3.7**[b]	**3.4**[a]	**3.8**[b]	61.6±16.9
Baker cysts							
No	143	3.6	4.0	3.9	3.6	4.0	63.5±15.5
Yes	41	3.2	3.6	3.6	3.2	3.8	61.9±16.9

Duration							
< 1year	77	3.7	4.1	3.9	3.6	4.1	65.0±14.0
1-5 years	79	3.5	3.9	3.9	3.5	3.9	61.6±18.0
>10 years	28	3.5	3.9	3.8	3.5	4.0	63.0±13.8

a: χ2, p<0.05; b: χ2, p < 0.01; c: Kruskall Wallis, p < 0.05

Patients who had the lower KOA stage had better HRQoL than those who had a higher stage of KOA, especially in mobility and pain dimensions. The VAS score had a significant difference in HRQoL, the smaller the VAS score, the better HRQoL. About the subclinical tests of KOA patients, the thickening synovial membrane had a significant difference with HRQoL in mobility, usual activities, and pain/discomfort dimensions. Patients with bone spurs had significant differences with HRQoL, only except mobility dimension.

Table 4. Spearman correlation coefficients between EQ-5D and WOMAC

	WOMAC pain	**WOMAC stiffness**	**WOMAC function**
Mobility	-0.442**	-0.187*	-0.413**
Self-care	-0.270**	-0.339**	-0.302**
Usual activities	-0.349**	-0.317**	-0.355**
Pain/Discomfort	-0.400**	-0.192**	-0.362**
Anxiety/Depression	-0.206**	-0.360**	-0.191**
EQVAS	-0.169*	-0.177*	-0.185*

Correlation is significant at the 0.01 level (2-tailed).

Correlation is significant at the 0.05 level (2-tailed).

All 5 dimensions of QoL in the EQ-5D-5L scale had moderate significant differences with WOMAC pain, stiffness, and function. The WOMAC pain and function scores best correlated with the mobility dimension in the EQ-5D-5L scale. All negative correlation coefficients showed that this is the opposite correlation, the higher WOMAC pain, stiffness, and function patients got, the worse QoL patients experienced.

4. DISCUSSION

Our study showed the characteristics of HRQoL and its associations with age and knee OA features among older patients with knee OA at the National Geriatric Hospital. We showed that the participant reported “mild or moderate problems” in a range of 21.7%-56.5% with highest rates for mobility, self-care, usual activities, pain and lowest rate for anxiety and pain dimension was 9.2% and 14.1%. The proportion of severe and extremely problems in 5 diemnsions QoL was so small: range from 0 to 9.2%.

The results showed that increased age was associated with lower HRQoL. To be more specific, patient aged from 70 to 79 years had more problems in mobility and ususal activities than those aged from 60 to 69 years (p <0.01, p< 0.05). This can be explained that getting older the patients have to face with functional impairment. The study of Zainal and his colluges also showed that: there was a significant negative correlation between physical functioning and age [5].

Overall, there was no significant difference between HRQoL and disease duration, also the side of KOA. This is so difference with the study of Gordana Nikolic, in this study, duration of disease significantly correlated with all scores, except for EQ-5D-5L mobility score [9]. Another study showed that the association with other medical characteristics showed negative correlation between duration of knee pain and all the HRQoL domains (except Role-Emotional) with significant negative correlation was found in the RP (Role-Physical) domain (r_s = -0.287, p < 0.005) [5]. This difference may be due to the sample size is not enough and the difference between 2 research subjects.

This study showed that patients who had the lower KOA stage, had better HRQoL than those who had higher stage of KOA, especially it is a significant difference in mobility and pain dimensions (p<0.05). The VAS score had significant difference in HRQoL, smaller VAS score, better QoL (p<0.05). Study conducted in Japan indicated that the EQ-5D-5L utility scores were not significantly associated with the KL grade of the knee after adjustment for age, BMI, and grip strength [10]. This difference may be due to the sample size is not enough and the difference between 2 research subjects.

About the subclinical tests of KOA patients, thickening synovial membrane had significant difference with HRQoL in mobility, usual activities and pain/discomfort dimensions. Patients with bone spurs had significant difference with HRQoL, only except mobility dimension. And patients with Baker cysts had no significant difference with HRQoL. This result showed that subclinical results of KOA had certains effects on patients HRQoL.

In this study, all 5 dimensions of HRQoL in EQ-5D-5L scale had significant difference with WOMAC pain, stiffness and function (p< 0.05, p<0.01). The WOMAC pain and function scores best correlated with the mobility dimension in the EQ-5D-5L scale (|r|= 0.442, |r|= 0.442). All negative correlation coefficients showed that this is opposite correlation, the higher WOMAC pain, stiffness and function patients got, the worse HRQoL patients experienced. The correlations between the WOMAC and EQ-5D-5L scales in our study were satisfactory, although the stiffness component on the WOMAC scale had the lowest correlation with the EQ-5D-5L, which is in accordance with other study [11].

5. CONCLUSIONS

More than two-thirds of the participants self-rated as having problems with one of the aspects of HRQoL among older patients with knee OA. Our results highlighted that aspects of the EQ-5D-5L are related to age, severe knee OA stages, severe VAS pain and WOMAC scores among this population.

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PREVALENCE OF FATIGUE IN ELDERLY POST-STROKE PATIENTS

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SUMMARY

This study aims to describe the prevalence of fatigue in elderly post-stroke patients. A cross-sectional study included 157 Subjects diagnosed with stroke (according to WHO) and treated as inpatients and outpatients at the National Geriatric Hospital, aged ≥ 60 years old examined or treated at National Geriatric Hospital from July to November 2021. Data were collected by using designed tools including characteristics of these patients. Entered data on Redcap and used SPSS version 22.0 for analysis. The mean age of the patients was 73.49 ± 9.58 years. The female/male ratio was 0.89. The percentage of patients with independence was 42.7% (n=67). 57.3% (n=90) of the participants depended on the ratio of the components: Minimally dependent, partially dependent, very dependent, and totally dependent, respectively 10.2% (n=16); 14% (n=22); 14.6% (n=23) and 18.5% (n=29). In one hundred and fifty-seven participants, the percentage of patients with sleep disturbances accounted for 81.5% (128), which is four times the percentage of patients with good sleep at 18.5% (29). The total score of the FSS test ranged from 7 to 63 with a mean score was 42.32 ± 11.50, in which, the greatest distribution was generated by post-stroke fatigue patients (<36 points), with the percentage of 74.5% and almost triple with normal group (25.5%). The majority of patients agreed with the statement that fatigue affects the patient's motor function, the patient is prone to fatigue but it is not a permanent problem for the patient. Early detection by performing FSS test to screen for fatigue in elderly post-stroke patients, thereby providing appropriate treatment can help improve treatment effectiveness as well as patient's quality of life.

Keywords: *fatigue, older patients, post-stroke*

1. INTRODUCTION

Stroke is a major cause of death and disability in many countries. It was reported that, in 2013, globally, there were nearly 25.7 million stroke survivors, 6.5 million deaths due to stroke [1]. Stroke is an especially serious problem in Asia, which has more than 60% of the world's population, and many of its countries are "developing" economies. Stroke mortality is higher in Asia than in Western Europe, the Americas or Australasia, except in the case of some countries such as Japan [1]. In Vietnam, according to information from the Ministry of Health, each year there are about 200,000 cases of stroke, it is the leading cause of death, with a rate of 10-20%, many times higher than some other common causes of death.

Fatigue is generally multidimensional, and is associated with physical, cognitive, emotional, and social factors. Fatigue is a normal condition, but it can also be medical and often mistaken for depression [2]. Fatigue is a common symptom after a stroke, with reported frequencies ranging from 38% to 77% [3], indicating that Post-stroke Fatigue (PSF) is a major problem after stroke. Forty percent of post-stroke patients report that fatigue is one of the worst consequences of stroke [4]. The causes of fatigue after stroke are complex and are thought to be multifactorial. It is not clear what medical and socio-demographic factors are associated with PSF as findings in earlier studies are contradictory [5]. However, PSF is often associated with pain and sleep disorders[8] and is closely associated with depression and anxiety [6].

After a stroke, patients often have reduced working capacity, hindering their reintegration. In addition, stroke affects their physical and mental health. After a stroke, patients often have a reduced ability to work, which hinders their reintegration. Besides, stroke also affects their physical and mental health. For the patient's family, stroke creates an economic burden through loss of work capacity, care and treatment as well as mental pressure. Currently, research on fatigue of patients after

stroke is limited, the research period is long, and the current reference value is still limited. For the above reasons, we conducted this research to describe the prevalence of fatigue in elderly post-stroke patients at the National Geriatrics Hospital.

2. SUBJECTS AND METHODS

2.1. Study subject

Patients at National Geriatric Hospital in age from 60 years old, who have been diagnosed and treated at National Geriatric Hospital from 12/07/2021 to 12/11/2021 volunteered to participate in this research. Informed consent was obtained from all participants.

Inclusion Criteria:

- Subjects participating in the study from 50 years and older.
- Subjects diagnosed with stroke (according to WHO) by doctor and are being treated as inpatient and outpatients at the National Geriatric Hospital.
- The patients have the physical and cognitive abilities to do a face-to-face interview.
- Patients and families of patients agree to participate in research.

Exclusion criteria:

- Subjects with a history of stroke more than 7 years.
- Cases of dementia severity, inaccessible.
- Aphasia case limiting in describing symptoms.
- Pharyngeal paralysis, severe quadriplegia limits communication.
- History of pre-stroke psychosis: shcizophrenia, majoir depression, bipolar disorder, substance abuse.
- Object and relatives did not agree to participate in research.

2.2. Methods

We performed the cross-sectional descriptive study with convenience sampling methods from July to November 2021

Tools and data collection method

General information including date of interview, contact information, full name, age, gender, level of education, occupation, living status, living area.

Data were collected by using designed tools included: Mini-Mental State Exam (MMSE), general information, Pittsburgh Sleep Quality Index (PSQI), Mini Nutritional Assessment Short Form (MNA-SF), Barthel Index (BI) for Activities of Daily Living (ADL), Instrumental Activities of Daily Living (IADL), Patient Health Questionnaire – 9 (PHQ-9).

❖ ***Fatigue assessment:*** Using Fatigue severity scale (FSS) to assess fatigue status of the participant [7].

- Performing: The original FSS is a nine-item unidimensional questionnaire developed by Krupp, LaRocca, Muir-Nash, and Steinberg (1989). The mean score of the items is used as the FSS score. FSS consists of 9 items, each is scored using a Likert-type scale ranging from 1 (strongly disagree) to 7 (strongly agree). The participants were asked to complete the version of FSS, regarding the previous week. Total score is the sum of these items divided by the number of items.

Evaluation: High scores indicate high levels of fatigue. A total score of less than 36 suggests that you may not be suffering from fatigue. A total score of 36 or more suggests that you may need further evaluation by a physician.

2.3. Process of data analysis

The process of data recording, entries into Redcap and analyzed by using Statistical Package for Social Science (SPSS) software version 22 with statically p less than 0.05. Descriptive statistics were adopted to examine characteristic data: frequency, percentage, mean. Inferential statistics was done to perform comparisons between groups, using χ^2.

2.4. Ethical consideration

All data collected was used for research. The results of the study were proposed for improving health of community, not for other purposes and ensure all ethical issues in biological research.

3. RESULTS

We conducted a cross-sectional study that included 157 post-stroke patients at National Geriatric Hospital. The interviews took place from July 1st, 2015 to November 7th, 2015

Table 1. Social demographic characteristics of the participants (n=157)

Characteristics	Frequency (n)	Percent (%)
Mean age	73.47 ± 9.58	
Age group		
<60	14	8.9
60 – 80	103	65.6
>80	40	25.5
Gender		
Male	83	52.9
Female	74	47.1
Marital status		
Married	134	85.4
Single/Widowed/Divorced	23	14.6
Educational level		
Secondary school and below	91	58
High school	31	19.7
College/university and above	35	22.3

The mean age of the patients was 73.49 ± 9.58 years. In which, the greatest distribution was generated by patients aged between 60 and 80, with percentage of 65.6%. Secondly, patients at over 80 years old accounted for 25.5%. Patients less than 60 years old represented only 8.9% in total number of the sample. A total of 157 individual female subjects made up 47.1% (n=74) of total number of the sample, which was smaller than distribution of male patients, namely 53.9% (n=83). The female/male ratio was 0.89. The majority 134 persons (85.4%) were married. The widowed, single and divorced persons were only 14.6% (n=23). Additionally, majority 91 respondents (58%) had formal education, only finished secondary school and lower than that, 31 others (19.7%) graduated from high school. Especially, 22.3% of total subjects were continued to study to higher level.

Physical function in the Post-stroke elderly

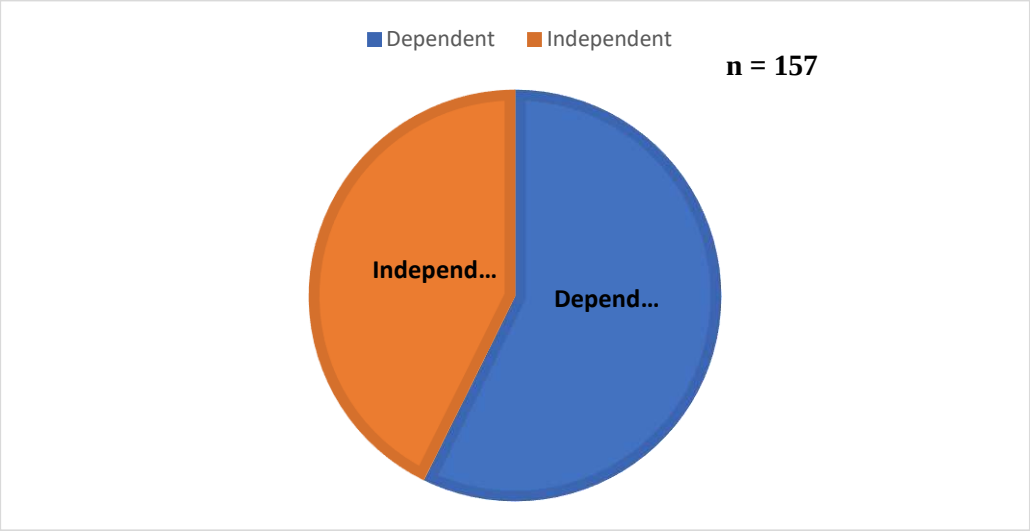

Figure 1. Physical function in the Post-stroke elderly

According to BI score, the percentage of patients with independence was 42.7% (n=67). 57.3% (n=90) of the participants depended with the ratio of the components: Minimally dependence, Partially dependent, Very dependent and Totally dependent, respectively 10.2% (n=16); 14% (n=22); 14.6% (n=23) and 18.5% (n=29).

- ***Cognitive status:***

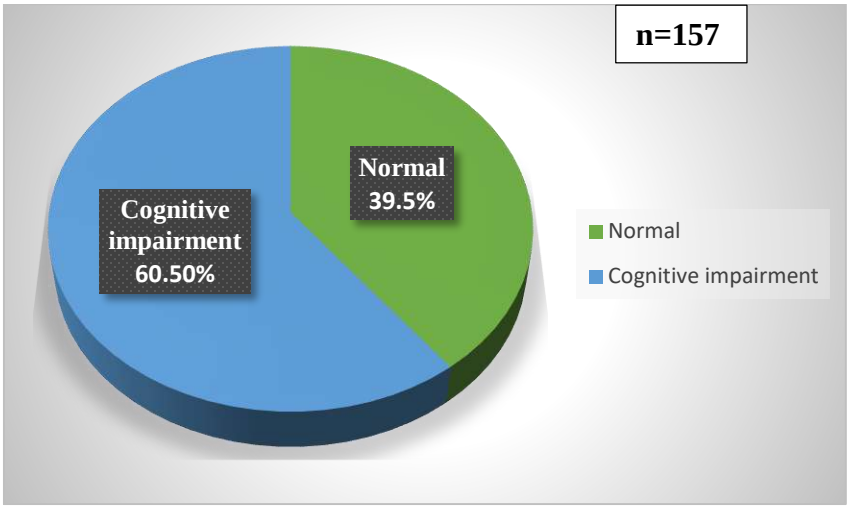

Figure 2. Mini – Mental State Examination (MMSE) in elderly post-stroke patients

Sixty-two patients (39.5%) were assigned to the group with no signs of cognitive impairment, whereas, ninety-five (60.5%) of the participants were in the group of patients with signs of cognitive impairment.

Table 2. Sleep disorders and depressive status in elderly post-stroke patients (n=157)

Characteristics	Frequency (n)	Percentage (%)
Sleep disorders (based on PSQI)		
Good sleep	29	18.5
Poor sleep	128	81.5
The severity of depression (based on PHQ-9)		
Normal-minimal depression	41	26.1
Mild depression	47	29.9
Moderate depression	40	25.5
Severe depression	29	18.5

In one hundred and fifty-seven participants, the percentage of patients with sleep disturbances accounted for 81.5% (128), which is four times the percentage of patients with good sleep at 18.5% (29).

Percentage of fatigue in elderly post-stroke patients

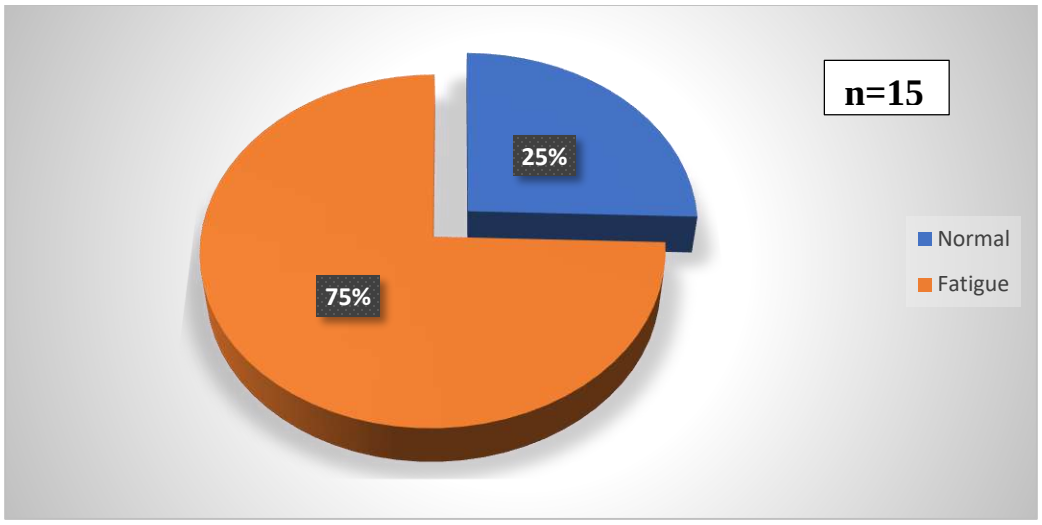

Figure 3. Fatigue in elderly post-stroke patient distribution.

The total score of FSS test was ranged from 7 to 63 with the mean score was 42.32 ± 11.50, in which, the greatest distribution was generated by post-stroke fatigue patients (<36 points), with percentage of 74.5% and which almost triple with normal group (25.5%).

