VIETNAM MEDICAL ASSOCIATION
VIETNAM ASSOCIATION OF PHYSIOLOGY

Vietnam Journal of PHYSIOLOGY

Volume 25, №3
9/2021

Vietnam Journal of Physiology
Volume 25, №3, September 2021

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VIETNAMESE GINSENG EXTRACT ATTENUATES OXIDATIVE STRESS IN COBALT CHLORIDE-SUBJECTED H9C2 CELLS

Ngo Thi Hai Yen[1,2,#], Vu Thi Thu[1,*,#]

ABSTRACT

Objective: *This study aimed to evaluate the protective effects of Vietnamese ginseng extract (VGE) on the formation of reactive oxygen species (ROS) in Cobalt chloride ($CoCl_2$)-exposed H9C2 cells.* ***Methods:*** *H9C2 cells were subjected to $CoCl_2$-induced hypoxia-reoxygenation (HR) model with or without treatment of VGE. The hydroperoxide (H_2O_2) and superoxide (O_2^-) productions in H9C2 cells in the experimental groups were assessed by suitable fluorescence kits.* ***Results:*** *The obtained data showed that VGE at a dose of 31.25 µg/ml significantly reduced ROS levels in HR-subjected H9C2 cells. In the HR group, the H_2O_2 and O_2^- content in H9C2 cells were sharply increased to 156.81±7.82% and 160.10±2.07% (of 100% control), respectively. Interestingly, in the VGE-treated HR group, these indicators were significantly decreased to 119.67± 3.37% and 124.72 ± 3.21% ($p < 0.05$), respectively.* ***Conclusion:*** *The results demonstrated that VGE effectively attenuated oxidative stress in cardiomyocytes under $CoCl_2$-induced HR condition.*

Key word: *H9C2, reactive oxygen species, $CoCl_2$.*

1. INTRODUCTION

Reactive oxygen species (ROS) such as hydroxyl (OH^-), superoxide (O_2^-), hydroperoxide (H_2O_2) are secondary products of chemical metabolism and are kept in balance by antioxidants in normal cells [1]. At low concentrations, ROS play a role in mediating the physiological effects. However, the excessive production of ROS will lead to oxidative stress, leading to ischemic diseases, neurodegenerative diseases, diabetes, and cancer [2]. Many studies have demonstrated that ROS overproduction has an important role in the mechanism of myocardial hypoxia-reoxygenation (HR) injury [3]. In myocardial HR injury, mitochondria are known to be the main source and target of ROS damages [4]. Thus, protecting mitochondria against oxidative stress is one of pharmacological approaches to reduced myocardial damage in HR conditions, therefore attenuating ischemic heart injury.

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Received date: 17/7/2021
Reviewed date: 02/8/2021
Accepted date: 09/8/2021

Vietnamese Ginseng also known as Ngoc Linh Ginseng is a precious medicinal herb in traditional remedies by enhancing health, reducing stress … [5]. In our previous study, total saponin extract of VG was shown to have the potential to protect H9C2 cardiomyocytes in HR injury through preserving cardiolipin content and mitochondrial membrane potential [6]. However, the mechanism of this effect of VG on oxidative stress in HR has not been elucidated yet. Therefore, in this study, we evaluated the effect of VGE on ROS level in H9C2 cells exposed to Cobalt chloride ($CoCl_2$)-HR condition. The obtained results showed that Vietnamese ginseng extract (VGE) exerts the ability to reduce ROS production in H9C2 cardiomyocytes under HR condition.

2. MATERIALS AND METHODS

2.1. Samples

H9C2 cell line derived from rat primary cardiomyocytes (ATCC©-USA) was provided by the Cardiovascular and Metabolic Disease Center, Inje University, Korea. VGE was prepared as described in the previous study [7]. The research was carried out 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 ($CoCl_2$, Sigma, USA); Inverted microscope 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); CO_2 Incubator (Shellab, USA); ApoTome Fluorescence Microscope (Zeiss, Germany); Microreader plate (Tristar, USA).