Characteristics of fatigue fields

Table 4. The result of components in FSS test in post stroke patients (n=157)

Components of FSS	Score	Reference (n)	Percentage (%)
1. My motivation is lower when I am fatigued.	1 – 4	45	28.7
	5 – 7	112	71.3
2. Exercise brings on my fatigue.	1 – 4	67	42.7
	5 – 7	90	57.3
3. I am easily fatigued	1 – 4	64	40.8
	5 – 7	93	59.2
4. Fatigue interferes with my physical functioning.	1 – 4	44	28
	5 – 7	113	72
5. Fatigue causes frequent problems for me.	1 – 4	83	52.9
	5 – 7	74	47.1
6. My fatigue prevents sustained physical functioning.	1 – 4	49	31.2
	5 – 7	108	68.8
	1 – 4	57	36.3

Components of FSS	Score	Reference (n)	Percentage (%)
7. Fatigue interferes with carrying out certain duties and responsibilities.	5 – 7	100	63.7
8. Fatigue is among my three most disabling symptoms.	1 – 4	109	69.4
	5 – 7	48	30.6
9. Fatigue interferes with my work, family, or social life.	1 – 4	73	46.5
	5 – 7	84	53.5

(Note: 1-4: Disagree; 5-7: Agree)

With the statements “Exercise gives me fatigue; I am easily fatigued; Fatigue interferes with my work, family, or social life”, the percentage of participants agreeing is almost equal with disagreeing group; Except for component 5, there are 83 (52.9%) participants agreeing with fatigue causes frequent problems for them and 74 (47.1%) disagreeing.

For sentence 8, the percentage disagreeing is 69.4% and which almost double with agreeing group (30.6%). The percentage disagreeing is nearly three times the percentage agreeing in components: 1, 4, 6, which reflects the effect of fatigue on the participants' physical performance, such as: My motivation is lower when I am fatigued; Fatigue interferes with my physical functioning; My fatigue prevents sustained physical functioning.

4. DISCUSSION

In this study, female subjects made up 47.1% (n=74) of total number of the sample and the male/female ratio was 1.12/1. However, in studies in Sweden, out of 91 people, 58% of women and 33% of men reported fatigue about FSS [8]. Another study by Lerdal et al., with 1893 participants aged 19 to 81 years old, had more women (26.2%) than men (19.8%) experienced high fatigue (p =0.004) [9]. Almost the study had the rate of female being higher than the rate of male. The reason may be explained by different research subject or sample size.

The prevalence rate decreased with age, from 35% (<70 years old), to 37.6% (70-79 years old) and dropped to 27.4% (≥ 80 years old). The average age of participants in this study was 73.47 years old (SD=9.58). It was similar finds in the research of Gillian, the average age of participants was 71.1 years (SD=10.8). And our result is also the same with study of Heruti and colleagues: stroke population characterized in the literature is in the age range of 60–74 years. One of the explanations might be older patients had more risk factors like comorbidity disease.

The percentage of patients completed secondary school and below took up 58%, 19.7% graduated from high school. Especially 22.3% of total subjects were continued to study to higher level. This result is similar to the study of Nguyen Thi Minh Tri and colleagues with the education level: low 99 patients (42.3%), average 104 (44.4%), high 31 patients (13 ,2%) [10]. This is similar to the results of a study in Norway where the most common level of education achieved was Middle school, equivalent to 8 years of education in 321 (55.2%) participants. But one of the disadvantages to this distribution is that the high proportion of patients with low education may affect their ability to answer the MMSE test.

Most of the post-stroke patients (85.4%) in this study were married and had family, a higher percentage than those who were single, divorced, and widowed. This result is reasonable given that most of the study's patients were 40 years of age or older. This finding is consistent with the results of a Norwegian study in patients with post-stroke fatigue, with the majority of participants being 169 married (52.6%).

In addition, post-stroke patients were discharged home with an average of 5.52 (SD=1,878) of drugs. There was a correlation between sustained users of >5 drugs and fatigue (OR: 3.43, p=0.03). this is lower than the drug’s

mean in Ostwald's study with 11.3 drugs (SD=4.94, range: 3-27) [11]. This can be explained by the age of the participants and the different sample sizes.

In this study, the percentage of dependent patients in daily activities was 70.1% (n=110) and the percentage of patients without dependence was 29.9% (n=47). There is similarity in the sensitivity study of 71 subjects recovering from stroke by Alexander, he found that 26% of the subjects scored > 95%, and 74% patients is from minimal dependent to totally dependent [12]. One hundred and twenty-six (80%) was classified as dependent in one or more IADL domains. But Thirty-one participants (19.7%) of the participants were independent of all Instrument activities of daily living (IADL). However, in the study of Badaru et al. (2013): the percentage of dependent patients accounted for 65% and independent patients accounted for 35% [13]. As the difference in age of subjects (younger and elderly patients), their ability to do IADLs is reduced.

The percentage of patients with cognitive impairment after stroke in this study was 60.5% (n=95). In the rehabilitation study of elderly stroke patients by Denti et al, it was shown that cognitive impairment was found in the 41% of patients, with only 9% showing a score less than 10/30. 94 patients (26.2%) had aphasia, while 71 (19.8%) showed neglect [14]. The difference is not too large, which can be explained by the different sample sizes and research subjects.

Of the 157 participants, 81.5% (128) had poor sleep. This rate is higher than the study in Turkey, it found that 53.3% of the subjects had poor sleep quality [15]. The difference can be explained by the different study sample size and subjects. In this study, the proportion of patients at risk of depression was 73.9%, of the total 157 participants, 29.9% of patients had signs of mild depression, 25.5% of patients had signs of moderate depression and 18.5% of patients. have symptoms of severe depression. This rate is higher than the prevalence in Hackett et al's study of 31% out of 25488 post-stroke patients [16]. The difference can be explained by different sample sizes (157 < 25488) and different study subjects.

In this study, we determined that the fatigue rate in patients after stroke was 74.5% with a limit of fatigue on the FSS scale of 36 points. This is higher than the prevalence in 155 stroke patients from acute to 18 months post-traumatic in the study of Anita et al., according to FSS, the fatigue level was 40.35% [17]. The difference in results between studies could be explained by the elderly population in this study who were post-stroke patients (ages 40 to 95 years), so sleep quality is affected. affected by many factors: age, comorbidities and geriatric syndromes. The mean value of the FSS scale is 4,702 ± 1,278, similar to the mean score in the previous Swiss study, which is 4.66 ± 1.64 (mean + SD) [18]. With the statements “Exercise gives me fatigue; I am easily fatigued; Fatigue interferes with my work, family, or social life”, the percentage of participants agreeing is almost equal with disagreeing group; Except that 83 (52.9%) participants agreeing with fatigue causes frequent problems for them and 74 (47.1%) disagreeing. With the statement “Fatigue is among my three most disabling symptoms”, the percentage disagreeing is 69.4% and which almost double with agreeing group (30.6%). The percentage disagreeing is nearly three times the percentage agreeing in components: 1, 4, 6, which reflects the effect of fatigue on the participants' physical performance, such as: My motivation is lower when I am fatigued; Fatigue interferes with my physical functioning; My fatigue prevents sustained physical functioning.

5. CONCLUSION

Percentage of patients with fatigue (using FSS): 74.5%. The mean value of FSS is 42.32 ± 11.50. The majority of patients agreed with the statement that fatigue affects the patient's motor function, the patient is prone to fatigue but it is not a permanent problem for the patient. Early detection by performing FSS test to screen for fatigue in elderly post-stroke patients, thereby providing appropriate treatment can help improve treatment effectiveness as well as patient's quality of life.

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QUALITY OF LIFE AND SOME RELATED FACTORS IN ELDERLY PATIENTS WITH DEMENTIA

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SUMMARY

Objectives*: This study aims to determine associated factors to health-related quality of life in dementia patients at the National Geriatrics Hospital.* ***Materials and Methods:*** *A cross-sectional study included 87 dementia patients aged ≥ 60 years old examined or treated at National Geriatric Hospital from July 2021 to November 2021. Data were collected by using designed tools including characteristics of these patients. Entered data on Redcap and used SPSS version 22.0 for analysis.* ***Results:*** *The mean age of the patients was 76.84 ± 8.38. The majority of the patients 57 people (65.5%) were females and 30 respondents (35.5%) were males. 42.5% (n=87) of participants had a high quality of life, and only 2.3% (n=87) of respondents had a poor quality of life. 54% of the sample reported slight or moderate problems in the dimension of mobility. 42.5% in the dimension of self-care. 44.8% in the dimension of usual activities. 69% in the dimension of pain/discomfort. Last one, the patients in the dimension of anxiety/depression reported slight or moderate problems was 70.1%. Besides, 22.9% of the sample reported severe or extreme problems in the dimension of mobility. 28.7% in the dimension of self-care. 49.4% in the dimension of usual activities. 11.4% in the dimension of pain/discomfort. And, finally, 9.1% of the sample reported severe or extreme problems in the dimension of anxiety/depression. The mean ± SD VAS score was 57.70 ± 18.518. There was a significant association between depression and health-related quality of life (p<0.05).* ***Conclusions:*** *Routine health check-ups should be recommended for elderly with dementia to improve their health-related quality of life*

Keywords: *dementia, quality of life, older patients*

1. INTRODUCTION

Elderly people may suffer from different chronic diseases and need special care that entails high expenditures. Dementia is one of the most important chronic diseases. The World Alzheimer Report 2009 report released for World Alzheimer's Day (2009) indicates that 35 million people are suffering from dementia and estimates that the number of cases will double every 20 years. During the next 20 years, this number will increase by 40% in Europe, 63% in North America, 77% in the Southern Cone of Latin America and 89% in the developed Asia Pacific countries. These figures are to be compared with the 117% growth in East Asia, 107% in South Asia, 134– 146% in the rest of Latin America, and 125% in North Africa and the Middle East. Dementia is a progressive, disabling, chronic disease affecting 5% of all people above 65 and over 40% of people over 85 years [1].

Dementia is a global epidemic– whilst many cases have been recorded in the world's richest and most aged countries, already the clear majority (63%) of people living with dementia live in low- and middle-income countries (LAMIC) where access to social protection, services, support and care are very limited.

This situation leads to the impacts on patients, care-givers, and society.

Dementia is the leading chronic disease contributors to disability, and, particularly, dependence among older people worldwide. While older people can often cope well, and remain reasonably independent even with marked physical disability, the onset of cognitive impairment quickly compromises their ability to carry out complex but essential tasks in daily life. In addition, people living with dementia will increasingly have difficulty to meet their basic personal care needs. The need for support from a caregiver often starts early in the dementia journey, intensifies as the illness progresses over time, and continues until death. There is a large literature attesting to the extent of the strain that caregivers experience, which is practical (hours spent caregiving detracting from other activities, particularly leisure and socializing), psychological (emotional strain, leading to a high prevalence of anxiety and depression), and economic (increased costs, coupled with giving up or cutting back on work to care).

Health-related quality of life (HRQoL) is "*an individual's or a group's perception of physical and mental health over time"*, it also includes emotions and social functioning [2]. On the personal level, this contains physical and mental health perceptions and others –including health risks and conditions, functional status, social support, and socioeconomic status. On the community level, HRQoL contains resources, conditions, policies, and practices that affect a population's health perceptions and functional status. Currently, more and more studies on the role of HRQoL in the treatment of dementia patients are being carried out, so improving the standard of care for dementia patients is also more important. However, most of it is research on caregivers, the reason is due to the difficulty in exploiting information from the patient because of the specificity of dementia. Therefore, we conducted this research to describe the Quality of life and some related factors in elderly patients with dementia at the National Geriatrics Hospital.

2. SUBJECTS AND METHODS

2.1. Study subject

Patients at National Geriatric Hospital in age from 60 years old, who have been diagnosed by neurologists at the National Geriatric Hospital, volunteered to participate in this research. All of them were assigned to perform the same task. Informed consent was obtained from all participants.

Excluded criteria:

- Acute and malignant diseases (advanced cancers, end-stage chronic diseases, acute myocardial infarction, and stroke).

- Symptomatic cardiovascular disease, coronary revascularization within 1 year.

- Clinical evidence of schizophrenia, severe depression, psychiatric or bipolar disorder (according to DSM-IV TR criteria [3]).

- Alcoholism or substance dependence (according to DSM--IV criteria [3]), currently, or within the past 2 years.

- Severe loss of vision, hearing or communicative ability (according to the interRAI Community Health Assessment).

- Participant or family unwilling to participate in the study.

2.2. Methods

We performed the cross-sectional descriptive study with convenience sampling methods from July to November 2021.

2.3. Tools and data collection method

General information including date of interview, contact information, full

name, age, gender, level of education, occupation, living status, living area.

Data were collected by using designed tools included: Mini-cog test, Mini-Mental State Exam (MMSE), general information, EuroQoL 5 Dimensions 5 levels (EQ-5D-5L), Pittsburgh Sleep Quality Index (PSQI), Mini Nutritional Assessment Short Form (MNA-SF), Barthel Index (BI) for Activities of Daily Living (ADL), Instrumental Activities of Daily Living (IADL), Patient Health Questionnaire – 9 (PHQ-9).

Dementia was assessed by using Mini – cog and the Mini – Mental State Exam (MMSE). Eligible patients to participate in the study were asked to sign an informed consent, then they did test Mini-cog. Mini-cog is a test which asked patient to recall three words and clock drawing with exactly time (11:00) [3]. If the mini-cog test is positive, the patient will be referred to the doctor for diagnosis, negative cases were excluded from the study. The Mini – Mental State Exam (MMSE) which use to test the cognitive function included memory, attention, language, orientation and visual – spatial skills [4]. Patients who do the MMSE test with 24/30 points or less will be allowed to take the test to collect data for the study, above 24 points were excluded from the study: score 30 – 25: normal, score 24 – 21: mild dementia, score 20 – 10: moderate dementia, score 0 – 9: severe dementia.

Health – Related Quality of Life (HRQoL) and EuroQol Visual Analogue Scale (EQ-5D-5L) in dementia patients were measured by EQ-5D HRQoL Questionnaire which is a standardized measure of health status developed by the EuroQoL Group. It includes the EQ-5D descriptive system and the EQ visual analogue scale (EQ VAS). The EQ-5D is a tool included 5 dimensions towards different aspects of health: mobility, self-care, usual activities, paint/discomfort and anxiety/depression. Each dimension has five levels of classification in regard to functional state: extremely problems (=1), severe problems (=2), moderate problems (=3), slight problems (=4) no problem (=5). Continuous visual analogue scale (VAS) ranging from 0 (worse health) to 100 (best health) and then compare the results with EQ-5D value.

Patient Health Questionnaire – 9 (PHQ-9) is a self-administered version of the PRIME-MD diagnostic instrument for common mental disorders". The scale has 9 items about problems during the past 2 weeks and every-item were marked: not at all = 0, several days = 1, a half of days = 2, every day = 3. With a maximum score of 27 points and a minimum score of 0 point, the result was classified as follows: 1 – 4: Minimal depression, 5 – 9: Mild depression, 10 – 14: Moderate depression, 15 – 19: Moderately severe depression, 20 – 27: Severe depression.

2.4. Process of data analysis

The process of data recording, entries into Redcap and analyzed by using Statistical Package for Social Science (SPSS) software version 22 with statically p less than 0.05. Descriptive statistics were adopted to examine characteristic data: frequency, percentage, mean. Inferential statistics was done to perform comparisons between groups, using χ^2.

2.5. Ethical consideration

All data collected was used for research. The results of the study were proposed for improving health of community, not for other purposes and ensure all ethical issues in biological research.

3. RESULTS

3.1. General characteristics

Of total 87 dementia patients, the mean age was 76.84 ± 8.38. The age

was separated into three groups: 18 persons (20.7%) from 60 to 69 years old, 36 persons (41.4%) from 70 to 79 and 33 people (37.9%) in and over the age of 80. The majority of the patients 57 people (65.5%) were females and 30 respondents (35.5%) were males. There were 51 people (58.6%) did not graduate from high school, 28 others graduated (32.2%), some continued to study higher level (8%).

Table 1. Characteristics of dementia (n=87)

Characteristics		Frequency (n)	Percentage (%)
Type of dementia	Vascular	12	13.8
	Mixed	18	20.7
	Alzheimer	47	54.0
	Others	10	11.5
Stage of dementia	Mild dementia	30	34.5
	Moderate dementia	38	43.7
	Severe dementia	18	20.7
Duration	≤ 1 year	33	37.9
	1 year – 5 years	48	55.2
	>5 years	6	6.9

More than half of the participants in the study had Alzheimer 54.0% (n=47/87). The number of vascular, mixed and others type was 13.8% (n=12/87), 20.7% (n=18/87) and 11.5% (n=10/87) respectively. There was 30 people (34.5%) diagnosed with mild dementia, another 38 participants (43.7%) were moderate dementia and 18 patients (20.7%) with severe dementia. A 55.2% of participants (n=48/87) had dementia in 1 year – 5 years. A 37.9% of patients (n=33/87) had dementia less than 1 year. Finally, only 6.9% of patients (n=6/87) had dementia in over 5 years.

3.2. Quality of life of dementia patients

EQ-5D descriptive system contains the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels in classification in regard to functional state (no problems, slightly problems, moderately problems, severely problems and extremely problems). The table below gives summary statistic information on these dimensions:

Table 2. Frequency and proportion of 5 levels by dimension

EQ-5D dimension	Mobility		Self-care		Usual activities		Pain/ Discomfort		Anxiety/ Depression	
	n	%	n	%	n	%	n	%	n	%
Level 1 No problems	20	23.0	25	28.7	5	5.7	17	19.5	18	20.7
Level 2 Slight problems	21	24.1	22	25.3	21	24.1	34	39.1	45	51.7
Level 3 Moderate problems	26	29.9	15	17.2	18	20.7	26	29.9	16	18.4
Level 4	17	19.5	16	18.4	27	31.0	9	10.3	7	8.0

Severe problems										
Level 5 Extreme problems	3	3.4	9	10.3	16	18.4	1	1.1	1	1.1
VAS score	Mean: 57.70 ± 18.518									

A 54% of the sample reported slight or moderate problems in the dimension of mobility. A 42.5% in the dimension of self-care. A 44.8% in the dimension of usual activities. A 69% in the dimension of pain/discomfort. Last one, the patients in the dimension of anxiety/depression reported slight or moderate problems was 70.1%. Besides, 22.9% of the sample reported severe or extreme problems in the dimension of mobility. A 28.7% in the dimension of self-care. A 49.4% in the dimension of usual activities. A 11.4% in the dimension of pain/discomfort. And, finally, a 9.1% of the sample reported severe or extreme problems in the dimension of anxiety/depression. The mean ± SD VAS score was 57.70 ± 18.518.

3.3. Association between quality of life (EQ-5D) and age

Table 3. Responses to EQ-5D by age (n=87)

EQ-5D DIMENSION		**60-69 yrs**		**≥ 70 yrs**		**p value**
		n	**%**	**n**	**%**	
Mobility	No problems	5	5.7	15	17.2	0.588
	Problems	13	14.9	54	62.1	
Self-care	No problems	3	3.4	22	25.3	0.204
	Problems	15	17.2	47	54.0	
Usual activities	No problems	0	0	5	5.7	0.239
	Problems	18	20.7	64	73.6	
Pain/discomfort	No problems	2	2.3	15	17.2	0.311
	Problems	16	18.4	54	62.1	
Anxiety/Depression	No problems	4	4.6	14	16.1	0.857
	Problems	14	16.1	55	63.2	
VAS Score (mean ± SD)		59.44 ± 19.54		57.25 ± 18.36		0.656

There was no significant difference in the QoL of patients with dementia compared to the age.

3.4. Association between quality of life (EQ-5D) and gender

Table 4. Responses to EQ-5D by gender (n=87)

EQ-5D DIMENSION		**Male**		**Female**		**p value**
		n	**%**	**n**	**%**	
Mobility	No problems	5	5.7	15	17.2	0.368
	Problems	24	27.6	43	49.4	
Self-care	No problems	5	5.7	20	23.0	0.094
	Problems	24	27.6	38	43.7	
Usual activities	No problems	2	2.3	3	3.4	0.745
	Problems	27	31.0	55	63.2	
Pain/Discomfort	No problems	7	8.0	10	11.5	0.444
	Problems	22	25.3	48	55.2	
Anxiety/Depression	No problems	6	6.9	12	13.8	1.00

	Problems	23	26.4	46	52.9	
VAS Score (mean ± SD)		56.03 ± 16.66		58.53 ± 19.47		0.556

There was no significant difference in the QoL of patients with dementia compared to the gender.

3.5. Association between quality of life (EQ-5D) and educational level

Table 5. Responses to EQ-5D by educational level (n=87)

EQ-5D DIMENSION		**< High school**		**High school**		**> High school**		**p value**
		n	**%**	**n**	**%**	**n**	**%**	
Mobility	No problems	12	13.8	7	8.0	1	1.1	0.834
	Problems	40	46.0	21	24.1	6	6.9	
Self-care	No problems	16	18.4	7	8.0	2	2.3	0.862
	Problems	36	41.4	21	24.1	5	5.7	
Usual activities	No problems	3	3.4	2	2.3	0	0	0.768
	Problems	49	56.3	26	29.9	7	8.0	
Pain/Discomfort	No problems	10	11.5	5	5.7	2	2.3	0.812
	Problems	42	48.3	23	26.4	5	5.7	
Anxiety/Depression	No problems	9	10.3	7	8.0	2	2.3	0.624
	Problems	43	49.4	21	24.1	5	5.7	
VAS Score (mean ± SD)		55.29 ± 18.98		60.54 ± 17.97		64.29 ± 16.18		0.301

There was no significant difference in the QoL of patients with dementia compared to the educational level.

3.6. Association between quality of life (EQ-5D) and marital status

Table 6. Responses by marital status (n=87)

EQ-5D DIMENSION		**Married**		**Widow**		**p value**
		n	**%**	**n**	**%**	
Mobility	No problems	12	13.8	8	9.2	0.059
	Problems	54	62.1	13	14.9	
Self-care	No problems	14	16.1	11	12.6	**0.006**
	Problems	52	59.8	10	11.5	
Usual activities	No problems	3	3.4	2	2.3	0.393
	Problems	63	72.4	19	21.8	
Pain/Discomfort	No problems	12	13.8	5	5.7	0.571
	Problems	54	62.1	16	18.4	
Anxiety/Depression	No problems	12	13.8	6	6.9	0.306
	Problems	54	62.1	15	17.2	
VAS Score (mean ± SD)		55.68 ± 17.67		64.05 ± 20.10		0.071

Widow respondents perceived a better QoL compared to married people, especially in the self-care dimension.

3.7. Association between quality of life (EQ-5D) and living status and living area

Table 7. Responses by living status and living area (n=87)

Variable		Quality of life								p value
		poor		average		high		Very high		
		n	%	n	%	n	%	n	%	
Living area	Urban	1	1.1	23	26.4	30	34.5	13	14.9	0.562
	Rural	1	1.1	6	6.9	7	8.0	6	6.9	
Living status	Family	2	2.3	26	29.9	33	37.9	17	19.5	0.665
	Care giver	0	0	2	2.3	0	0	1	1.1	
	Alone	2	2.3	1	1.1	4	4.6	1	1.1	

There was no significant difference in the QoL of patients with dementia compared to the living area and living status.

Table 8. Association between depression and quality of life in dementia patients

Depression		Quality of life				p value
		Poor	Average	High	Very high	
PHQ-9	Mild depression	0 0.0%	11 12.6%	24 27.6%	14 16.1%	**0.005**
	Moderate depression	2 2.3%	8 9.2%	11 12.6%	3 3.4%	
	Severe depression	0 0.0%	10 11.5%	2 2.3%	2 2.3%	

PHQ-9 scale shown that the number of patients with mild depression, moderate depression and severe depression was 0 (0%), 2 (2.3%) and 0 (0%) respectively in poor quality of life. Besides, in average quality of life patients, had 11 people (12.6%), 8 people (9.2%) and 10 persons (11.5%) were mild depression, moderate depression and severe depression respectively. A 27.6% of participants (n=24/87) with mild depression, a 12.6% of ones (n=11/87) with moderate depression and a 2.3% of ones (n=2) with severe depression, had a high quality of life. Finally, in a very high quality of life, the respondents had mild depression, moderate depression and severe depression were 14 people (16.1%), 3 people (3.4%) and 2 persons (2.3%) respondents. Thus, there was association between depression and quality of life. This association was statistic significant (p=0.005).

4. DISCUSSION

The study was conducted at the National Geriatric Hospital in patients 60 years old and above. People aged between 60 and 69 accounted for 20.7% of participants. The greatest proportion of participants, 41.4% was people in the 70-79 age group, and there was 37.9% of the total number of the sample in the oldest age group, 80 years old and above. The mean age of respondents was 76.84 years old. This result was lower to mean age in the study of Juanita Hoe et al: 85.8 years [5]. It also lower than others: study of S. Miguel et al (83.2 years old) [6] and study in US (83.7 years old) [7]. The reason may be explained by sample size. 65.5% of participants were females, males represented 34.5% of total number of subjects. The female/male ratio was 1.90. This distribution was similar to a previous study conducted in Spain: 70% of the patients were woman [6]. The study of Barca M.L et al also showed 34% of respondents was male and 66% was female [8]. Another study in UK reported the majority were female (79.2%) [5].

The percentage of patients completed secondary school and below was 59.8%, and highly education patients made up 40.2%. It shows that people with higher education tend to have lower rate of dementia. It is similar to the statement in the study of T. Ngandu et al [9].

We showed that the dementia patients used the “extreme problems” option, with response rates ranging from 1.1% to 18.4%, with highest rate for usual activities, self-care (10.3%), mobility (3.4%) and lowest rate for pain and anxiety. At “severe problems” selection, the participants reported with highest rate for usual activities (31.0%), lowest rate for anxiety/depression (8.0%); mobility, self-care and pain/discomfort were respectively 19.5%, 18.4% and 10.3%. At “moderate problems” selection, the patients reported with mobility, self-care, usual activities, pain/discomfort and anxiety/depression were 29.9%, 17.2%, 20.7%, 29.9% and 18.4% respectively. A 24.1% all participants had slight problems with mobility, 25.3% had ones with self-care, 24.1% with usual activities, 39.1% with pain/discomfort, and 51.7% of patients had anxiety/depression. The mean score of HRQoL was 17.03 ± 4.00. It showed that health-realted quality of life in dementia patients in our study was high.

According to the research of P. Hancock et al, the mean of PHQ-9 in demented patients was 4.1 (SD=5.4) (n=49) [10]. In our study, that number was 9.64 (SD=4.79), much higher than P. Hancock’s study. The reason is the sample in this study is different from P. Hancock’s research (87>49). In other study, depression had a significant effect on the HRQoL in dementia patients [11]. Our study also showed that level of depression was associated with HRQoL ($p<0.05$). To be more specific, dementia patients with mild depression had better quality of life than those with moderate depression and severe depression. This finding were also defined in Maria Crespo’s research [12].

The study has some strength and limitation. The strengthen was that the questionnaires used were high internal consistency and a reliability coefficient. We used this questionnaire by interviewing the participants face-to-face instead of telephone interview. All of the clinical disorders were obtained and verified from medical records rather than from self-reported simple questions. Moreover, the data collected was reliable and well controlled. Besides, the sample size was rather small (n=87) due to the impact of the Covid 19 pandemic, it was difficult to access and collect data. Therefore, the results can be generalized only to the study area due to the power of the sample size calculation. Second, HRQoL was measured by self-reporting only and no objective measures, such as a polysomnography test, were included. Thirdly, the cross-sectional study design limits the degree to which conclusions can be drawn regarding the causal relationship between the factors analyzed in this study and HRQOL among the dementia patients.

5. CONCLUSION

After studying on 87 dementia patients, we noticed that the health-related quality of life among older people was quite high. Routine health check-ups should be recommended for elderly with dementia to improve their health-related quality of life

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THE PREVALENCE OF ANTIPHOSPHOLIPID ANTIBODIES AND THEIR CORRELATION WITH CLINICAL, LABORATORY FINDINGS IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

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ABSTRACT

Objective. *To evaluate the prevalence of antiphospholipid antibodies (aPL Abs) in systemic lupus erythematosus (SLE) patients and the relationship between these Abs with clinical and laboratory findings.* ***Methods.*** *Cross-sectional descriptive study on 60 patients newly diagnosed with SLE according to SLICC 2012 criteria, at the Center for Allergy - Clinical Immunology, Bach Mai Hospital from October 2022 to August 2023. The aPL group were defined as positive for at least one aPL. The clinical manifestation and laboratory findings of the positive and negative aPL groups were compared.* ***Results****. 37/60 patients (61.67 %) had positive for at least one aPL Abs. The percentage of groups positive for single antibody aCL, LAC, β2GPI, aPL, double and triple antibodies are 46.67%, 30%, 20%, 11.67%, 35%, 18.33%, respectively. We found that positive aPL and LA Abs significantly correlated with chronic kidney disease as compare with negative group. Unfortunately, no significance was found in hematology, neurology, thromboembolism, obstetric pathology, or livedo manifestations between the two groups.* ***Conclusion.*** *61.7% newly SLE diagnosis was positive for at least one aPL Abs and the presence of these Abs was independently associated with the risk chronic kidney disease in SLE patients.*

Keywords: *systemic lupus erythematosus, antiphospholipid syndrome, lupus anticoagulation, anti-cardiolipin antibody, anti-beta2-glycoprotein-I antibody*

1. INTRODUCTION

Systemic lupus erythematosus (SLE) is the most common systemic autoimmune diseases characterized by the presence of several autoantibodies (Abs) and organ damage related to immune complex-mediated microvascular injuries. Clinical features are variable from mild joint and skin involvement to life-threatening renal, hematologic, and/or central nervous system manifestations.[1] SLE patients have been shown to have an increased risk of blood clotting formation, venous and arterial thromboembolism, and high risk of fetus loss.[2] Several factors that have been demonstrated to increase these risks in SLE patients such as immune complex-related vascular damage, the presence of lupus anticoagulants (LA) and antiphospholipid antibodies (aPL Abs), and acquired or inherited thrombophilic defects as well as placental dysfunction.[2-4]

Antiphospholipid syndrome (APS) is mainly classified into primary and secondary; in which secondary APS is often closely related to autoimmune diseases, the most common is systemic lupus erythematosus.[5] Clinical manifestations of APS are veins, arteries, and small blood vessels thrombosis as well as obstetric events such as gestational gestation, recurrent early miscarriages, intrauterine growth restriction, or severe preeclampsia.[5,6]

aPL Abs are a heterogenous group of autoantibodies, including LA, anticardiolipin antibodies (aCL), and/or anti-beta2-glycoprotein-I antibodies (β2GPI).[7] Although up to 40% of patients with SLE have positive for at least one aPL Ab, however, less than 40% will eventually experience thrombotic events.[7] APS has been demonstrated to develop in 50%~ 70% of patients with both SLE and positive for at least one aPL Abs after 20 years of follow up. Moreover, positive aPL Abs have been associated with the risk of mortality and visceral organs damage, such as thrombocytopenia, valvular heart disease and/or neuropsychiatric manifestations.[8-10]

In Vietnam, the clinical and laboratory findings of APS in SLE were reported. aCL positive is commonly found in SLE patients.[11,12] However, the aPL antibody profiles in newly SLE diagnosis have not been shown. Therefore, we conducted this research with two goals:

1. To clarify the prevalence of aPL antibodies in newly SLE diagnosed.
2. To evaluate the relationship between aPL antibody profiling with clinical and laboratory findings of these patients.

2. MATERIALS AND METHODS

1. Study population

This was a cross-sectional enrolled 60 newly SLE patients at the Center for Allergy - Clinical Immunology, Bach Mai Hospital from October 2022 to August 2023. SLE patients were diagnosed according to the criteria of Systemic Lupus International Collaborating Clinics (SLICC) with ≥ 4 criteria (at least 1 clinical criterion and 1 laboratory criteria) or LN proven on biopsy accompanied by the appearance of antinuclear antibodies (ANA) or anti-double-stranded DNA antibodies (anti-DsDNA). The patients who did not consent to participate or concomitant with other autoimmune diseases such as mixed connective tissue disease, scleroderma, rheumatoid arthritis, Sjogren's syndrome, primary APS, pregnant or taking any anticoagulants, were excluded from the study. The participant characteristics was shown in Table 1.

Table 1. General characteristics of the study subjects

Characteristics (n=60)	N	Mean/%
Age	60	41.82 ± 17.59 (years)
Female	52	86.7
Fever	41	68.3
Hair loss	14	23.3
Malar rash	22	36.7
Vasculitis	9	15
Oral ulcer	5	8.3
Arthritis	21	35
Serositis	34	56.7
Renal disorder	34	56.7
Neurologic disorder	10	18.3
Hemolytic disorder	51	85
Positive anti-ANA	57	95
Positive anti-dsDNA	60	100
SLEDAI – 2K	52	12.46 ± 5.98

2. Sampling data

Variables were collected according to the medical record form with biometric variables (age, gender, height, weight); symptoms and signs in SLE (butterfly rash, discoid rash, photosensitivity, oropharyngeal ulcers, hair loss, seizures, membrane effusion, SLEDAI index), kidney damage. Antibody tests were performed at the Center for Allergy and Clinical Immunology laboratory, Bach Mai Hospital, using ELISA Sandwich technique. In another hand, lupus anticoagulation test was performed on the ACLTOP 700 machine at the Center for Hematology and Blood Transfusion, Bach Mai Hospital.

3. Statistical analysis

Collect and process data using SPSS software version 20.0. Quantitative data were expressed as mean ± standard deviation. The threshold of statistical significance is determined when the index $p < 0.05$.

4. Research ethics

Research subjects were informed and voluntarily participated in our study. The information collected will be kept confidential and provided for research purposes. The study was approved by the Medical Ethics Committee of the Hanoi Medical University.

3. RESULTS

1. Characteristics of APS in research subjects

We firstly evaluated the clinical manifestations of APS in study subjects. We found that only 3 patients had history of thrombosis events (1 venous and 2 arterial) and 1 patient had ≥ 1 unexplained fetal death ≥ 10^{th} week of gestation. Regarding antibody profiling, LA positive was found in 30% patients; 61.7%, 35%, 18.33% patient positive for single, double and triple antibodies, respectively. The highest prevalence of positive antibody was 35.6% of aCL-IgM (Table 2 and Figure 1).

Table 2. Clinical manifestations and laboratory findings of APS

Clinical findings	**N**	**%**
Thrombosis	3	5
Venous thromboembolism	1	1.7
Arterial thromboembolism	2	3.3
Pregnancy morbidity	1	1.7
≥ 1 unexplained fetal death ≥ 10^{th} week of gestation	1	1.7
≥ 1 premature birth <34^{th} week of gestation because of:		
• *Eclampsia or severe pre-eclampsia*	0	0
• *Features of placental insufficiency*		
≥ 3 unexplained consecutive abortions < 10th week of gestation	0	0
Laboratory findings		
Single positive	37	61.7
Double positive	21	35
Triple positive	11	18.33
LA (+)	18	30

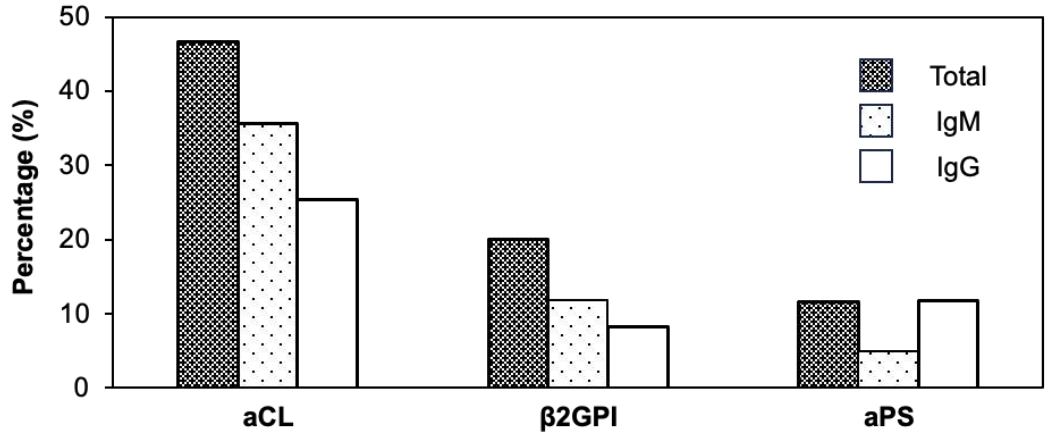

Figure 1. Antibody profiling of APS in study subjects

2. Relationship between antiphospholipid antibodies and some clinical and laboratory characteristics in SLE pateints

To evaluate the relationship between APS antibody profiling and other clinical and laboratory findings, the positive and negative group of each antibody were compared. Interestingly, patients with LA or any APS antibody positive were at high risk of having stage III to V as compared with negative group. We also found that the aPL (+) group showed a significantly higher number of malar rash as compared with aPL (-). Moreover, aCL-IgG (+) group had lower C3, but not C4 concentration than in negative group. There was no difference in pulmonary hypertension, thrombocytopenia, hemolytic anemia, arterial hypertension, 24-hour proteinuria between any positive and negative group (Table 3).

Table 3. Relation between vascular comorbidities, renal manifestations, and obstetric complications and aPL abs

Variable	Any aPL	LA	aCL -IgM	aCL -IgG	aβ2GPI-IgM	aβ2GPI-IgG	aPL -IgM	aPL -IgG
Pulmonary hypertension	ns	ns	ns	ns	ns	ns	Ns	Ns
Thrombocytopenia	ns	ns	ns	ns	ns	ns	Ns	Ns
Hemolytic anemia	ns	ns	ns	ns	ns	ns	Ns	Ns
Arterial hypertension	ns	ns	ns	ns	ns	ns	Ns	Ns
Chronic kidney disease, stage ≥ 3	0.03	0.03	ns	ns	ns	ns	Ns	Ns
24-hour proteinuria	ns	ns	ns	ns	ns	ns	Ns	Ns
Malar rash	0.012	ns	ns	ns	ns	ns	Ns	Ns
C3	ns	ns	ns	0.00	ns	ns	ns	Ns
C4	ns	ns	ns	ns	ns	ns	ns	Ns
SLEDAI	ns	ns	ns	ns	ns	ns	ns	Ns

4. DISCUSSION

In our study, we firstly estimated the prevalence of APS antibody profiling in newly SLE diagnosis in Vietnam. 61.7% SLE patients have positive for at least single APS antibody. Among that aCL was the most commonly found in our study subjects. Moreover, the relationship between these antibodies and the clinical, laboratory findings were also shown. Similar to epidemiological studies, SLE is commonly found in women and mainly in reproductive age, accounted for 86.7% of cases with an average age of 41.82 ± *17.59* years in our study. This may be explained by the important role of sex hormones, especially estrogen in the pathogenesis of the disease of SLE. Estrogen is reported to enhance CD8 levels, increase CD4 cytolytic activity, and the production of interferon-γ and interleukin-10 from helper T cells (Th)1 and Th2.[13] Kidney involvement is the most common clinical manifestation and mostly present with exacerbations with an average SLEDAI score of 12.46, which is explained by the fact that we only enrolled inpatients, but not outpatients.