2.3. Methods

Cell culture, hypoxia-reoxygenation in vitro model and treatments

H9C2 cells were maintained in DMEM supplemented with 1% PS and 10% FBS at 37^0C with 5% CO_2. Culture medium was changed every 2-3 days. H9C2 cells were grown in a 96-well black plate, glass bottom at a density of 5.10^3 cells/well or in a confocal dish at a density of 5.10^3 cells/well at 37^0C, 5% CO_2. After 24 h, the cells were then subjected to HR using $CoCl_2$ (300 µM) as described in a previous publication [8]. Experimental cells were divided into normal control group and HR groups as follows:

1) The normal control group: the cells continue to be grown under normal condition (DMEM, 10% FBS, 1% PS, 37^0C and 5% CO_2) for 48 h;

2) The HR groups: the cells were grown in DMEM medium supplemented with $CoCl_2$ 300 µM for 24 h (simulating the hypoxia stage) at 37^0C and 5% CO_2. Then, the medium containing $CoCl_2$ was removed, the cells continue to be grown for 24 h in new media (simulating the reoxygenation stage), with the further treatments: HR group (HR, HR-$CoCl_2$): the reoxygenation stage normal culture medium contained DMEM, 10% FBS, 1% PS; DMSO group (DMSO, HR-$CoCl_2$+DMSO): the reoxygenation stage medium contained DMEM, 10% FBS, 1% PS, 0.1% DMSO; VGE group (VGE, HR-$CoCl_2$+DMSO+VGE): the reoxygenation stage medium contained DMEM, 10% FBS, 1% PS, DMSO 0.1% and VGE 31.25 µg/ml.

At the end of the experiment, the amount of H_2O_2 and O_2^- was determined indirectly through the analysis of fluorescent indicators CM-H_2DCFDA and MitoSOX Red. Experiments were performed in triplicate.

Measurement of hydroperoxide production

H9C2 cells were seeded at a density of 5.10^3 cells/well in 96-well black, glass bottom plates or in confocal dishes and subjected to the above-described HR model. After being subjected to different conditions, cells were stained with 5 µM CM-H_2DCFDA (ex/em 485/525 nm) at 37^0C for 30 min at room temperature to detect changes in H_2O_2 levels. After washing twice with PBS 1X, the different fluorescence intensities were measured using the microplate reader. The total CM-H_2DCFDA intensity in each well was expressed as a percentage value relative to the normal control. In another experimental set, CM-H_2DCFDA-stained cells were captured using the ApoTome 2 and the images were then reconstructed from individual tiles (X:6, Y:9) using ZEN Blue 2.5 software (Carl Zeiss). Experiments were performed in triplicate.

Measurement of superoxide production

H9C2 cells were seeded at a density of 5.10^3 cells/well in 96-well black, glass bottom plates or in confocal dishes and subjected to the above-described HR model. After being subjected to different conditions. After being subjected to different conditions, cells were stained with 1 µM MitoSox Red (ex/em: 510/580 nm) at 37^0C for 30 min at room temperature to detect changes in mitochondrial O_2^- levels. After washing twice with PBS 1X, the different fluorescence intensities were measured using the microplate reader. MitoSox Red-stained cells were captured using the ApoTome 2 and the images were then

reconstructed from individual tiles (X:6, Y:9) using ZEN Blue 2.5 software (Carl Zeiss). The total MitoSox Red intensity was expressed as a percentage value relative to the normal control. Experiments were performed in triplicate.

Statistical analysis

Data are presented as means ± standard error (SD). Differences between the two groups were evaluated by one-way analysis of variance (ANOVA) and Tukey test. Differences with a p-value ≤ 0.05 was considered significant.

3. RESULTS

Hypoxic or reoxygenated mitochondria may produce excess superoxide (O_2^-) and H_2O_2. In this research, to clear the mitochondrial protective mechanism of VGE, we assessed O_2^- and H_2O_2 production in HR-subjected H9C2 cells with or without supplement of this extract.

3.2. VGE significantly reduces H_2O_2 level in HR-exposed H9C2 cells

H_2O_2 is lipid-soluble and can diffuse freely across membranes, acting as a physiological second messenger signaling molecule [9]. The redox-regulated molecular targets are proteins that respond to redox states with a change in conformation, stability, ... In this study, the changes in H_2O_2 level were determined indirectly through the changing in fluorescent intensity of CM-H_2DCFDA kit in Figure 1, Figure 2, Table 1.

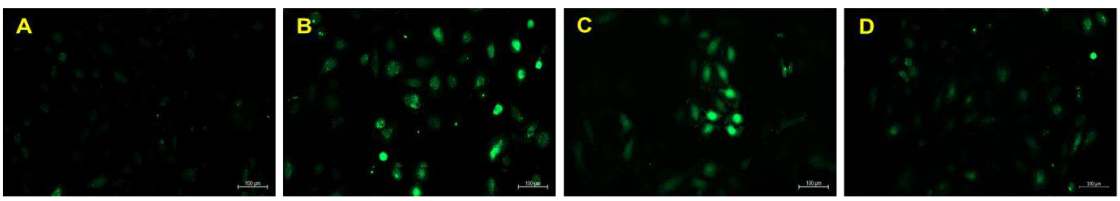

Figure 1. The images of H9C2 cells-stained CM-H_2DCFDA dye

A. Normal control: The cells were cultured in normal; B. HR: The cells were grown in HR condition; C. DMSO: The cells were grown in HR and supplied DMSO 0.1%, D.VGE: The cells were grown in HR and supplied DMSO 0.1% and VGE at dose of 31.25 µg/ml in reoxygenation period. The green color represents CM-H_2DCFDA. The images were taken by Apoptome 2; Magnification: 20x; scale bar: 100 µm.