In SLE, 30%-40% of patients are positive for aPL; when each aPL is investigated individually, the prevalence of a positive LA test and aCL varies between 11%-30% and 17%-40%, respectively.[14-17] In our study, the prevalence of patients positive for at least single antibody was 61.67%. The higher prevalence of positive APS antibodies might due to our study sampling design. Similarity, aCL positive were the most commonly found in APS antibody profiling comparable to other study in Vietnamese and worldwide population.[11,12,18] The relationship of APS antibody profiling with clinical manifestations and laboratory findings were also reported. Studies showed that aPL-positive has much higher prevalence of thrombocytopenia, autoimmune hemolytic anemia, arterial and/or venous thrombosis, pulmonary hypertension than aPL-negative SLE patients.[19-22] Unfortunately, we found no difference between these 2 groups in pulmonary hypertension, thrombocytopenia, hemolytic anemia, arterial hypertension, 24-hour proteinuria. This can be explained due to the sampling size and design. Interestingly, LA or any APS antibody positive were at high risk of having stage III to V as compared with negative group in our research. That might indicate that the presence of these antibodies would contribute to renal diseases in SLE patients. However, we could not explain whether the stage III to V due to lupus nephritis or APS nephropathy. APS nephropathy is a separate renal disease entity with different underlying pathogenic mechanisms compared to LN, the prevalence of APS nephropathy in SLE patients was reported as 32% and 23.2% in two studies.[23,24] According to a recently reported study encompassing a prospective cohort of 64 biopsy-proven active LN patients without concomitant APS nephropathy and cross-sectional analysis of 498 SLE patients with or without LN, both aPL positivity and level were similar in patients with active LN and non-renal SLE. However, IgG aCL or IgG aβ 2GPI positive patients had higher creatinine levels compared with patients without those IgG aPL both at active LN and after induction therapy, but not in the long-term data analyses. The authors concluded that aPL are not associated with occurrence of LN, and IgG aPL might contribute to an impaired renal function during a LN flare in the absence of APS nephropathy, probably due to their own pathogenic roles.[24] We suggest that aPL antibodies act as an independent factor associated with

increased the risk of chronic kidney disease.

CONCLUSION

Our study showed that aPL antibodies positive in 61.67% patients suffered from newly SLE diagnosis at the Center for Clinical Allergy and Immunology, Bach Mai Hospital. Among these, aCL antibody is the most common when identified in the blood of our research subjects. Positive aPL antibodies was independently associated with risk chronic kidney disease in SLE patients.

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Collagen10a1 GEN EXPRESSION AND BONE MINERALIZATION IN THE *col10a1*:nlGFP TRANSGENTIC MEDAKA FISH LARVAE

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ABSTRACT

Objective*: To assess the expression level of the osteoblast-specific collagen10a1 gene and the degree of bone mineralization during the early larval stages of col10a1:nlGFP transgenic medaka fish to identify a specific developmental time window suitable for conducting bone anabolic effect evaluations of bioactive substances.* ***Methods:*** *Homozygous and hemizygous col10a1:nlGFP fish larval groups at 7, 9, 10, 11, and 14 days post fertilization (dpf) were used in the study (n=…/group). The fish were subjected to live staining with alizarin red S (ARS) dye to visualize their mineralized bone structures. Fluorescent images were captured using a fluorescent stereoscope to document both the GFP and ARS signals in the fish. The density of the GFP signal was utilized to assess the expression level of the collagen10a1 gene in osteoblasts, while the density of the ARS signal was used to evaluate the extent of bone mineralization. ImageJ software was employed for this analysis.* ***Results****: The intensity of GFP signal exhibited an upward trend as the fish grew from 7 to 9 dpf, followed by a gradual decline from 9 to 14 dpf. Along with that, the intensity of ARS signal increased as the fish grew from 7 to 10 dpf, followed by a gradual decrease from 10 to 14 dpf. These findings indicate that the optimal developmental window for conducting tests on the fish with bioactive compounds, utilizing a stereo fluorescence microscope, is between 7 and 9 dpf.*

Key words: *medaka, transgenics, col10a1, osteoblasts, mineralization, GFP.*

1. INTRODUCTION

In vivo studies conducted on bone and bone disease typically utilize mammalian models such as mice, rabbits, dogs, and primates [1]. However, more recently, the use of medaka fish *(Oryzias latipes)* has gained momentum and has emerged as a promising alternative to mammalian models [4, 5]. Medaka fish exhibit bone metabolism mechanisms at both the molecular and cellular levels with striking similarities to those found in humans [4, 5]. Moreover, the fish offers various advantages as laboratory animals, including ease of care, prolific egg production, rapid growth period, external fertilization, transparent embryos, and the ability to perform techniques and observe bone cell activity in live fish in real time [4, 5].

In 2013, researchers at the National University of Singapore generated the transgenic medaka fish using the *col10a1*:nlGFP construct. This construct enabled the expression of the *colagen10a1 (col10a1)* gene through the production of green fluorescent protein (GFP) in osteoblasts, the bone forming cells [3]. The transgene construct consisted of a 5.865 kb promoter region from the medaka *col10a1* gene, followed by the GFP coding sequence and a nuclear localization signal (nlGFP). The construct was inserted into the medaka genome using the meganuclease transgenic technique. It was observed that the *col10a1* gene is expressed early during the differentiation of osteoblastic cells in the mineralized bone structures of the fish. It appears both before and during the mineralization of the vertebral bodies, thus suggesting that it could serve as an indicator for bone formation in fish [6]. Osteogenic cells appear as individually spaced cells located on the outer surface of the notochord and

mineralized structures. Therefore, the *col10a1*:nlGFP transgenic medaka fish model is used in studies on bone formation and osteoblasts in relation to bone diseases and to evaluate the bone protective and bone anabolic effects of bioactive compounds for drug development [2].

The *col10a1*:nlGFP fish has been raised and maintained in our laboratory since 2013. Recently, our group has examined and identified the stability of the *col10a1* transgene of the fish and test crossed them to obtain homozygous and hemizygous fish with the transgene [6]. Given that investigations examining the impacts of substances on bone formation are commonly carried out on fish larvae, which refers to the period from post-hatching (around 7 days) to approximately two weeks of age, our study aimed to determine the optimal time frame during larval development for evaluating the effects of substances. To this end, we measured the level of expression of the *col10a1* gene via the density of GFP signal and assessed the level of bone mineralization through ARS signal of the fish at various time points during the early larval stages, seeking to identify the developmental time window suitable for conducting bone anabolic effect evaluations of bioactive substances.

2. MATERIAL AND METHODS

2.1. Fish lines and fish maintenance

In this study, homozygous and heterozygous *col10a1*:nlGFP were used [6]. Fish were maintained following established procedures [2] with temperature set at 28ºC using an air conditioner and LED lighting cycles of 14-h light-10-h dark. Fish embryos were raised in E3 medium as previously reported [2].

2.2. Fish crossing and embryo collection

Male and female fish are kept together in the same tank with a ratio of 1 male: 3 females. After the fish spawns, embryos were collected into a petri dish. Wash the embryos with 1X E3 medium with a little methylene blue added to avoid mold growth, unfertilized and dead embryos were removed.

2.3. Live bone staining with Alizarin red S and fluorescent imaging

Fish were stained with ARS (0.03%, 3 hours) in a 24-well plate at a density of 5 fish per well. After staining, the fish was washed with E3 water, left to rest for 1 hour and then taken for imaging. Before taking photos, fish were anesthetized with tricaine (0.03%) and mounted in a 35mm glass-bottomed petri dish with low melting agarose (0.15%) [4, 6].

col10a1:nlGFP expression and ARS stained mineralized bone in fish larvae were imaged by a fluorescent stereoscope (STEREO Discovery V8) using GFP and RFP filter settings, respectively. Images were captured at 8X magnification using BUC5F-2000C Microscope Digital Camera (Bestcope) and analyzed using ImageJ software [6].

2.5. Statistical analysis

One-way Anova was used to evaluate differences in GFP density as well as ARS density between fish groups. The level of significance was set as follows: *0.01 < p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. Graphs were created using Graphpad Prism 9 software.

3. RESULTS

3.1. Osteoblastic GFP intensity in *col10a1*:nlGFP homozygous and hemizygous fish at 7, 9, 10, 11, 14 days of age

Figure 1 shows representative fluorescent images taken for *col10a1*:nlGFP homozygous (+/+) and hemizygous (+/-) fish of 7, 9, 10, 11, 14 dpf. GFP signal was observed in bone

structures of the head, the vertebral column and the tails of both (+/+) and (+/-) tested fish. The vertebral column consists of vertebrae, each when fully developed has a vertebral body and a neural arch and a hemal arch. In 7dpf fish, GFP was seen only in vertebral bodies and neural arches of some anterior vertebrae, while the signal was present in these bone structures of all vertebrae in 14dpf fish.

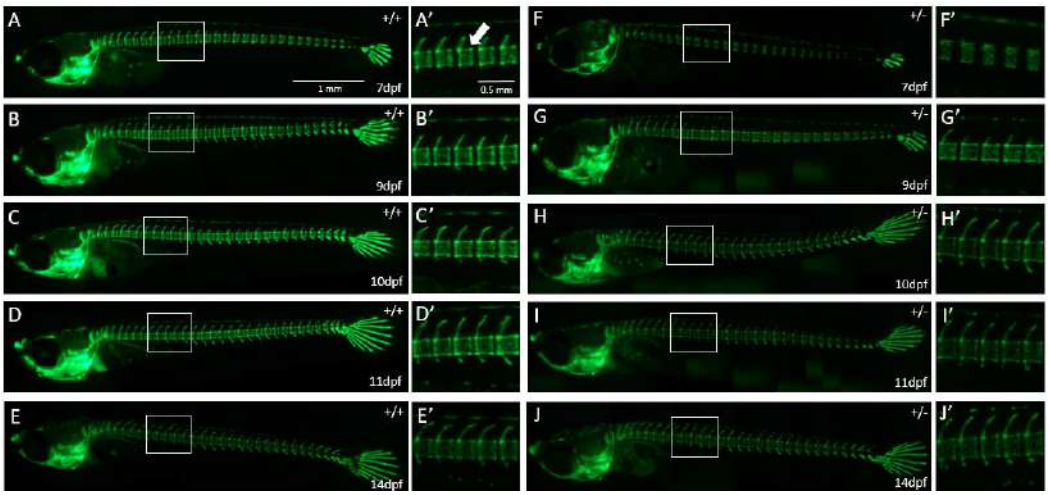

Figure 1. GFP signals of homozygous and hemizygous col10a1:nl GFP fish larvae at 7, 9, 10, 11, 14 dpf.

Fluorescent images of homozygous (+/+, A-E) and heterozygous (+/-, F-J) fish at days of age indicated by dpf; A'-E'; F'-J': Magnified images of five vertebrae (8-12) boxed in A-E; F-J, respectively.

Intensity of GFP signal of five vertebrae (vertebrae 8-12; boxed in Figure 1 A-E, A'-E' for homozygous fish; F-J, F'-J' for hemizygous fish) (n=7/group) was measured and chosen as representative for the expression of *col10a1* gene of each fish. Statistical analysis of GFP intensity of all tested fish groups (n=7/group) is shown in Figure 2. It reveals that GFP signal intensity of homozygous fish is significantly higher than that of hemizygous fish of the same age and in both homozygous and hemizygous fish, GFP signal intensity increased when the fish grew from 7 to 9dpf ($p < 0.0001$ for homozygous and $p < 0.001$ for hemizygous fish) and then gradually decreased when the fish grew older to 10, 11, and 14dpf.

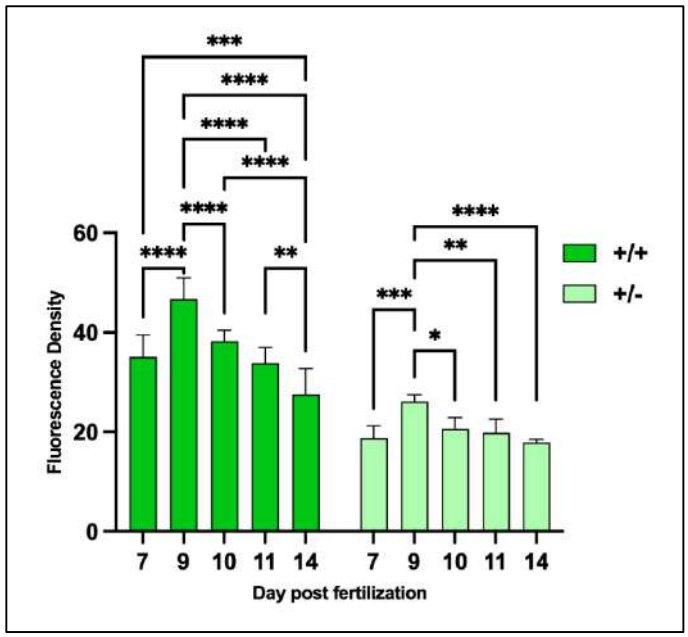

Figure 2. *Average GFP intensity of homozygous (+/+) and hemizygous (+/-) fish groups at 7, 9, 10, 11, 14 dpf (n=7/group).*

**0.01 < p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001.*

3.2. Bone mineralization level of homozygous and hemizygous fish at 7, 9, 10, 11, 14 days of age

To evaluate the level of bone mineralization, we stained live fish larvae with the fluorescent dye alirazin red S (ARS). Representative fluorescent images taken for ARS signal of *col10a1*:nlGFP homozygous (+/+) and hemizygous (+/-) fish groups of 7, 9, 10, 11, 14 dpf are presented in **Figure 3.**

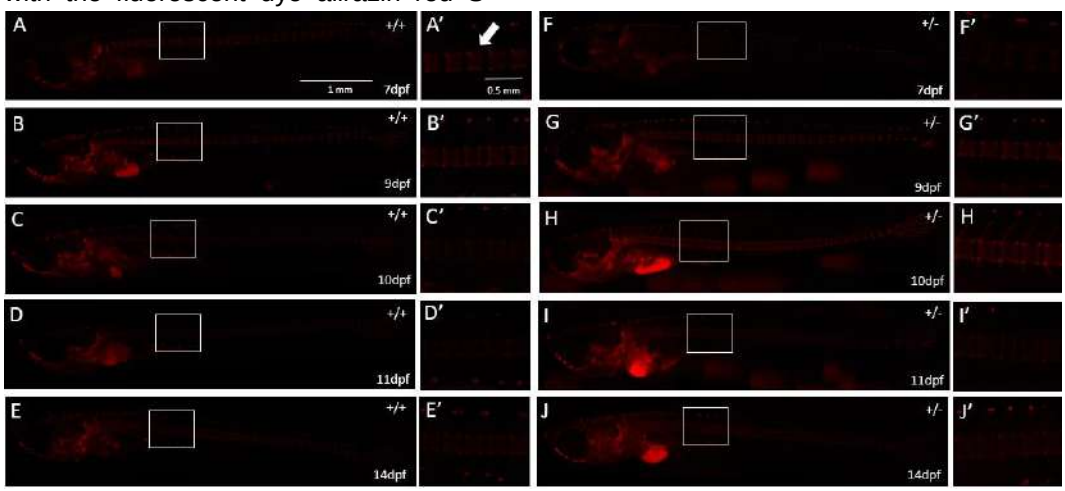

Figure 3: ARS fluorescence signals of homozygous and hemizygous col10a1:nl GFP fish larvae at 7, 9, 10, 11, 14 dpf.

Fluorescent images for ARS signal of homozygous (+/+, A-E) and heterozygous (+/-, F-J) fish at days of age indicated by dpf; A'-E'; F'-J': Magnified images of five vertebrae (8-12) boxed in A-E; F-J, respectively.

As expected, ARS signal was seen in mineralized bone structures in the head, the vertebral column and the tail of

fish of all tested groups. The ARS intensity seemed to correspond to the level of bone development in fish larvae (**Figure 3**).

We also chose to measure ARS signal intensity of 5 vertebrae (vertebrae 8-12) as representative for the level of mineralization of each fish. Average ARS density of fish groups (n=7/group) and statistical analysis are presented in **Figure 4**.

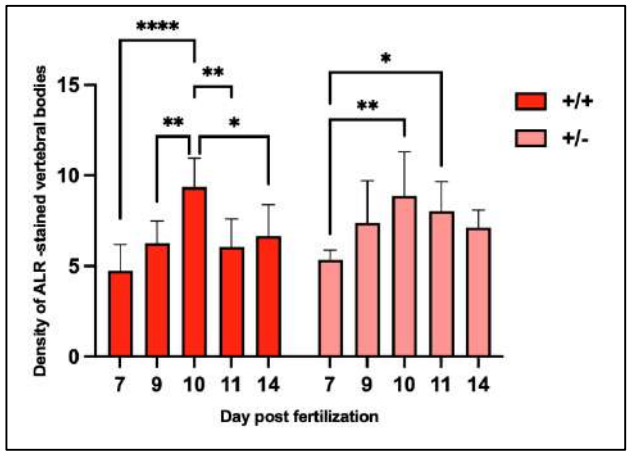

Figure 4. Average ARS signal level of homozygous (+/+) and hemizygous (+/-) fish groups at 7, 9, 10, 11, 14 days of age (n=7/group)

**0.01 < p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001.*

The results show that the level of ARS signal increased as fish grew from 7 to 10 dpf ($p < 0.0001$ for homozygous fish and $p < 0.01$ in hemizygous fish), followed by a decreasing trend when the fish grew older to 11 and 14 dpf. When comparing the ARS density between homozygous and hemizygous fish of the same age, there is no significant difference.

4. DISCUSSION

In this study, we evaluated the level of *col10a1* expression in osteoblasts and level of bone mineralization via the intensity of GFP and ARS signals, respectively, of both homozygous and hemizygous *col10a1*:nlGFP transgenic medaka at larval stages of 7, 9, 10, 11, 14 dpf.

GFP signal was observed in the fish as previously described [3, 6], indicating the stability of the *col10a1*:nlGFP transgene in the fish.

Quantification of GFP and ARS intensity of the representative vertebrae of the fish groups indicates that the GFP intensity increased when the fish developed from 7 to 9 days of age and then decreased in the following days as the fish gets older. Along with that, the ARS intensity also increased in fish during the period of 7 - 10 days of age and decreased as the fish gets older.

The decrease in GFP intensity in fish 10 days old or older can be explained by the fact that GFP signal is the reporter for the expression of *collagen10a1,* which is expressed in osteoblast precursors, the early stage of osteoblast differentiation, so its expression decreases as osteoblasts become more differentiated and the fish gets older [3]. However, it is possible that this result is due to technical limitations when using a fluorescence stereoscope to image the fish. The fish grew and became thicker, which made it difficult for the fluorescence stereoscope to capture the GFP signal from deep within the vertebrae. The limitation of the imaging technique is a likely explanation for this decrease in GFP intensity, as the

measured ARS density also showed a similar decrease in fish older than 10 days old. This seems unreasonable, as ARS density represents the level of mineralization of the fish and bone mineralization should increase during larval growth. A confocal microscope using a laser light to scan deep tissue and provide high-resolution images may help to clarify this issue [4]. However, fluorescence stereoscope was the only way to image live fish larvae in our laboratory. Therefore, our results suggest to assess intensity of GFP and ARS signal of the *col10a1*:nlGFP larvae from 7-9 dpf to evaluate bone anabolic effect of bioactive substances when using a fluorescence stereoscope.

5. CONCLUSION

Osteoblast-specific *collagen10a1* expression increases in the *col10a1*:nlGFP fish larvae as the fish grow from 7 to 9 days post-fertilization (dpf), and the mineralization level of the fish increases as they grow from 7 to 10 dpf. The bone anabolic effect of bioactive substances can be tested on the fish during the period of 7 to 9 dpf using a fluorescence stereoscope.

Acknowledgments

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EVALUATING THE EFFECT OF PRODUCTS EXTRACTED FROM PANAX BIPINNATIFIDUS SEEM ON MULTIPLE MYELOMA CELLS

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ABSTRACT

Panax bipinnatifidus Seem (PB) is a precious medicinal plant in Vietnam. Although known for its many biological activities, the impact of this herb on multiple myeloma has not been elucidated. ***Objective:*** *The study was conducted to evaluate the effects of total saponin extract from PB on the viability, reactive oxygen species (ROS), and mitochondrial cardiolipin content of multiple myeloma cells.* ***Methods:*** *Human myeloma cell line (KMS-20) cells were divided into 2 groups: control group and experimental group. In the control group, cells were grown in medium containing DMSO 0.05%. In the experimental group, cells were grown in medium containing total saponin extract (PB-TS) at different concentration ranges. Taxol is an anti-cancer drug used as a positive control in the study. Cell viability, ROS, and cardiolipin content were determined using suitable kits.* ***Results:*** *IC50 values of PB-TS for KMS-20 cells were 4.43±0.78 (µg/ml), higher than the IC50 value of Taxol (2.88±0.51 ng/ml). Besides, the CM-H_2DCFDA fluorescence density of the cell group treated with Taxol and PB-TS increased to 121.52 ± 5.16% and 134.27 ± 3.59% compared to the control group ($p<0.05$). Moreover, the NAO fluorescence density of the cell group treated with Taxol and PB-TS also decreased to 62.73±6.87% and 41.56± 8.52%, respectively ($p<0.05$ compared with the control).* ***Conclusion:*** *The results of the study showed that total saponin from PB exhibited strong toxicity to KMS-20 cells through stimulation of ROS production and reduction of mitochondrial cardiolipin content.*

Keywords: *KMS-20 cells, Panax bipinnatifidus Seem, cardiolipin, ROS.*

1. INTRODUCTION

Multiple myeloma is an incurable cancer of mature B lymphocytes [8] and accounts for about 10% of all blood cancers [6]. The disease also accounts for about 1% of cancer deaths and nearly 20% of deaths from hematological malignancies [2]. Although the understanding of the disease and the discovery of novel therapies have resulted in a significant increase in overall survival time, almost all patients relapse and eventually succumb to their condition [4]. In fact, many studies have shown that mitochondria play an important role in the development and progression of multiple myeloma [6, 9]. Therefore, the search for mitochondrial-targeting drugs or/and natural compounds to inhibit the growth of cancer cells has paid attention to scientists.

In Vietnam, Panax bipinnatifidus Seem (PB, Vu Diep ginseng) is a precious herb with identified biological activities such as anti-aging, neurological disorders, enhancing immunity, and improving cardiovascular system activity [14]. However, in multiple myeloma subjects, the role of Vu Diep ginseng as well as products extracted from Vu Diep ginseng have not yet been studied. Therefore, in this study, we evaluated the effects of total saponin extract from Panax Bipinnatifidus Seem on the human multiple myeloma cell line (KMS-20) by evaluating the rate of viable cells, the changes in mitochondrial inner membrane cardiolipin content and the production of ROS free radicals.

2. METHODS

2.1. Object

The KMS-20 cell line (ATCC® - USA) was donated by the Center for

Cardiovascular Metabolic Disease Research, Inje University, Korea. Ethanol extract and SVD total saponin extract were provided by Phenikaa University, Vietnam.

2.2. Materials

RPMI 1640 (RPMI, Gibco, USA); Fetal bovine serum (FBS, Gibco, USA); Penicillin-Streptomycin (PS, Gibco, USA); Phosphate buffered saline (PBS, Gibco, USA); MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide; Sigma, USA); Cell Counting Kit-8 (CCK-8, Dojindo, Japan); Dimethyl sulfoxide (DMSO, Sigma, USA); 10-Nnonyl acridine orange (NAO, excitation/emission: 495/519 nm, Invitrogen, USA); 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA, 495/5169 nm, Invitrogen, USA); Culture dishes 90x20 mm (SPL, Korea); 96-well black, glass bottom plates (SPL, Korea); CO_2 Incubator (Shellab, USA); and Microplate reader (Tristar, USA). The study was carried out at the Animal Cell Biotechnology Laboratory, Life Science Research Center, Faculty of Biology, VNU University.

2.3. Methods

2.2.1. Cell cultured and treatments

KMS-20 cells were grown in RPMI medium containing 10% FBS, and 1% of PS at 37°C with 5% CO_2. The culture medium was changed every 2-3 days. For drug treatment, KMS-20 cells were further transferred to 96-well at the density of 2.10^4 cells per well and three cells per group at 37°C, 5% CO_2. After 24 h, the cells were further divided into three groups:

+ The control group (RPMI, control): KMS-20 cells were continuously cultured in RPMI medium plus DMSO 0.05% for 48 h;

+ The positive control drug: KMS-20 cells were continuously grown for 48 h in new media containing Taxol at the range of concentration from 0.1 to 30 ng/ml;

+ The PB-TS group: KMS-20 cells were continuously grown for 48 h in new media containing PB-TS at the range of concentration from 0.1 to 50 µg/ml.

2.2.2. Measurement of cell viability

Cell viability was determined using CCK-8. KMS-20 cells were seeded at a density of 2.10^4 cells per well in 96-well plates. After different treatments, CCk-8 was added to each well with a ratio of 10% v/v at 37°C for 1.5 h as our previous study [12]. The optical density (OD) was determined at 450 nm using a microplate reader (Spectramax plus 384, Molecular Devices, USA). The live cell number in each well (3 wells per group) was expressed as a value relative to the normal control well. Experiments were repeated three times.

2.2.3. Measurement of reactive oxygen species

Reactive oxygen species (ROS) were measured by CM-H_2CMDCFDA fluorescence kits following the previous reports [13]. KMS-20 cells were seeded at a density of 2.10^4 cells per well in 96-well black, glass bottom plates. After treatments, the cells were stained with 5 µM NAO (ex/em: 495/516 nm) at room temperature for 30 min. After treatments, the cells were stained with either 0.1 µM NAO (ex/em: 495/519 nm) at room temperature for 30 min. The cells were washed twice with PBS before measuring fluorescence intensity [10]. The CM-H_2CMDCFDA intensity in each well was expressed as a percentage value relative to the normal control. There were three wells per group for each repeat. Experiments were repeated three times.

2.2.4. Measurement of mitochondrial cardiolipin

The mitochondrial cardiolipin content was measured by NAO fluorescence kits following the previous reports [13]. KMS-20 cells were seeded at a density of 2.10^4 cells per well in 96-well black, glass bottom plates. After treatments, the cells were stained with either 0.1 µM NAO (ex/em: 495/519 nm) at room temperature for 30 min. The cells were washed twice with PBS before measuring fluorescence intensity [10]. The NAO intensity in each well was expressed as a percentage value relative to the normal control. There were three wells per group for each repeat. Experiments were repeated three times.

2.2.5. Statistical Analysis

Data are presented as means ± Standard deviation (SD) by using Excel 2016, Origin 8.5. Statistical significance was evaluated by one-way analysis of variance followed by T-test. P<0.05 was considered to be a statistically significant difference.

3. RESULTS AND DISCUSSION

3.1. The toxicity of PB-TS on KMS-20 cells

The effect of PB-TS at different concentrations on KMS-20 cells was evaluated through the change in cell survival by CCK-8 kit. The results are illustrated in Figure 1, and Table 1.

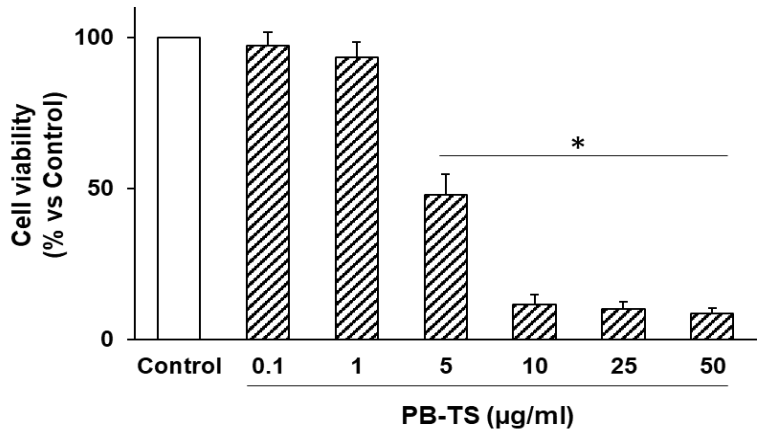

Figure 1. The cell viability of KMS-20 cells in medium supplemented with PB-TS

*Control: KMS-20 cells cultured in normal condition; PB-TS (tested compound): KMS-20 cells cultured in medium containing PB-TS at range of concentration from 0.1 to 50 µg/ml. *p<0.05 of Control*

Results from Fig.1 show the survival rate of cell groups supplemented with PB-TS at concentrations of 0.1 µg/ml (97.43±4.28%) and 1 µg/ml (93.31±5.27%) was not different from the Control group (p>0.05). However, this ratio in the group treated with PB-TS at concentration from 5 to 50 µg/ml decreased sharply to 46.21±7.04% at 5 µg/ml, and lowest at 8.06±1.63% at the dose of 50 µg/ml. The difference was statistically significant compared to the control group (p<0.05). This suggests that PB-TS exhibits strong cytotoxicity against KMS-20 cells. Based on this data, the IC50 value of PB-TS for KMS-20 cells was also determined and compared with the Taxol positive control. The results are shown in Table 1.

Table 1. IC50 value of PB-TS for KMS-20 cells

Compounds	IC50 value			
	1st	2nd	3rd	Mean ± SD
PB-TS (µg/ml)	4.80	3.53	4,97	4.43±0.78
Taxol (ng/ml)	2.95	2.34	3.26	2.88±0.51

The results from Table 1 show that the mean IC50 value of PB-TS for KMS-20 cells is 4.43±0.78 (µg/ml). This value is higher than the IC50 of Taxol (2.88±0.51 ng/ml), proving that PB-TS extract exhibits weaker toxicity to KMS-20 cells than Taxol. The results from Table 1 show that the mean IC50 value of PB-TS for KMS-20 cells is 149.49±1.98 (µg/ml). This value is higher than the IC50 of Taxol (0.89±0.14 µg/ml), proving that PB-TS extract exhibits weaker toxicity to KMS-20 cells than Taxol. However, this IC50 value shows that PB-TS has great potential in the treatment of multiple myeloma.

3.2. PB stimulates ROS production in KMS-20 cells

One of the pathways by which substances exhibit anticancer effects is by activating the apoptotic process in cancer cells, leading to cell death [7]. Many studies have shown that several signaling pathways promote cancer cell apoptosis by activating or inhibiting ROS production [5]. In this study, the impact of PB-TS and Taxol at IC50 value on ROS production was determined through the change in the CM-H_2DCFDA fluorescence signal. The results are presented in Fig 2.

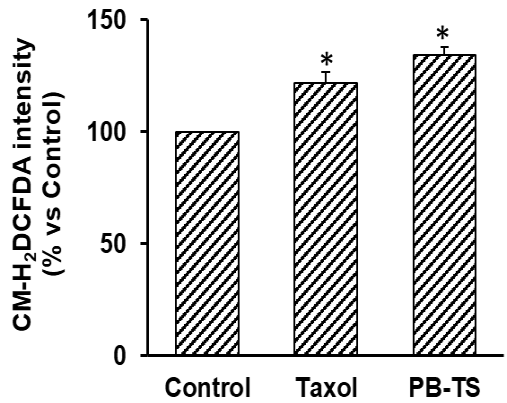

Figure 2. The CM-H_2DCFDA fluorescence intensity of KMS-20 cells under different conditions

*Control: KMS-20 cells cultured in normal condition; Taxol: KMS-20 cells cultured in medium containing Taxol 3 ng/ml; PB-TS: KMS-20 cells cultured in medium containing PB-TS 5 µg/ml; *p<0.05 of Control.*

Figure 2 shows that the CM-H_2DCFDA fluorescence density of reflecting ROS production of Taxol and PB-TS treated cells increased to 121.52 ± 5.17% and 134.27±3.59% compared with the Control group ($p<0.05$). This shows that, similar to Taxol, PB-TS stimulates ROS production in KMS-20 multiple myeloma cells. This result is consistent with the results on the percentage of living cells

above. Overproduction of ROS is responsible for the oxidation of macromolecules, which in turn activates a series of reactions that lead to cancer cell death [1].

3.3. PB-TS reduces the mitochondrial cardiolipin content of KMS-20 Cells

Cardiolipin is a phospholipid specific to the inner mitochondrial membrane, accounting for 13–15% of the total mitochondrial phospholipids. Depletion of cardiolipin content as well as structural changes lead to mitochondrial dysfunction. Cardiolipin is closely related to the opening of the inner mitochondrial membrane pore and leads to the release of cytochrome C, thereby stimulating apoptosis. Therefore, cardiolipin is the target of many studies to inhibit the growth of cancer cells [3]. In this study, the effects of PB-TS and Taxol on the mitochondrial cardiolipin content of KMS-20 cells were evaluated through changes in NAO fluorescence density. The results are shown in Figure 3.

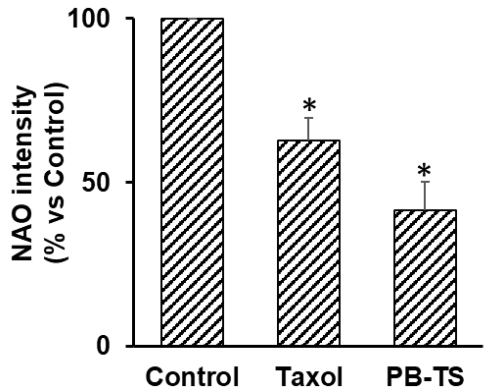

Figure 3. The NAO fluorescence intensity of KMS-20 cells under different conditions *Control: KMS-20 cells cultured in normal condition; Taxol: KMS-20 cells cultured in medium containing Taxol 3 ng/ml; P-TS: KMS-20 cells cultured in medium containing PB-TS 5 µg/ml; *p<0.05 of Control.*

The results in Figure 3 show that the KMS-20 cell group treated with Taxol and PB-TS had a fluorescence density of NAO reflecting a sharp decrease in the mitochondrial membrane cardiolipin content to 62.73±6.87 % and 41.56±8.52% ($p<0.05$ compared with Control). This result is consistent with the ROS content results above because stimulation of ROS production can cause increased cardiolipin oxidation, thereby reducing phospholipid content. A decrease in cardiolipin levels can lead to mitochondrial damage and cell death [15]. The effect of PB-TS is quite similar to the effect of Curcurmin and Naringin in the privious studies of Vu Thi Thu et al [11, 12]. In particular, Curcumin extracted from turmeric and Naringin extracted from citrus fruit peels have the ability to reduce mitochondrial membrane cardilipin, thereby limiting the growth ability of KMS-20 cells.

4. CONCLUSSION

The current findings indicate that PB-TS is potential to inhibit the growth of multiple myeloma cell by stimulating ROS production, and reducing cardiolipin levels. This might be the basis for further studies on the mechanism of action of PB-TS on multiple myeloma.

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ARALOSIDE A METHYL ESTER FROM PANAX BIPINNATIFIDUS SEEM PROTECTS H9C2 CARDIOMYOCYTES AGAINST CHEMICAL HYPOXIA/REOXYGENATION-INDUCED INJURY BY PRESERVING MITOCHONDRIAL FUNCTION

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ABSTRACT

Panax bipinnatifidus Seem (PB) is a precious herb used in many traditional remedies in Vietnam. Previous studies have proven that the saponin-rich extract of this herb has a protective effect on cardiomyocytes in hypoxia-reoxygenation injury (HR). However, which compound in PB plays a major role in this effect has not been elucidated. Therefore, this study was conducted to evaluate the effects of saponin extracted from PB extract, Araloside A Methyl Ester (AAME) on H9C2 cardiomyocytes in HR injury. Cells were grown in normal conditions (control) and HR-$CoCl_2$ conditions, and AAME was added to the culture medium during reoxygenation. Cell viability and some mitochondrial indices (cardiolipin content, mitochondrial membrane potential, ROS) were determined by suitable kits. The results showed that AAME at concentrations of 10 μM significantly reduced the cell death rate by limiting mitochondrial dysfunction (preserving cardiolipin content and mitochondrial membrane potential, reducing mitochondrial ROS) of H9C2 cells in HR-$CoCl_2$ injury ($p<0,05$). This result demonstrated the potential of AAME to protect cardiomyocytes from HR injury.

Keywords: *Araloside A Methyl Ester, mitochondria, H9C2.*

1. INTRODUCTION

Hypoxia-reoxygenation (HR) injury is damage caused by reperfusion /reoxygenation to tissue or cells that were ischemic/hypoxic previously. HR is a key mechanism responsible for cellular and tissue harm in various pathological scenarios, notably encompassing ischemic heart disease (IHD) [3].

As a powerhouse, mitochondria have been shown to play an important role in the mechanism of this disease [4]. Limiting mitochondrial dysfunction to minimize HR-induced injury has been the target of many studies [7]. In recent decades, research on screening and searching for natural active ingredients with cardioprotective effects has achieved many achievements, but clinical applications are still limited. Therefore, this research direction still needs to be further expanded.

Ginseng is a precious medicinal herb widely used in traditional medicine in many countries and Viet Nam. For ischemic diseases, the efficacy of ginseng and its ginsenosides on stroke outcomes with the anti-oxidant property has been described [6]. Also, ginseng extracts have been shown to possess beneficial effects in hypoxia/reoxygenation injury [1, 14]. In which, Panax bipinnatifidus Seem. has anti-platelet activity with its major saponin component, araloside A methyl ester [10, 11]. In our previous study, the total saponin extract of Panax bipinnatifidus Seem. in hab the ability to protect H9C2 cells in HR injury by modulating mitochondrial function during the hypoxia phase. However, which compound in PB plays a major role in this effect has not been elucidated. Therefore, this study was conducted to evaluate the effects of saponin extracted from PB extract, Araloside A Methyl Ester (AAME) on H9C2 cardiomyocytes in HR injury.