Figure 1 shows that HR and DMSO cell groups have a significant increase in CM-H_2DCFDA fluorescence signal compared to the control group, shown in bright, sharp blue color and a greater number of cells carrying green color. The group of cells supplemented with VGE at dose of 31.25 µg/ml at the time of reoxygenation had a decrease in CM-H_2DCFDA fluorescence signal (greener green, fewer green-bearing cells) compared to those in either HR or DMSO cell group.

Corresponding to the fluorescence image, an increase in CM-H_2DCFDA fluorescence density is clearly shown in Figure 2. In which, HR condition highly increased the CM-H_2DCFDA fluorescence density of H9C2 cells to 156.81±7.82% (HR group) and 155.03±4.15% (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 [10]. However, supplementation of VGE 31.25 µg/ml to the reoxygenation phase, the CM-H_2DCFDA fluorescence density of the VGE cell group was strongly decreased to 119.67±3.37% compared to the control, which was lower about 30% than the HR group ($p<0.05$). The similar results were also found in total fluorescence intensity assay acquired in 96 well plates (Table 1). This demonstrates that VGE at a dose of 31.25 µg/ml is capable of inhibiting H_2O_2 production in H9C2 cells in HR-

$CoCl_2$ injury; further confirming the described protective effect of VGE in our previous study [6, 7]. The effect of VGE might be explained by the fact that this ginseng contains antioxidant compounds such as Majonoside R2 [7] and Vina-ginsenoside [11]. The potential role of VGE might also be similar to Red ginseng extract [12].

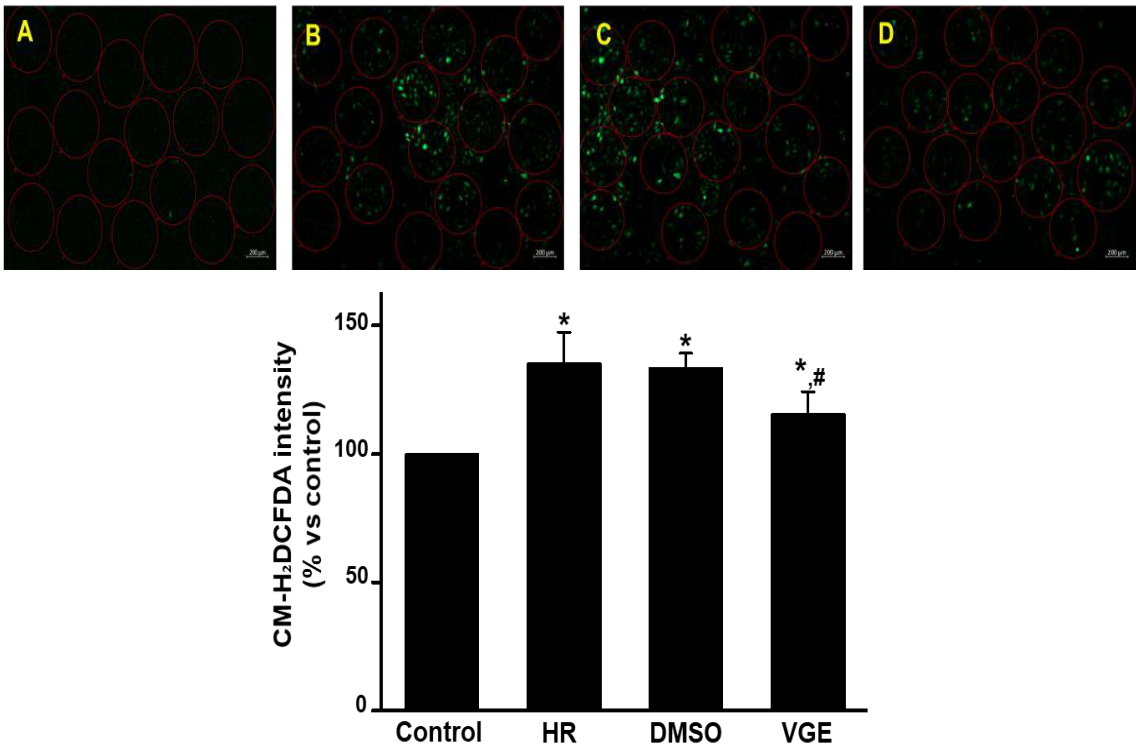

Figure 2. The CM-H_2DCFDA intensity in different conditioned-H9C2 cells

*A, B, C, and D: The representative cell images which are cultured in normal, HR, DMSO, and VGE conditions. Graph indicates fluorescence intensities analyzed in the region of interest of A, B, C, D groups. The green color presented the CM-H_2DCFDA; The red circle: region of interest; the images were taken by Apoptome 2; scale bar: 200 µm. *p<0.05 vs Control. #p<0.05 vs HR.*