2. METHODOLOGY

2.1. Samples

The H9C2 cell line (ATCC©-USA) was provided by the Cardiovascular and Metabolic Disease Center, Inje University, Korea. AAME was provided by Phenikaa University. The research was conducted at the Animal cell biotechnology laboratory, Life science research center, Faculty of Biology, VNU University of Science.

2.2. Materials

Dulbecco's Modified Eagle Medium 4,5g/l glucose (DMEM, Gibco, USA); Phosphate buffered saline (PBS, Gibco, USA); Fetal bovine serum (FBS, Gibco, USA); Cell Counting Kit-8 (CCK-8, Dojindo, Japan); 2′,7′-dichlorodihydrofluorescein-diacetate (CM-H_2DCFDA; ex/em 485/525 nm, Invitrogen, USA); MitoSox Red (ex/em: 510/580 nm, Invitrogen, USA); Penicillin-Streptomycin (PS, Gibco, USA); Dimethyl Sulfoxide (DMSO, Sigma, USA); Cobalt chloride (CoCl2, Sigma, USA); Inverted microscopy Axiovert (S100, Carl Zeiss, Germany); Culture dishes 90x20, 60x15, 35x15 mm (SPL, Korea); 96-well black, glass bottom plates (CAT. 33196, SPL), confocal dishes (CAT. 100350, SPL); CO2 Incubator (Shellab, USA); ApoTome.2 Fluorescence Microscope (Zeiss, Germany); Microreader plate (Tristar2, USA).

2.3. Cell Culture

H9C2 cells were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% FBS and 100 U/mL penicillin, 100 µg/mL streptomycin at 37 °C, 5% CO_2. The culture medium was changed every 2-3 days.

2.4. Inducing hypoxia-reoxygenation by $CoCl_2$ and treatment

H9C2 cells were grown in a 96-well plate at a density of 5.10^3 cells/well at 37°C, 5% CO_2 for 24 h. Then, the cells were divided into 2 groups: In the normal control group, the H9C2 cells were continuously cultured in normal media (DMEM, 10% FBS, 1% PS, 37°C, and 5% CO_2) for 48 h. In the HR-$CoCl_2$ group, the cells were subjected to $CoCl_2$ 300 µM for 24 h. Then, the medium containing $CoCl_2$ was removed, and the cells were continued to be grown for 18-24 h in normal media containing NEC 10 µM or AAME at the range of concentrations (1-100 µM). At the end of the experiment, the values of cell viability, mitochondrial membrane potential, cardiolipin content, and mitochondrial stress were determined indirectly through the analysis of CCK-8, fluorescent indicators. Experiments were performed in triplicate.

2.5. Measurement of cell viability

After treatments, cell viability was assessed by using CCK-8 (Dojindo) as previously described [13]. For each group, H9C2 cells were further incubated for 1 h with CCK-8. The absorbance value indicating cell viability was measured at 450 nm using a microplate reader. The number of alive cells in each well was expressed as a value relative to the normal control. Experiments were repeated in triplicates.

2.6. Measurement of mitochondrial cardiolipin and mitochondrial membrane potential

H9C2 cells were seeded at a density of 5.10^3 cells/well in 96-well black, glass bottom plates or 3.10^5 cells/well in a 60 mm dish and subjected to $CoCl_2$ treatments. After treatment, the cells were stained with either NAO 0.1 µM (ex/em: 495/519 nm, Invitrogen, USA) or TMRE 0.1 µM (ex/em: 535/570 nm) for 30 min at room temperature [13]. The cells were washed twice with PBS before measuring fluorescence intensity using a microplate reader or flow cytometry. The NAO or TMRE intensity in each well was expressed as a percentage value relative to the normal control. NAO or TMRE-stained cells were captured using an Axio observer

combined with the ApoTome.2. Experiments were repeated 3 times.

2.7. Measurement of oxidative stress

After being treated with different conditions, H9C2 cells were stained with either 5 µM CM-H2DCFDA (ex/em: 485/525 nm) or 5 µM MitoSox Red (ex/em: 510/580 nm) at 37°C for 30 min at room temperature to detect changes in H_2O_2 or O_2^- levels [12]. After washing twice with PBS 1X, the different fluorescence intensities of the dyes were measured using the microplate reader or flow cytometry. The total intensity in each well was expressed as a percentage value relative to the normal control. CM-H_2DCFDA and MitoSox Red-stained cells were captured using an Axio observer combined with the ApoTome.2. Experiments were performed in triplicate.

2.8. Statistical Analysis

Data are presented as means ± standard error (SD) by using Excel 2016, Origin 8.5 software. Differences between the two groups were evaluated by ANOVA and Tukey test; a p-value ≤ 0,05 was considered significant.

3. RESULTS AND DISCUSSION

3.1. The cytotoxicity of AAME on H9C2 cells in normal condition

The effect of AAME on the viability of H9C2 cells under normal conditions was assessed by the CCK-8 kit. The results are shown in Figure 1.

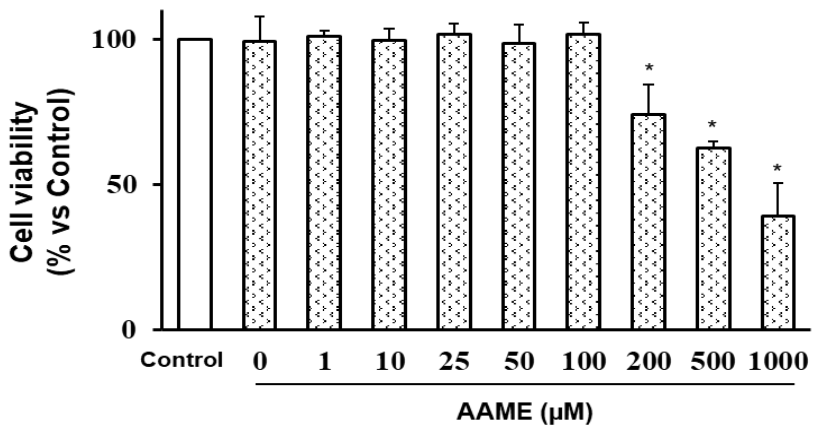

Figure 1. The viability of H9C2 cells in normal condition

**p<0,05 vs control, n=3.*

The results in Figure 1 show that, the group of cells incubated with AAME at concentrations from 0 to 100 µM had no difference compared with the control group (p>0.05). The cell viability started to decrease in the AAME 200 µM group with the cell survival rate of 81.35±7.06% compared with the control group (p<0.05). When the concentration of AAME increased to 200, 500, and 1000 µM, the percentage of viable cells decreased sharply to 62.71±2.28%, 39.25±11.15%, and 13.64±2.07, respectively, (p<0.05 compared with the control). Thus, AAME at concentrations of ≤ 100 µM did not show cytotoxicity to H9C2 cells and was selected to be used for further analyses.

3.2. AAME enhances the cell viability in HR-$CoCl_2$ condition

The effect of AAME on the viability of H9C2 cells under HR-$CoCl_2$ conditions was shown in Figure 2.

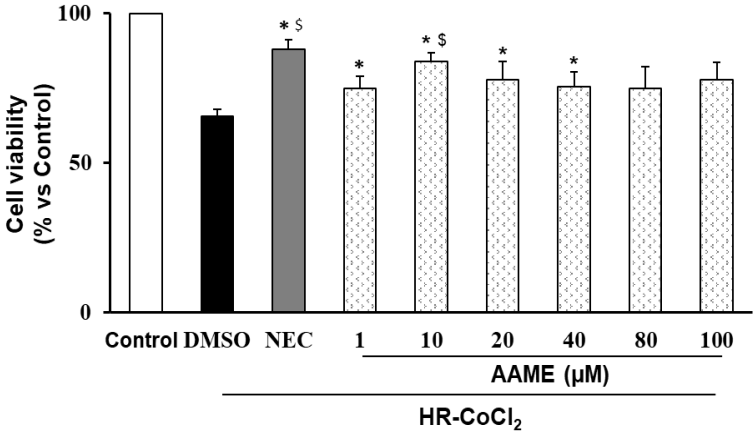

Figure 2. The viability of H9C2 cells in HR-$CoCl_2$ condition

**p<0,05 vs HR, $p<0,05 vs HR-$CoCl_2$+AAME 1 μM; n=3.*

The results shown in Figure 2 showed that the HR-$CoCl_2$+DMSO group had a sharp decrease in the percentage of viable cells to 65.68±2.35% (compared to 100% of the control, p<0.05). Compared with the HR-$CoCl_2$+DMSO group, this ratio increased significantly in the groups of cells supplemented with NEC or AAME (at concentrations of 1, 10, 20, and 40 μM, p < 0.05). In which, the percentage of viable cells was highest in the group supplemented with AAME at the concentration of 10 μM. On this basis, a concentration of 10 μM of AAME was selected for further studies.

3.3. AAME limited mitochondrial dysfunction in HR-$CoCl_2$ condition

3.3.1. AAME preserved mitochondrial cardiolipin content and membrane potential

In this study, the change in mitochondrial inner membrane cardiolipin (CL) content of H9C2 cells was determined indirectly through the blue fluorescence signal of NAO.

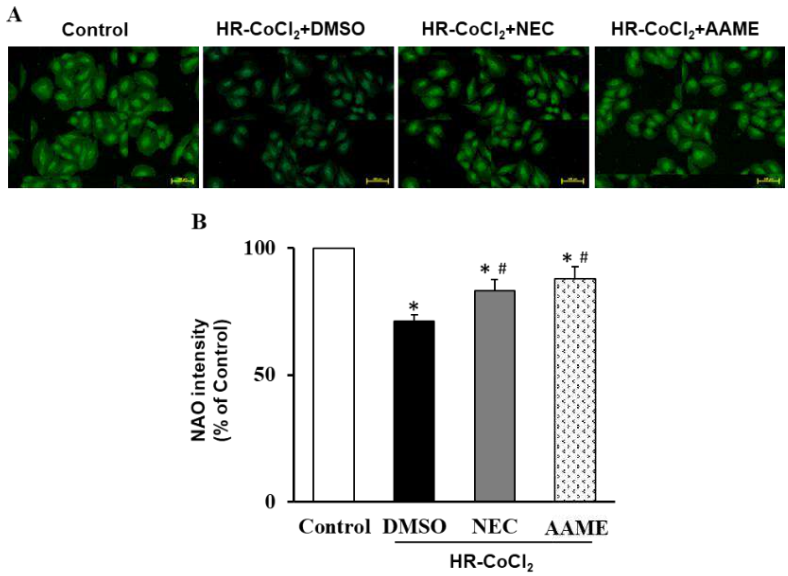

Figure 3. The NAO intensity in different conditioned-H9C2 cells

A. NAO fluorescence images were taken by the Axio observer system combined with Apo Tome.2; B. The graph of NAO fluorescence intensity values (%) was analyzed by a

*fluorescence microplate reader; Objective lens: 20X, Scale bar: 100 µm; *p<0,05 vs Control, #p<0,05 vs HR-$CoCl_2$+DMSO; n=3.*

The results in Figure 3 show that NAO fluorescence density in the HR-$CoCl_2$+DMSO group had a strongly reduced ratio of NAO fluorescence density and only reached about 71.06±2.80% (compared to 100% of control, p<0.05). This percentage in NEC-supplemented and AAME cell groups increased significantly with values of 83.25±4.36%, and 87.93±4.62%, respectively, (p<0.05 compared with HR-$CoCl_2$+DMSO group, Figure 3). This suggests that like NEC, AAME has the ability to preserve CL content, limit mitochondrial structural abnormalities, and stabilize electron transport chain activity [8], thereby limiting the rate of cell death under hR conditions.

Besides, to investigate the effect of AAME on mitochondrial function, the mitochondrial membrane potential was also determined indirectly through TMRE fluorescence kit. The results are shown in Figure 4.

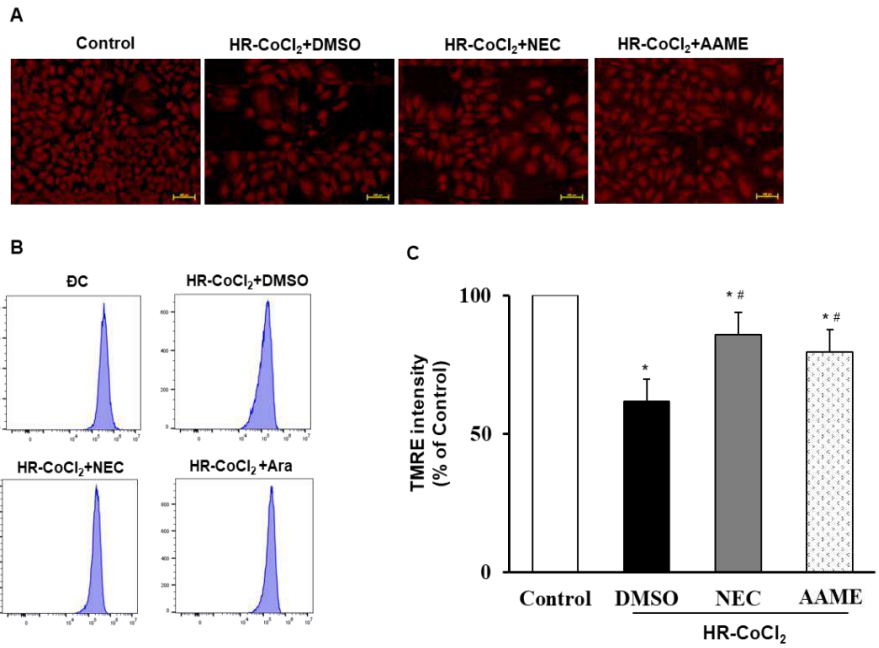

Figure 4. The TMRE intensity in different conditioned-H9C2 cells

*A. TMRE fluorescence images were taken by the Axio observer system combined with Apo Tome.2; B, C. Histograms, and the graph of TMRE fluorescence intensity (%) values analyzed by flow cytometer; Objective lens: 20X, Scale bar: 100 µm; *p<0,05 vs control; #p<0,05 vs HR-$CoCl_2$+DMSO; n=3.*

The results in Figure 4 show the HR-$CoCl_2$+DMSO group had the TMRE fluorescence density ratio reduced to 61.77±3.49% (compared to 100% of the control, Figure 4). This percentage in the group of cells subjected to the disease model supplemented with NEC, and AAME during the reoxygenation phase increased significantly, with values of 85.99±5.49%, and 79.63±7.17% respectively (p<0.05 compared with HR-$CoCl_2$+DMSO group). This result is consistent with the results of CL content and the rate of viable cells above. In particular, the preservation of CL content contributed to stabilizing membrane potential, limiting mitochondrial dysfunction, and increasing cell viability

under HR conditions. The effect of AAME on mitochondrial cadiolipin and membrane potential is quite similar to the effect of Panax bipinnatifidus Seem [14] extract and saponin MR2 extracted from Panax Vietnamese (Ngoc Linh) ginseng in our previous studies [13]. This suggests that AAME may protect H9C2 cell mitochondria in HR injury induced by $CoCl_2$. And to further explore the role of this active ingredient, the effect of AAME on the ros amount of cells in the disease model was evaluated.

3.3.2. AAME inhibited mitochondrial stress

In HR pathological conditions, mitochondria are both the major source and target of ROS [9]. Mitochondrial oxidative stress is expressed in the excessive proliferation of H_2O_2 and O_2^-. In this study, the H_2O_2 and O_2^- contents were assessed indirectly through the change in the blue fluorescence signal of CM-H_2DCFDA and the red color of MitoSOX Red. The results are shown in Figures 5 and 6.

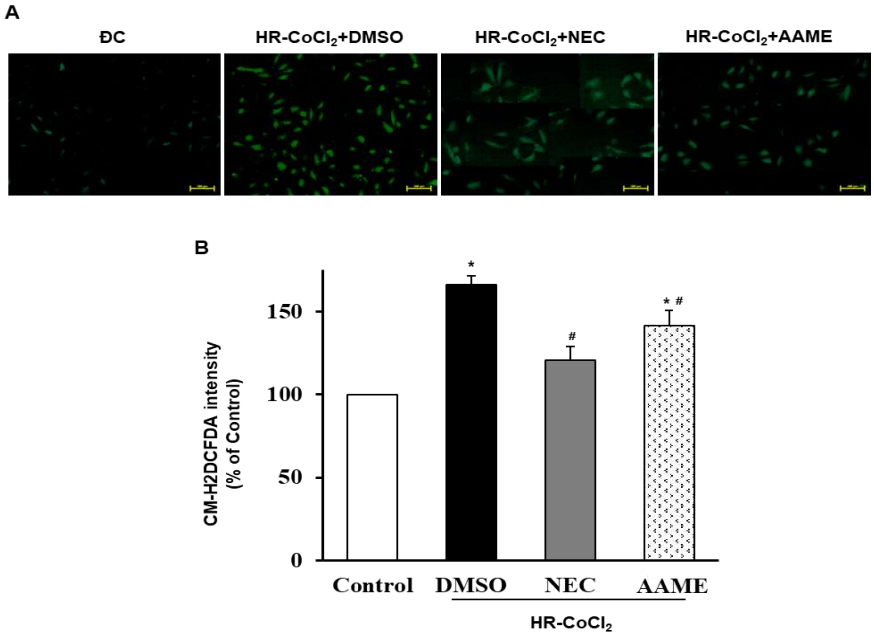

Figure 5. The CM-H_2DCFDA intensity in different conditioned-H9C2 cells

*A. CM-H_2DCFDA fluorescence images were taken by the Axio observer system combined with Apo Tome.2; B. The graph of CM-H_2DCFDA fluorescence intensity values analyzed by a fluorescence microplate reader; Objective lens: 20X, scale bar: 100 µm; *p<0,05 vs control, #p<0,05 vs HR-$CoCl_2$+DMSO; n=3.*

Figure 5A shows that HR-$CoCl_2$+DMSO cell groups have a significant increase in CM-H_2DCFDA fluorescence signal compared to the control group, shown in bright blue color and a greater number of cells carrying green color. The group of cells supplemented with AAME at the dose of 10 µM at the time of reoxygenation had a decrease in CM-H_2DCFDA fluorescence signal (greener green, fewer green-bearing cells) compared to those in HR-$CoCl_2$+DMSO cell group. Corresponding to the fluorescence image, an increase in CM-H_2DCFDA fluorescence density is clearly shown in Figure 5B. In which, HR condition highly increased the CM-H_2DCFDA fluorescence density of H9C2 cells to 166,03±5,49% (HR-$CoCl_2$+DMSO group) compared to 100% of the control group (p<0,05). This result showed that $CoCl_2$ 300 µM causes

overproduction of H_2O_2, thereby causing cell damage and death, which was consistent with a previous report [15]. However, supplementation of AAME 10 µM to the reoxygenation phase, the CM-H_2DCFDA fluorescence density of the AAME cell group was strongly decreased to 141,65±8,82% compared to the control, which was lower by about 20% than the HR group ($p<0.05$).

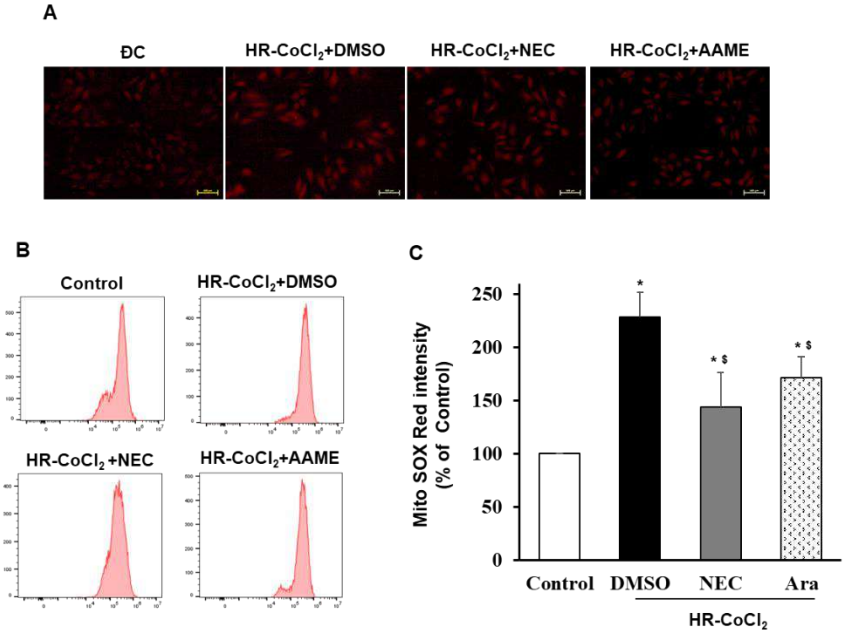

Figure 6. The MitoSOX Red intensity in different conditioned-H9C2 cells

*A. MitoSOX Red fluorescence images were taken by the Axio observer system combined with Apo Tome.2; B, C. Histograms, and the graph of MitoSOX Red fluorescence intensity (%) values analyzed by flow cytometer; Objective lens: 20X, Scale bar: 100 µm; *p<0,05 vs control, $p<0,05 vs HR-$CoCl_2$+DMSO, n=3.*

As mentioned above, the superoxide radical is a byproduct of a deficient transferring the electrons from mitochondrial respiratory chain complexes I–IV to oxygen. In fact, complexes I and III are reported as the central sites to form mitochondrial superoxide radicals [5]. Superoxide is unstable and dismutation of O_2^- to H_2O_2 can be occured spontaneously or through a reaction catalyzed by superoxide dismutase [2]. Thus, to find out the relationship between the above alterations in H_2O_2 level with O_2^-, we determined the change in O_2^- level. The results in Figures 6 A, B, and C show that MitoSOX Red fluorescence signal and density of the cells in the HR-$CoCl_2$+DMSO group increased to 228.55±23.55% compared with the control ($p<0.05$). The addition of NEC or AAME 10 µM during the reoxygenation phase reduced this ratio to 144.25±31.98% or 171.47±20.18%, respectively. Compared with HR-$CoCl_2$+DMSO, the value of MitoSOX Red fluorescence density in the remaining model groups were significantly different with $p<0.05$.

4. CONCLUSION

Taken together, the present study documents the ability of AAME in protecting cardiomyocytes in $CoCl_2$-induced HR injury *in vitro* via preserving mitochondrial function.

ACKNOWLEDGMENTS

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PREVALENCE OF FALL AND SOME FACTORS ASSOCIATED WITH FALLS IN OLDER PEOPLE WITH DEMENTIA

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ABSTRACT

Background: *Falls are common in people with dementia. Examining the fall rate and the multiple factors is necessary to inform fall-risk screening, caregiver education and support, and prevention strategies for this high-risk population of older adults.* ***Aim:*** *The aim of the study was to assess the prevalence of fall and identify some factors associated with falls in older people with dementia.* ***Methods:*** *A cross-sectional study was conducted on outpatients with dementia aged ≥ 60 years old. Dementia was diagnosed by neuropsychiatrists following DSM-5 criteria.* ***Results:*** *A total of 87 participants was recruited in the study (mean age: 76.84±8.38 years; female: 65.52%). 26 (29.89%) participants experienced a fall at least once in the previous year. 11 participants (12.65%) had two or more falls. Falls in older adults with dementia were associated with gender (female) and polypharmacy (p<0.05).* ***Conclusion:*** *The prevalence of falls among people with dementia was quite high, especially in female and participants with comorbidities. This highlights the need for effective fall preventions that target people with dementia.*

Keywords*: Fall, dementia, risk factors*

1. INTRODUCTION

Falls are common in people over the age of 65, with an increase in prevalence in people with dementia[6]. The annual incidence of falls in this patient group is about 70–80%, at least twice the incidence of falls in cognitively normal older people[2]. Dementia can increase falls rate by impairing judgment, gait, visual-spatial perception, and the ability to recognize.

Previous studies showed that 65.7% of participants with dementia had at least one fell during 12 months[3]. Sharma (2018) had shown that 48.8% of first fell were accompanied by a fracture. The falls are the result of the interaction between the factors of life, medical care, the behavior of the subject and the environment. Many risk factors can be changed or modified to help prevent falls. They include: history of falls; gait disorder; dizziness and vertigo; confusion; muscle weakness; vitamin D deficiency; environment-related; vision problems; foot pain or poor footwear; use of medicines (such as tranquilizers, sedatives, or antidepressants); even some over-the-counter medicines can affect balance and how steady you are on your feet; medical history (such as postural hypotension, Parkinson, cardiovascular disease, diabetes)[4]

Examining the fall rate and the multiple factors is necessary to inform fall-risk screening, caregiver education and support, and prevention strategies for this high-risk population of older adults. Therefore, this study was conducted to assess the prevalence of fall and identify some factors associated with falls in older people with dementia

2. SUBJECTS AND METHODS

2.1. Study design

The cross-sectional study

2.2. Study subject

Patients who are 60 years old and over have been diagnosed with dementia according to DSM V criteria[5] by the

neurologist at the National Geriatrics Hospital

Inclusion criteria: participants aged 60 years old and over, exam and treatment at National Geriatrics Hospital, have a caregiver who either lives with the participant or visits for at least four hours per week.

*Exclusion criteria: a*cute and malignant diseases (advanced cancers, end-stage chronic diseases, acute myocardial infarction, stroke);; clinical evidence of schizophrenia, severe depression, psychiatric or bipolar disorder (according to DSM-IV TR criteria); alcoholism or substance dependence (according to DSM-V criteria), currently, or within the past 2 years; severe loss of vision, hearing or communicative ability (according to the interRAI Community Health Assessment); participant or caregiver unwilling to participate in the study

2.3. Sample size.

This is a cross-sectional study. The sample size was calculated following the formula:

$$n = Z^2_{(1-\alpha)}.\frac{p.(1-p)}{d^2}$$

- n: the smallest sample to study have significance

- $Z_{(1-\alpha)}$ =1.96 with 95% confidence intervals

- p=27.6% the rate falls among dementia patients in the past study[6].

- d=0.1 is random error

Thus, the study's sample size was calculated to be at least 77 participants, and with an allowable margin of error of 10%, the required sample size was determined to be at least 85 participants.

2.4. Location.

The National Geriatric Hospital.

2.5. Time.

The study was conducted from July to December in 2021.

Variables:

- Demographic characteristics: gender, age, educational level, living status

Fall characteristic: falls within the last 12 months, number of falls in the last 12 months, falls location, falls injury. Fall characteristic was obtained through interview the caregiver.

Dementia: was diagnosed by neuropsychiatrists following DSM-5 criteria.

The type of dementia include: vascular, Alzheimer, Mix and Others

The factors associated with falls: age (60-69, 70-79, ≥80), gender, living status, polypharmacy (the concurrent use of 5 or more than 5 medications at the same time), comorbidities (the co-occurence of more than one diseases in the same individual)

2.6. Data analysis

The process of data coding, entry was done by using Redcap software and data analysis was done by SPSS software version 22. Descriptive statistics were adopted to examine characteristics data: frequency, percent, mean. Statistical significance was defined as any p-value less than 0.05.

Ethical consideration

Ethical approval has been performed in accordance with the Declaration of Helsinki and have been approved by the Hanoi Medical university (IRB00003121) on December 31, 2020

3. RESULTS

Table 1. Demographics characteristic (N=87)

Variables		**Numbers of participants (n)**	**Percentage (%)**
Age Mean ±SD: 76.84±8.38	60-69	18	20.69
	70-79	36	41.38
	≥ 80	33	37.93
Gender	Male	30	34.48
	Female	57	65.52
Living status	Family	78	89.69
	With caregivers	3	3.45
	Alone	6	6.86
Dementia type	Vascular	13	14.94
	Alzheimer	46	52.88
	Mix	18	20.69
	Others	10	11.49

In the total 87 participants, female subjects accounted for 65.52%, higher than the male subjects accounted for 34.48%. The ratio of female/male is 1.9. The age of sample was ranged from 60 to 96 with the mean age was 76.84±8.38 years old. The greatest distribution was generated by people aged from 70 to 79, with percentage of 41.38%. There was 89.69% of participants lived with their family; 3.45% older people lived with caregivers and 6,86% older people lived alone. Of the 87 participants, a high percentage of dementia were Alzheimer with 52.88%; followed by mix dementia with 20.69%, vascular dementia with 14.94% and the other with 11.49% respectively.

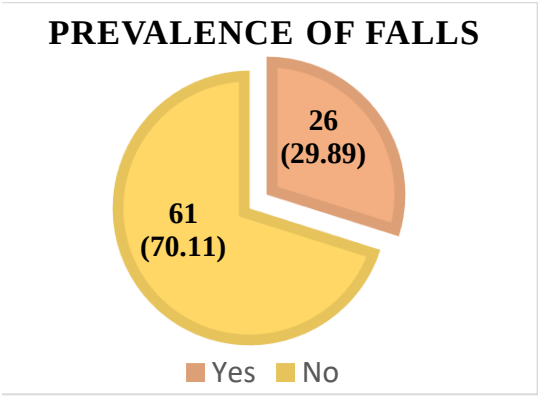

Figure 1. Prevalence falls (N=87)

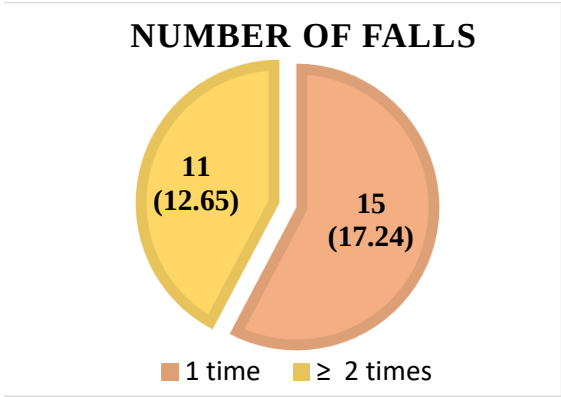

Figure 2. Number of falls (N=87)

Of the 87 total participants, 26 (29.89%) participants experienced a fall at least once in the previous year. Firgue 2 showed the number of falls of the participant in the previous 12 months. 11 participants (12.65%) had two or more falls.

Table 2. Characteristics of falls in older participants (N=87)

Variable		Numbers (n)	Percentage (%)
Falls location	In the bedroom	14	41.17
	Bathroom/ toilet	9	26.47
	Stairs	3	8.82
	Floor or outside the house	6	17.65
	The other	2	5.88
Falls injury	No injury	11	40.74
	Mild injury	10	37.04
	Fracture bone	4	14.81
	Hospitalization	2	7.41

Table 2 showed the characteristics of falls among the participants. The majority of falls occurred at home (41.17%). Moreover, a high percentage of falls were caused by a losing balance, followed by slippery floor and as a consequence of dizziness. Regarding the consequences of falls, the majority were no injury or mild injury and hospitalization rate was only 7.41%.

Table 3. Assocaition between demographic and falls risk (N=87)

Variable		Falls		p
		Yes (n=26)	No (n=61)	
Gender	Male	5 (19.23%)	25 (40.98%)	0.03
	Female	21 (80.77%)	36 (59.02%)	
Age	60-69	4 (15.38%)	14 (22.95%)	0.12
	70-79	10 (38.46%)	26 (42.62%)	
	≥ 80	12 (46.16%)	21 (34.43%)	
Living area	Urban	20 (76.92%)	47 (77.05%)	0.05
	Rural	6 (23.08%)	14 (22.95%)	
Living status	Family	22 (84.62%)	56 (91.81%)	0.15
	The other	4 (15.38%)	5 (8.19%)	
	No	24 (92.31%)	50 (81.97%)	
Polypharmacy	Yes	14 (53.85%)	29 (47.54%)	<0.01
	No	12 (46.15%)	32 (52.46%)	
Comorbidities	Yes	14 (53.85 %)	18 (29.51%)	0.069
	No	12 (46.15%)	43 (70.49%)	

There was an association between gender and fall, in which the prevalence of falls in female was higher than in male (p=0.03). There was an association between polypharmacy and fall. In which, people who with polypharmacy had the prevalence of falls higher than those without polypharmacy (<0.01).

DISCUSSION

The average age of participants in this study was 76.84 years old (SD=8.38). It was similarly found in the previous research in Japan with the average age of participants was 77.5 years (SD=8.1) and research in Tawai with mean age 77.3 ± 9.0 years[7].

The prevalence of falls in older patients with dementia was 29.89% (single fall 17.24% and multiple falls 12.65%), which was similar to the result

of Asada et al. conducted in community-dwelling from Yamanashi (Japan)[7]. However, the prevalence of falls in our study was lower than the study of Allen in the UK outpatients (65.7% of participants with dementia had at least one fall during 12 months)[3]. Malaysia's research has shown that 65% of participants fell one or more times in the follow-up year, with 43% reporting multiple falls[8]. The differences could be due to differences in the sample size, culture and medical care conditions across countries.

We observed that the majority of falls occurred in the bedroom (41.17%). This result was in line with the results of the previous study: the majority of falls occurred at the bedside while they were getting up from their beds (72.2%)[9]. However, another research found that the bathroom was the place where participants fell the most. The bathroom was found to be a hazardous space carrying risks of falls, such as a slippery floor, poor lighting and a shortage of handrails and non-slip mats. Imbalance, dizziness, slipping were frequent causes of falls. This is also the main reason pointed out from previous research[93–95]. The fracture rate in our research was higher than in the research of Van Doorn, which showed the fracture rate was 2.8% [10].

There was a significant difference between gender in fall risk ($p<0.05$). It was the similar found in Malaysia's research, had significant differences were found between males and females in falls rate. The studies indicated that the fall rate in females was higher than in males. A finding in Maryland's research reported that female elderly people had fallen risk higher compared to male counterparts[10].

There was a significant difference between polypharmacy in fall rate. It was consistent with the research in the UK on the association between falls and polypharmacy (4 and over) in people 60 and over[11].

CONCLUSION

The prevalence of falls among people with dementia was quite high, especially in female and participants with comorbidities. This highlights the need for effective fall preventions that target people with dementia.

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TỶ LỆ NGÃ VÀ MỘT SỐ YẾU TỐ LIÊN QUAN TRÊN NGƯỜI CAO TUỔI CÓ SA SÚT TRÍ TUỆ

TÓM TẮT

Đặt vấn đề: *Ngã thường gặp ở người có sa sút trí tuệ. Việc xem xét tỷ lệ té ngã và các yếu tố liên quan là cần thiết để cung cấp thông tin sàng lọc nguy cơ ngã, giáo dục và hỗ trợ người chăm sóc cũng như các chiến lược phòng ngừa cho nhóm người cao tuổi có nguy cơ cao này.* ***Mục tiêu:*** *Mục tiêu của nghiên cứu là đánh giá tỷ lệ ngã và xác định một số yếu tố liên quan đến ngã ở người cao tuổi có sa sút trí tuệ.* ***Phương pháp nghiên cứu:*** *Nghiên cứu cắt ngang được thực hiện trên bệnh nhân ngoại trú có sa sút trí tuệ ở độ tuổi ≥ 60. Sa sút trí tuệ được chẩn đoán bởi các bác sĩ tâm thần kinh theo tiêu chuẩn DSM-5.* ***Kết quả:*** *Tổng cộng có 87 người tham gia được tuyển dụng trong nghiên cứu (tuổi trung bình: 76,84 ± 8,38 tuổi; nữ: 65,52%). 26 người tham gia (29,89%) đã bị ngã ít nhất một lần trong năm trước. 11 người tham gia (12,65%) bị ngã từ hai lần trở lên. Ngã ở người cao tuổi có sa sút trí tuệ có liên quan đến giới tính (nữ) và việc sử dụng nhiều thuốc (p<0,05).* ***Kết luận:*** *Tỷ lệ ngã ở người cao tuổi có sa sút trí tuệ khá cao, đặc biệt là ở phụ nữ và những người có đa bệnh lý. Điều này nhấn mạnh sự cần thiết của các biện pháp ngăn ngừa ngã hiệu quả trên người cao tuổi có sa sút trí tuệ.*

Từ khóa: *Ngã, sa sút trí tuệ, yếu tố nguy cơ*

RESULTS OF IRON SUPPLEMENTATION FOR REGULAR VOLUNTARY NON-REMUNERATED BLOOD DONORS AT NATIONAL INSTITUTE OF HEMATOLOGY AND BLOOD TRANSFUSION

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SUMMARY

Objective: *To evaluate the results of iron supplementation for RRVBD at the National Institute of Hematology and Blood Transfusion.* ***Subjects and methods:*** *Including 279 RRVBD with serum ferritin below 26 ng/ml taking iron tablets containing 35 mg of elemental iron. Cross-sectional description, prospective study.* ***Results****: The proportion of RRVBD taking iron tablets and testing on time is 43.4%, the proportion of RRVBD taking enough iron tablets and not coming to the test on time is 18.6%. Hematological parameters of RRVBD taking iron tablets are within the normal range. The group of RRVBD taking enough iron tablets and testing on time: the mean serum ferritin concentration before taking iron tablets is 15.8 ± 5.5 ng/ml and after taking iron tablets is 24.7 ± 14.9 ng/ml, the proportion of RRVBD with ferritin returning to normal range after taking iron tablets is 39.7%. In group of RRVBD not taking iron tablets or not taking enough iron tablets, serum ferritin before iron intake is 18.1 ± 5.3 ng/ml and after iron intake is 14.2 ± 10.9 ng/ml, the proportion of blood donors having serum ferritin returning to normal range after iron intake is 7.8%. In group of RRVBD taking enough iron tablets and not coming to the test on time, the mean serum ferritin concentration before iron intake is 16.5 ± 5.9 ng/ml and after iron intake is 28.8 ± 17.1 ng/ml, the proportion of RRVBD with serum ferritin concentration returning to normal range after blood donation is 51.9%.* ***Conclusion****:The RRVBD group, upon consuming sufficient iron and undergoing timely examinations, displayed an increased average serum iron concentration compared to their levels prior to iron supplementation, whereas the subgroup that either lacked adequate iron intake or took insufficient iron exhibited a decreased average serum ferritin concentration compared to their levels prior to initiating iron intake.*

Keywords*: regular repeated blood donors, serum ferritin.*

1. INTRODUCTION

Blood and blood products are invaluable medicines, essential for emergency and specialized treatment of diseases that require blood transfusion. Diseases such as blood clotting disorders, congenital bleeding disorders, blood cancers, and others necessitate blood transfusions. Blood can only be obtained from voluntary blood donors, as there is currently no substitute source. Therefore, caring for and maintaining a steady supply of blood donors is of utmost importance, especially focusing on iron supplementation for regular repeated blood donors (RRVBD) [1], [2].