Table 1. The total CM-H_2DCFDA intensity

Groups	CM-H_2DCFDA intensity (% vs control)			
	1st repeat	2nd repeat	3rd repeat	Mean ± SD
Control	100	100	100	100
HR	150.133	154.8593	165.4238	156.8054±7.82*
DMSO	155.08	151.01	159.31	155.1333±4.15*
VGE	116.2426	122.9701	119.8102	119.6743±3.36*,#

** p<0.05 vs control, #p<0.05 vs HR*

3.2. VGE markedly reduces O_2^- level in H9C2 cell in HR injury

As mentioned above, mitochondria are the major source and target of ROS production under pathophysiological condition, such as HR [9]. The superoxide radical is a byproduct of a deficient transferrin of 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 [4]. Superoxide is unstable and dismutation of O_2^- to H_2O_2 can occur spontaneously or through a reaction catalyzed by superoxide dismutase [13]. 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 using MitoSOX Red fluorescence kit. The results are shown in Figure 3 and Figure 4.

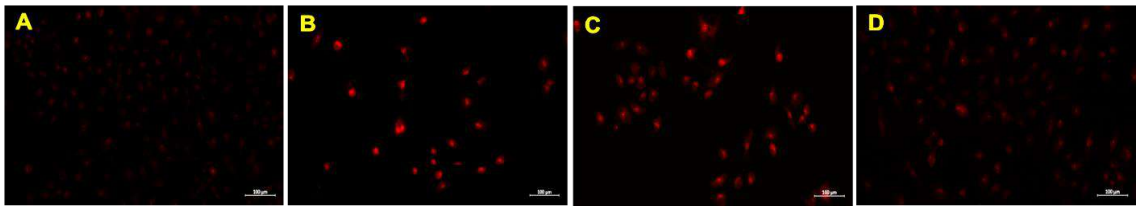

Figure 3. The images of H9C2 cells stained MitoSOX Red dye

*A. Normal control: The cells were cultured in normal; B. HR: The cells were grown in HR condition; C. DMSO: The cells were grown in HR and supplied DMSO 0.1%; D.VGE: The cells were grown in HR and supplied DMSO 0.1% and VGE at dose of 31.25 µg/ml in reoxygenation period. The red color is Mito*SOX *Red. The images were taken by Apoptome 2; Magnification: 20x; scale bar: 100 µm.*