Each milliliter of blood contains about 0.5 mg of iron. Consequently, with each blood donation, a certain amount of iron is lost by the blood donor. Many countries

around the world are concerned about providing iron supplementation for RRVBD. However, as of now, this practice has not been implemented in our country [1], [2].

Therefore, the research topic is pursued with the following objective: "Assessment of iron supplementation outcomes for regular repeated blood donors at the National Institute of Hematology and Blood Transfusion."

2. 2. SUBJECTS AND METHODS

2.1. Patient cohort

Including 279 RRVBD with serum ferritin below 26 ng/ml taking iron tablets containing 35 mg of elemental iron.

2.2. METHODS

***2.2.1. Research design**:* Cross-sectional description, prospective study.

2.2.2. Method of procedure

- Selection criteria: The eligible blood donors are those who meet the blood donation eligibility criteria outlined in Circular 26/BYT/2013 [3]. The potential blood donors' comprehensive blood cell analysis, serum iron concentration, and serum ferritin levels are examined. Donors with serum ferritin levels < 26 ng/ml are chosen for advising on taking iron supplements after blood donation.
- RRVBD for male donors is a minimum of 3 times per year, and for female donors, it's a minimum of 2 times per year.
- According to Joseph E. Kiss in 2015, blood donors with ferritin levels < 26 ng/ml are considered as having decreased ferritin (iron deficiency), while those with ferritin levels ≥ 26 ng/ml are considered normal (not iron deficient) [4].
- Sample size for blood donors receiving iron supplements: Based on the formula estimating a proportion: $n = Z^2_{1-\alpha/2} \times p(1-p) \times d^2$
  - n: is the sample size
  - p: is the proportion of individuals with decreased ferritin (according to the AABB report in 2017, the proportion of decreased ferritin is 0.22).
  - d: is the desired margin of error between the proportion obtained from the study sample and the population proportion, with d = 0.05 chosen.
  - α: is the significance level, Z 1- α/2 is the confidence coefficient, and the value of Z obtained from the Z-table corresponding to the chosen α value, α = 0.05 (95% confidence interval), the Z value being 1.96.
  - Using the formula, the calculated sample size for blood donors using iron supplements is n ≥ 263.

2.2.3. Research content

- RRVBD with serum ferritin below 26 ng/ml are advised to take iron supplementation:
  - Use a type of iron tablet: Take a tablet containing 300 mg of ferrous gluconate, equivalent to 35 mg of elemental iron, take 1 tablet on an empty stomach within 60 days.
  - RRVBD is invited to the Institute of Hematology and Blood Transfusion for a follow-up test after 85 days from the start of medication, and the maximum sampling time is 120 days.
- The follow-up tests after taking iron tablets include: Complete blood cell analysis, serum iron concentration, and serum ferritin.
- Classification of RRVBD subjects:
  - Group 1: Blood donors who took iron tablets and came for a follow-up test including complete blood cell analysis, serum iron, and serum ferritin within the timeframe from day 85 to under 120 days from the donation date.
  - Group 2: Blood donors who did not take iron or took insufficient iron supplementation, and came for a follow-up test including complete

blood cell analysis, serum iron, and serum ferritin, with the testing time starting from day 85 after the donation date.

+ Group 3: Blood donors who took sufficient iron tablets and came for a follow-up test including complete blood cell analysis, serum iron, and serum ferritin after 120 days from the donation date.
+ Group 4: Blood donors who withdraw from the study, do not take iron tablets, and do not repeat the tests.

– Evaluation of RRVBD's test results after taking supplemental iron.

2.3. Data processing: SPSS 20.0

3. RESULTS

Table 1. Some features of RRVBD using iron tablets at NIHBT

The individuals who donate blood take iron tablets	Male		Female		Total	
	n	%	n	%	n	%
Group 1 (RRVBD taking iron tablets and testing on time)	25	***9.0***	96	***34.4***	121	***43.4***
Group 2 (RRVBD not taking iron tablets or not taking enough iron tablets)	4	***1.4***	47	***16.8***	51	***18.3***
Group 3 (RRVBD taking enough iron tablets and not coming to the test on time)	5	***1.8***	47	***16.8***	52	***18.6***
Group 4 (RRVBD dropping out of the study)	2	***0.7***	53	***19.0***	55	***19.7***
Total	36	***12.9***	243	***87.1***	279	***100***

There were 279 regular repeated blood donors who agreed to participate in an iron supplementation program, among whom 121 RRVBD took the iron supplements fully and had their tests done on time, resulting in a rate of 43.4%. RRVBD who did not take the iron supplements or took them incompletely and underwent retesting were 51 individuals, accounting for a rate of 18.3%. RRVBD who took the iron supplements fully but had their tests done late were 52 individuals, making up a rate of 18.6%. RRVBD who received the iron supplements but neither took them nor underwent the test were 55 individuals, representing a rate of 19.7%.

Table 2. Characteristics regarding age, number of blood donations, and post-iron supplementation testing time of the RRVBD subject taking iron tablets

RRVBD subject taking iron tablets	Age	Number of blood donations	Post-iron supplementation testing time
	Av ± SD	Av ± SD	Av ± SD
Group 1 (n = 121)	28.9 ± 7.7	12.6 ± 8.9	96.6 ± 9.7
Group 2 (n = 51)	25.8 ± 7.4	11.7 ± 8.6	322.2 ± 274.9
Group 3 (n = 52)	27.2 ± 7.1	9.8 ± 8.6	189.5 ± 115.3
Group 4 (n = 55)	27.6 ± 7.1	7.6 ± 4.2	0
Total (n= 279)	27.8 ± 7.5	11.0 ± 7.7	169.5 ± 168.4
p	p1-2 < 0.05 p1-3, p2-3 ≥ 0.05	p1-2, p2-3 ≥ 0.05 p1-3, p1-4< 0.05	p1-2, p1-3, p2-3 < 0.05

RRVBD subjects taking iron tablets have an average age of 27.8 ± 7.5 years, an average number of blood donations of 11.0 ± 7.7 times, and an average post-iron

supplementation testing time of 169.5 ± 168.4 days. The RRVBD group that fully complied with iron intake and participated in testing at the correct average time was 96.6 ± 9.7 days. In contrast, the RRVBD group that did not fully comply with iron intake or did not take iron had the longest average testing time of 322.2 ± 274.9 days, with statistically significant differences at $p < 0.05$.

Table 3. Certain hematological parameters, serum iron levels, and ferritin levels of RRVBD prior to iron tablet supplementation

Parameters (n=279)	Group 1	Group 2	Group 3	Group 4	p
	Av ± SD (n=121)	Av ± SD (n=51)	Av ± SD (n=52)	Av ± SD (n=55)	
RBC (T/l)	4.63 ± 0.44	4.61 ± 0.38	4.56±0.44	4.57±0.58	≥ 0.05
HBG (g/l)	130.4 ± 11.2	128.9 ± 6.9	128.1 ± 8.7	128.6±8.7	≥ 0.05
MCV (fl)	86.4 ± 5.6	86.4 ± 5.6	85.9 ± 6.0	87.7±4.9	≥ 0.05
MCH (pg)	28.2 ± 2.2	28.1 ± 2.1	28.1 ± 2.3	28.3±2.8	≥ 0.05
Fe (µmol/L)	12.8 ± 6.1	15.1 ± 8.2	14.9 ± 6.6	13.2 ±7.2	≥ 0.05
Ferritin (ng/ml)	15.8 ± 5.5	18.1 ± 5.3	16.5 ± 5.9	15.4 ± 5.4	≥ 0.05

Some hematological indices, average serum iron of RRVBD, lie within the range of normal individuals, while the average ferritin concentration of RRVBD is 15.4 ± 5.4 ng/ml, lower than the normal range (selected standard below 26 ng/ml). Hematological indices, serum iron concentration, and average serum ferritin levels showed no significant differences among RRVBD groups before iron tablet supplementation.

Table 4. The results of examining certain hematological indices, serum iron concentration, and ferritin levels of RRVBD after iron supplementation

Parameters (n=279)	Group 1	Group 2	Group 3	p
	Av ± SD (n=121)	Av ± SD (n=51)	Av ± SD (n=52)	
RBC (T/l)	4.75 ±0.51	4.58 ± 0.39	4.67 ± 0.46	≥ 0.05
HBG (g/l)	132.6 ± 11.6	126.2 ± 6.7	132.4 ± 9.9	< 0.05
MCV (fl)	85.3 ± 5.4	85.0 ± 5.5	85.7 ± 5.7	≥ 0.05
MCH (pg)	27.9 ± 2.0	27.6 ± 2.1	28.3 ± 2.2	≥ 0.05
Fe (µmol/L)	16.0 ± 9.3	13.9 ± 10.5	15.4 ± 7.1	≥ 0.05
Ferritin (ng/ml)	24.7 ± 14.9	14.2 ± 10.9	28.8 ± 17.1	< 0.05

The quantities of red blood cells, HBG, MCV, MCH, and serum iron concentration in the RRVBD groups of blood donors after iron tablet supplementation all fall within the normal range. The average serum ferritin concentration in the RRVBD group that used complete iron tablets (group 1) and adhered to scheduled testing, as well as the RRVBD group that did not take iron or took incomplete doses (group 2), is lower than that of normal individuals. The average hemoglobin concentration and average serum ferritin level of RRVBD subjects who did not use iron or used incomplete iron supplementation are lower than those of other RRVBD groups, with statistically significant differences at $p < 0.05$.

Table 5. Recovery outcomes of ferritin in RRVBD subjects after iron supplementation

	Normal and elevated ferritin levels	Decreased ferritin levels < 26 ng/ml	Total

	≥ 26 ng/ml					
	n	%	n	%	n	%
Group 1 (RRVBD taking iron tablets and testing on time)	48	***39.7***	73	***60.3***	121	100
Group 2 (RRVBD not taking iron tablets or not taking enough iron tablets)	4	***7.8***	47	***92.2***	51	100
Group 3 (RRVBD taking enough iron tablets and not coming to the test on time)	27	***51.9***	25	***48.1***	52	100
p	***< 0.05***					

The proportion of RRVBD subjects in the group of full iron intake and timely testing with normal and elevated ferritin levels is 39.7%, while for RRVBD subjects who do not take iron or take it irregularly, it is 7.8%. The group of RRVBD subjects taking full iron supplementation but not adhering to scheduled testing constitutes 51.9%. There is a statistically significant difference among the RRVBD groups with regards to iron tablet intake, with $p < 0.05$.

4. DICUSSION

Table 1 results show that out of the 279 RRVBD subjects with serum ferritin levels below 26 ng/ml, 121 RRVBD subjects adhered to both full iron tablet intake and timely testing, constituting a rate of 43.4%. Among the RRVBD subjects who did not take iron or took it inadequately and still underwent testing (51 individuals), the rate was 18.3%. For RRVBD subjects who took full iron supplementation but did not adhere to scheduled testing (52 individuals), the rate was 18.6%. Meanwhile, among the RRVBD subjects who neither took iron nor repeated the testing (55 individuals), the rate was 19.7%. Consequently, despite being advised and provided with free iron tablets for blood donation, a number of individuals did not provide information or failed to take the iron tablets adequately. According to the study by Walter Bialkowski et al. conducted in 2017, where 139 blood donors were supplemented with 38 mg of iron element within 60 days, there were 3 individuals who did not participate in the study and 17 blood donors dropped out, resulting in a dropout rate of 14.4%. In the case of supplementing 19 mg of iron element to 139 blood donors within 60 days, 6 individuals dropped out and 22 blood donors dropped out mid-study, with a dropout rate of 20.1% [5]. Therefore, the implementation of iron supplementation for RRVBD encounters a certain proportion of blood donors who either do not participate in the study or drop out mid-study, with various reasons including busy work schedules, medication forgetfulness, and occurrences of complications such as constipation, skin rashes, and others.

Table 2 results indicate that the mean age of RRVBD participants engaged in iron supplementation is 27.8 ± 7.5 years. The overall mean number of blood donations for RRVBD is 11.0 ± 7.7 times. Among these, blood donors who take complete iron supplementation and adhere to scheduled testing have an average of 12.6 ± 8.9 blood donation occurrences, which is higher than the group of blood donors who do not take iron or take it inadequately, with an average of 9.8 ± 8.6 blood donation occurrences. The group of blood donors who neither take iron nor undergo testing has an average of 7.6 ± 4.2 blood donation occurrences, and there is a statistically significant difference with $p < 0.05$. Therefore, RRVBD

individuals with more frequent blood donations are more likely to show an interest in iron supplementation compared to those with fewer blood donation occurrences.

Table 3 results demonstrate that the hematological indices including RBC, HBG, MCV, MCH, and average serum iron concentration all fall within the range of normal individuals, with no significant differences observed among the RRVBD subject groups taking iron tablets ($p \geq 0.05$). The average serum ferritin concentration among the RRVBD subject groups taking iron tablets is below 26 ng/ml, and there are no significant differences among the groups ($p \geq 0.05$). Therefore, several hematological indices and average serum iron and ferritin concentrations before blood donation in the various RRVBD subject groups prior to iron tablet usage exhibit no differences.

Table 4 results reveal that several hematological indices and serum iron concentrations of RRVBD after iron supplementation fall within the range of normal individuals. However, in the group of RRVBD subjects who did not use iron or used it inadequately and underwent testing after iron supplementation, the HBG was 126.2 ± 6.7 g/l, which is lower than the group of RRVBD subjects using complete iron supplementation and adhering to scheduled testing, with an HBG of 132.6 ± 11.6 after iron supplementation, and the group of RRVBD subjects using complete iron supplementation but not adhering to scheduled testing, with an HBG of 132.4 ± 9.9 g/l. There is a statistically significant difference among the RRVBD subject groups taking iron tablets with $p < 0.05$. Regarding the average serum ferritin concentration in the group of RRVBD subjects using complete iron supplementation but not adhering to scheduled testing, the average ferritin concentration after testing following iron supplementation is 28.8 ± 17.1 ng/ml, which is within the normal range for serum ferritin levels in normal individuals. This is in contrast to the average ferritin level before iron supplementation, which was 16.5 ± 5.9 ng/ml. In the group of RRVBD subjects using complete iron supplementation and adhering to scheduled testing, the average ferritin concentration after iron supplementation testing is 24.7 ± 14.9 ng/ml, which is not within the normal range (≥ 26 ng/ml); however, there is a significant improvement compared to the average ferritin level before iron supplementation, which was 15.8 ± 5.5 ng/ml. For the group of RRVBD subjects not taking iron tablets or taking them inadequately, the average serum ferritin concentration is 14.2 ± 10.9 ng/ml, which is even lower than the average ferritin level before iron supplementation, which was 18.1 ± 5.3 ng/ml. Therefore, there is a significant recovery in average serum ferritin concentration for RRVBD subjects using complete iron supplementation, while RRVBD subjects not taking iron or taking it inadequately do not exhibit improvement. According to a study by author Walter Bialkowski and colleagues in 2017, there was an improvement in total body iron content for RRVBD individuals in the group using 19 mg and 38 mg of iron element supplementation daily for 60 days. The author also suggested that using lower iron doses for blood donors compared to iron-deficient anemia treatment for patients (with a dose of 65 mg of iron element daily) reduces gastrointestinal side effects, leading to better adherence to iron supplementation [5]. According to author Moretti Diego and colleagues in 2015, continuous daily iron tablet use may not be as effective in absorption, and the author recommended that iron tablets can be taken every other day or even weekly [6].

Table 5 results show that the proportion of RRVBD individuals with ferritin concentrations ≥ 26 ng/ml in the group of blood donors who took complete iron supplementation and adhered to scheduled

testing is 39.7%. In the group of blood donors who did not use iron or used it inadequately, this proportion is 7.8%, and in the group of blood donors who took complete iron supplementation but did not adhere to scheduled testing, the proportion is 51.9%. The proportion of RRVBD individuals with ferritin concentrations ≥ 26 ng/ml is higher in the first group compared to the other two groups, with a statistically significant difference of $p < 0.05$. For RRVBD subjects who used complete iron supplementation but did not adhere to scheduled testing, the average time to retest after supplementation was 189.5 ± 115.3 days, whereas the group of RRVBD subjects who took complete iron supplementation and adhered to scheduled testing had an average time to retest of 96.6 ± 9.7 days, and the group of RRVBD subjects who did not use iron or used it inadequately had an average time to retest of 322.2 ± 274.9 days. Therefore, RRVBD individuals who used complete iron supplementation and had a longer interval to retest exhibited a higher proportion of serum ferritin recovery compared to the other two groups. According to a study by author Alan E. Mast and colleagues in 2020, the effect of taking iron tablets with 37.5 mg of iron element helped recover iron stores and iron in red blood cells at a minimum in blood donors with serum ferritin levels ≥ 50 ng/ml, but significant recovery occurred when serum ferritin was < 50 ng/ml. The study found that initial erythropoietin levels increased rapidly, nearly doubling after whole blood donation. After reaching the initial peak, erythropoietin levels decreased to baseline levels. This decrease was slowed down in the group of blood donors with serum ferritin levels below 12 ng/ml and between 12-50 ng/ml in those not taking iron compared to the iron-taking groups. As erythropoietin responds to blood oxygen deficiency, the slower decrease in those who donated blood may reflect lower HBG levels and a relatively prolonged cellular oxygen deficiency in the kidneys. The initial increase in erythropoietin was related to a corresponding increase in reticulocyte count. Reticulocytes remained elevated for a longer duration in the iron-taking groups, indicating an immediate reticulocyte response dependent on available iron and erythropoietin. Reticulocyte count then began to rise again around day 100 in individuals with serum ferritin levels ≥ 12 ng/mL. It's unclear why a similar response wasn't observed in individuals with ferritin levels < 12 ng/mL, but it could be related to continued low iron reserves in these blood donors. The authors indicated that blood donors will fully recover iron after 100 days of iron supplementation [7].

5. CONCLUSION

RRVBD subjects who took complete iron supplementation and adhered to scheduled testing constituted 43.4% of the group, while those who did not take iron or took it inadequately accounted for 18.3%. RRVBD individuals who took complete iron supplementation but did not adhere to scheduled testing made up 18.6%, and those who did not take iron and did not undergo testing comprised 19.7%.

Hematological indices including RBC, HBG, MCV, MCH, and serum iron concentrations of various RRVBD groups before and after iron supplementation all fell within the normal range.

Results of ferritin recovery in RRVBD after iron supplementation: Among RRVBD individuals who took complete iron supplementation and adhered to scheduled testing, the average serum iron concentration after supplementation was 24.7 ± 14.9 ng/ml, showing an increase compared to before iron intake. The proportion of blood donors with ferritin levels above 26 ng/ml was 39.7%. In the group of RRVBD individuals who did not take iron or took it inadequately, the average ferritin concentration was 14.2 ± 10.9 ng/ml, which was lower compared to

before iron intake, and the proportion of blood donors with ferritin levels above 26 ng/ml was 7.8%. For RRVBD individuals who took complete iron supplementation but did not adhere to scheduled testing, the average ferritin concentration was 28.8 ± 17.1 ng/ml, within the range of normal values, and the proportion of blood donors with ferritin levels above 26 ng/ml was 51.9%.

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