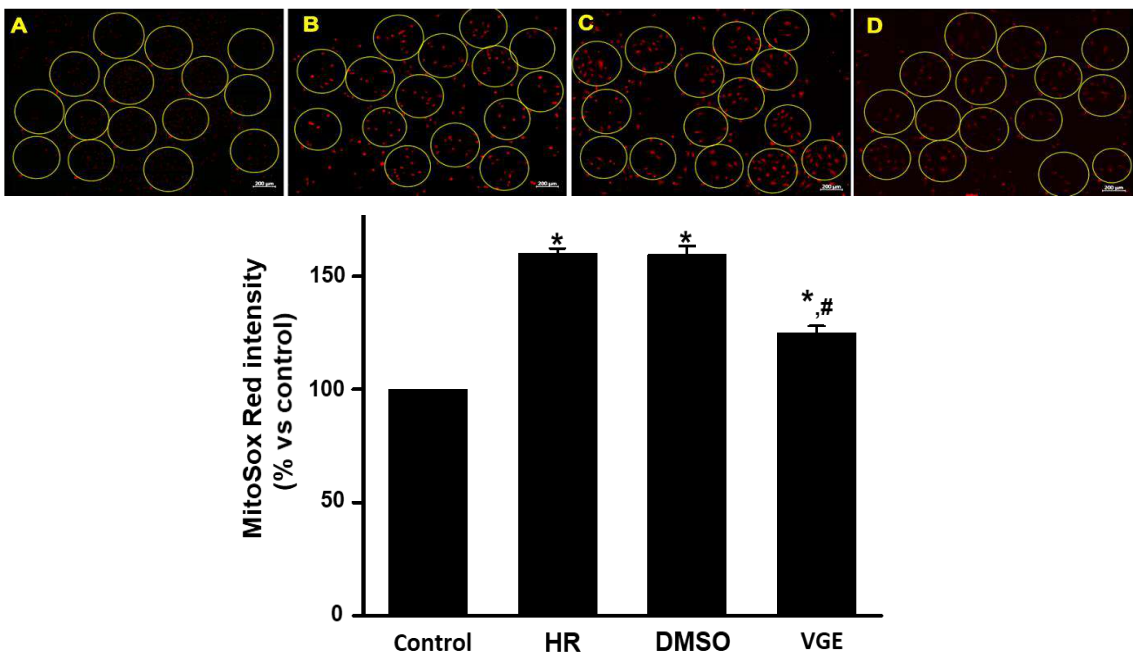

Figure 4. The MitoSOX Red intensity in different conditioned-H9C2 cells

*A, B, C, D: The representative cell image cultured in normal, HR, DMSO, VGE conditions. Graph indicates fluorescence intensities analyzed in the region of interest of A, B, C, D groups. The green color presented the color of MitoSOX Red. The yellow circle: region of interest; the images were taken by Apoptome 2; scale bar: 200 µm. *p<0.05 vs Control. #p<0.05 vs HR.*

The results on Figure 3 show that the red fluorescence signal of MitoSOX Red in the HR and DMSO cells group is significantly increased compared to the control group. This is reflected in a greater number of red cells and a darker, brighter red. The VGE cell group has a fainter red fluorescence signal, and the number of red cells is less.

O_2^- levels corresponded to the change of MitoSOX Red fluorescence intensity was presented in Figure 4. O_2^- levels in HR group and DMSO group were significantly increased to 160.10±2.06% and 161.09±2.67% compared to those in control group (100%). This result is consistent with previous study documenting that $CoCl_2$ 300 µM causes overproduction of ROS, thereby causing cell damage and death [14]. However, post-hypoxic treatment of VGE at a dose of 31.25 µg/ml strongly reduced MitoSox Red intensity levels to 124.72±3.22%. This showed that VGE exerts ability to attenuate the formation of O_2^- in a $CoCl_2$-induced HR injury. This is consistent with the results of reducing H_2O_2 level above (Figure 1, 2 and Table 1). In this study, the protective effect of VGE could be a result of its major saponin as proved in previous study [7], thereby reducing mitochondrial and cellular oxidative stress, subsequently decreased H9C2 death under HR conditions [6, 7].

5. CONCLUSION

Taken together, the present study documents the ability of VGE in attenuating oxidative stress in $CoCl_2$-induced HR injury in vitro via reducing H_2O_2 and O_2^- levels.

Financial support

This work was supported by the Hanoi Pedagogical University 2 Foundation for Sciences and Technology Development via grant number: C.2020.07 and the National Foundation for Science and Technology Development via grant number 106-YS.06-2016.23.

Acknowledgements

We thank Associate Professor Nguyen Lai Thanh, Professor Han Jin, Associate Professor Nguyen Huu Tung, Dr Pham Thi Bich for help and support.

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VALUE OF PERIPHERAL BLOOD COUNT FOR DENGUE SEVERITY PREDICTION

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ABSTRACT

Objective: *Dengue virus infection has diverse clinical manifestations often with unpredictable clinical evolution and outcome. This study was to evaluate the value of the peripheral blood cell in association with the severity of Dengue Infection.* ***Method:*** *This was an observational study on 480 adult patients admitted to Bach Mai Hospital (Vietnam) with Dengue illness between August and October 2017. The diagnosis and classification of Dengue were based on WHO 2009 criteria. Blood samples were collected from patients during acute febrile phase time in hospital and the blood cell indices were analyzed.* ***Results:*** *A total 480 patients participated in the study including 223 patients of Dengue without warning signs (A), 212 patients of Dengue with warning signs (B), and 45 patients of severe Dengue (C). B and C were considered as the severe group, and A was classified as the non-severe group. Compared to the non-severe group, the severe group had significantly lowered hemoglobin, WBC, lymphocytes count and percentage of lymphocyte during acute febrile phase on day 5 of illness. The percentage of neutrophil ≥45%, the percentage of lymphocyte <25% was associated with the severe group.* ***Conclusion****: The percentage of lymphocytes, percentage of neutrophil are likely to predict for severity.*

Keywords: *Peripheral blood count, Dengue with warning signs, severe Dengue*

1. INTRODUCTION

Dengue is a mosquito-borne disease caused by the Dengue virus. In Vietnam Dengue outbreak was first recorder in 1958, in Hanoi by C. Mihov et al [1] and in Southern in 1963 by Scott B Halstead et al [2]. Due to tropical monsoon climate, Dengue appears annually in Vietnam, usually peaks from June to October. Therefore, Dengue is a disease which has been concerned in Vietnam [3, 4].

Dengue virus (DENV) comprises four different serotypes and patients with the disease show diverse clinical manifestations, often with unpredictable clinical evolution and outcome. The clinical features of Dengue illness are ranging from undifferentiated fever, self-limiting to more severe, life threatening forms with hemorrhage or shock syndrome, which are characterized by plasma leakage as a result of increased vascular fragility and permeability[5]. According to the 2009 WHO criteria, Dengue was divided into three severity levels: (A) Dengue without warning signs; (B) Dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increasing hematocrit with decreasing platelets); and (C) severe Dengue (Dengue with severe plasma leakage, severe bleeding, or organ failure) [5, 6], Sooner than that in 1997 WHO classified Dengue into the following three categories: undifferentiated fever, Dengue fever (DF) without hemorrhage or with unusual hemorrhage and Dengue hemorrhagic fever (DHF) with plasma leakage may lead hypovolaemic shock (Dengue shock syndrome, DSS) [6, 7].

The peripheral blood index changes during the illness. Dengue is characterized by leucopenia (White Blood Cells (WBC) ≤5000 cells/mm^3), thrombocytopenia (≤150,000 cells/mm^3), rising hematocrit (5–10%) [8]. The changing of peripheral blood cell index especially platelet during the illness causes the patient's tendency to hemorrhage or increasing of severity. The plasma leakage may be characterized by thrombocytopenia and rising of hematocrit [9]. Clinical risks and laboratory

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Received date: 15/7/2021
Reviewed date: 02/8/2021
Accepted date: 09/8/2021

tests have been studied to forecast the severity of infection [10-12].

This study was conducted with the aim of evaluating the value of peripheral blood cell in predicting the severity of Dengue.

2. MATERIALS AND METHODS

2.1. Study design

We performed an observational study on adult patients admitted to Bach Mai Hospital (Hanoi- Vietnam) with Dengue virus Infection (DI) between August and October 2017.

2.2. Study population

480 adult patients with DI confirmed by a positive NS1 antigen or positive Dengue IgM (SD Bioline Dengue Duo) were included in this study. Patients with a known history of hematologic malignancy, immunosuppression, HIV positive status, known infection with other viral pathogens, pregnancy, used anti coagulation were excluded.

Classification according to 2009 WHO criteria [5][8] there were 223 patients of (A) Dengue without warning signs; 212 patients of (B) Dengue with warning signs, and 45 patients of (C) severe Dengue. B and C were considered as the severe group, and A was classified as the non-severe group.

Based on hemorrhagic manifestations, the patients were also divided into 2 groups: 272 patients without hemorrhage and 208 patients with hemorrhage.

2.3. Data collection

Blood samples were collected from patients with diagnosis Dengue positive during their hospital stay in acute phase (day 2, 3) and day 5 of illness and were analyzed by ADVIA 2120i (SIEMENS) to estimate the blood cell indices. Blood smear were performed to evaluate atypical lymphocytes (lymphocytes were stimulated during viral infection).

2.4. Data analysis

SPSS version 22.0 statistical package was used for the statistical analysis. In the descriptive analysis, the quantitative variables were presented as mean. Two categorical variables were created in the statistical analysis: the non-severe group and the severe group. To test the difference between the variables, the t- test was used. P values of <0.05 were considered significant. To analyze the associated factors, we used a logistic regression model. The odds ratios and respective 95% confidence intervals were estimated.

All of the patients were managed according to the national guidelines on management of Dengue fever in Vietnam, and all patients were provided informed consents.

3. RESULTS

3.1. Patient characteristics

480 patients' data were used in the study. Ratio male/female was 1.33. Mean age was 35.6 ± 14.7, ranged from 16 to 87 years.

3.2. Associated factors of severity

Analytical results (Table 2) showed that hemoglobin, WBC (white blood cell), lymphocyte count and percentage of lymphocyte values were significantly lowered among the severe group compared to the non-severe group on day 5, while in the first 3 days the difference was in the percentage of lymphocyte.

In the univariate analysis, there was a significant association between the severe group and the percentage of neutrophil ≥ 45%, percentage of lymphocyte <25% (Table 1).

Table 1. Analysis of factors associated with the severe group

Variables	OR	95% CI	P value
Percentage of neutrophil (%) <45	1	1.17 – 2.47	0.005
Percentage of neutrophil (%) ≥45	1.7		
Percentage of lymphocyte (%) ≥25	1	1.05 – 2.23	0.03
Percentage of lymphocyte (%) <25	1.52		

OR = odds ratio, CI = confidence interval

Table 2. Distribution of the Index of blood cell during the acute febrile phase according to severity of Dengue

Variables		Day 2-3 of illness				Day 5 of illness		
	A/ B+C	No.	Mean (min=max)	SD	p value	Mean (min=max)	SD	p value
Hemoglobin (g/L)	A	223	144.9	13.8	0.34	138.9	12.9	**0.018**
	B+C	257	143.6	16.5		135.8	15.7	
Hematocrit (L/L)	A	223	0.43	0.04	0.14	0.44	0.04	0.65
	B+C	257	0.42	0.05		0.44	0.05	
Platelet (10^9/L)	A	223	67(14– 316)		0.93	44(10 – 316)		0.22
	B+C	257	76(9 – 361)			42(6 – 329)		
White blood cell (10^9/L)	A	223	3,6(1.1- 14.1)		0.99	3.1(1.12– 9.6)		**0.042**
	B+C	257	3.5(1.2-15.2)			2.8(0.8– 11.0)		

Neutrophil (10^9/L)	A	223	1.63(0.33 – 9.02)		0.26	1.3	0.7	0.23
	B+C	257	1.76(0.31 – 10.65)			1.3	0.9	
Lymphocyte (10^9/L)	A	223	1.01(0.25 – 8.97)		0.12	0.97(0.25-5.13)		**0.03**
	B+C	257	0.95(0.20 – 8.37)			0.83(0.12-5.25)		
Percentage of neutrophil (%)	A	223	50.8	18.9	0.17	28.4	10.8	0.96
	B+C	257	52.9	17.9		28.8	10.8	
Percentage of lymphocyte (%)	A	223	33.9	15.6	**0.013**	31.7	14.1	**0.015**
	B+C	257	30.5	14.8		28.7	13.1	
Percentage of monocyte (%)	A	223	8.9	3.1	0.77	7.3	2.5	0.66
	B+C	257	8.8	2.8		7.2	2.1	

A: Dengue without warning signs, B: Dengue with warning signs, C: severe Dengue

4. DISCUSSION

Some studies have examined the predictive factors for hemorrhage in Dengue patients [13, 14], though bleeding is just one of the manifestations when assessing the severity of Dengue. Besides, there are other signs such as plasma leakage, shock [6]. However, hemorrhage can also be a warning sign of severe Dengue or DHF. The simple blood tests such as peripheral blood cells can help early assessment of hemorrhage but these tests can also help in predicting the severity of the Dengue [10, 15]. The most interested index are usually the hematocrit and platelets. A rapid thrombocytopenia in combination with an increase in hematocrit occurs in patients with plasma leakage causing possibly hemorrhage and shock [9]. In our study there was a statistically significant difference in the WBC, lymphocyte count and percentage of lymphocyte among the Dengue with warning signs and severe Dengue (B+C) compared to the Dengue without warning signs (A). We found an association between the severe group with the higher percentge of neutrophil (≥ 45%), the lower percentage of lymphocyte (<25%). Ralapanawa U et al showed that acute phase neutrophil values were arithmetically higher among DHF patients [13]. Eu-Ahsunthornwattana N et al suggested that those with severe infection (Dengue hemorrhagic fever grade II or worse) also had a lower percentage of lymphocytes [15]. Thus, the change in the composition of white blood cells is also valuable in predicting the severity of Dengue fever.

5. CONCLUSION

Our objective of this study was to predict severity of Dengue using peripheral blood count test in the acute febrile phase of the illness. Hemoglobin, WBC, lymphocyte count and percentage of lymphocytes are lowered during acute febrile phase of the Dengue with warning signs and the severe Dengue. The percentage of lymphocytes, percentage of neutrophil are likely to predict for severity.

Acknowledgments

The authors thank all the technicians in the Center of Hematology and Blood Transfusion – Bach Mai Hospital for their efforts in supporting research.

This study was performed at the Center of Hematology and Blood Transfusion, Center of Tropical Disease, Bach Mai Hospital, 78 Giai Phong street, Hanoi, Vietnam

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THE ROLE OF GLYCATION GAP IN PATIENTS WITH DIABETIC NEPHROPATHY

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ABSTRACT

Objective:*The glycosylated indexes are the essential criteria for monitoring and treating chronic complications in patients with diabetes, especially in diabetic nephropathy. The present study aims to investigate the role of a new index, which is the glycation gap.* ***Results:****The research comprised 50 patients shows that the mean glycation gap is 0.004 ± 0.714 %, ranging from -1.8 to 1.4%. The degree of proteinuria and estimated glomerular filtration rate between two groups of patients with glycation gap <+1 and ≥ +1 are different with statistical significance (p <0.05).* ***Conclusion:*** *The finding showed there is a clear correlation between male and female students with psychological instability - sensitivity and smartphone addiction.*

Keyword: *glycation gap, estimated glomerular filtration rate, albumin-to-creatinine ratio*

1. INTRODUCTION

The prevalence of diabetic nephropathy complications has been rapidly increasing recently globally, producing increased mortality rates and a significantly reduced health-related quality of life in these patients [1]. Early diagnosis and close monitoring of the progression of kidney complications posed particular problems to healthcare providers. Many factors are known to influence HbA1c, including various erythrocytic processes.

Fructosamine may more readily be influenced by very short-term changes in blood glucose levels. Many current research works in the world are focusing on the glycation gap (GG). However, no studies evaluate the role of this index on diabetic nephropathy patients in Vietnam.

2. MATERIALS AND METHODS

Subjects**:** The study population comprised 50 subjects diagnosed with diabetes mellitus based on ADA 2019 standards [1]. The diabetic subjects were selected from outpatients attending from May 2019 to May 2020 at Nephrology and Endocrinology Clinic, University Medical Center at Ho Chi Minh. Exclusion criteria: patients with diagnosed hemoglobinopathies (Hb < 11.8 g/dL), or hypoproteinemia (total serum protein < 6.6 mg/dL or serum albumin < 3.4 g/dL).

Research designs: an analytical cross-sectional study

Research variables:

+ Variables evaluate glycosylation status: fasting blood glucose, fructosamine, HbA1c, the glycation gap.

+ Creatinine clearance based on Cockcroft-Gault Equation (Ccr) [2], estimated glomerular filtration rate (eGFR) based on serum creatinine concentration using Modification of Diet in Renal Disease (MDRD) Study formula and the CKD-EPI equation (Chronic Kidney Disease Epidemiology Collaboration Study) recommended by National Kidney Disease Education Program (NKDEP) guidelines [3, 4].

- *Research methods:* The weight and height of these patients were measured, and laboratory tests were done at the Department of Biochemistry, University Medical Center at Ho Chi Minh city.

+ Measuring of serum HbA1c by high-performance liquid chromatography (HPLC) method on a BioRad D10 analyzer and serum fructosamine by

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Received date: 16/7/2021
Reviewed date: 28/7/2021
Accepted date: 09/8/2021

spectrophotometry assay on a Cobas 8000 analyzer from Roche Diagnostics.

Glycation gap (GG) = measured HbA1c – predicted HbA1c [5].

Predicted HbA1c = a x fructosamine + b [3]

+ Measuring of serum creatinine by the kinetic Jaffe's method with HITACHI 917 automatic analyzer, using IVD-certified reagents kit. Measuring of urine albumin and creatinine by Acon Mission U500 analyzer detects the level of microproteinuria by the urinary albumin-to-creatinine ratio (ACR).

- *Statistical analysis:* Data evaluation and analysis were carried out using Stata 10.0 statistical software, and $p < 0.05$ was considered to indicate statistical significance.

3. RESULTS

The biochemical characteristics of the study population

Table 1-4 showed the data examined in the subjected patients.

Table 1. Demographic and biochemical characteristics of the study population

Parameters	Total (n = 50)	Male (n=25)	Female (n=25)	p
Age (year)	60,2 ± 11,1	58,0 ± 10,0	62,3 ± 11,1	> 0,05
BMI (kg/m^2)	23,3 ± 2,3	23,1 ± 2,0	23,5 ± 2,6	> 0,05
Systolic BP (mmHg)	133,0 ± 10,1	134,3 ± 8,7	131,6 ± 11,4	> 0,05
Diastolic BP (mmHg)	77,2 ± 6,4	78,0 ± 4,8	76,5 ± 7,6	> 0,05
Hemoglobin (g/dL)	12,4 ± 1,5	12,8 ± 1,8	12,0 ± 1,0	> 0,05
Fasting blood glucose (mg/dL)	145,9 ± 56,8	136,2 ± 50,8	155,6 ± 61,7	> 0,05
HbA1c (%)	7,5 ± 0,9	7,4 ± 0,7	7,5 ± 1,1	> 0,05

Table 2. Means of glycosylated indexes in the study population

Parameters	Means
Measured HbA1c (%)	7,5 ± 0,9
Fructosamine (µmol/L)	275,8 ± 66,3
Predicted HbA1c (%)	7,5 ± 0,6
GG (%)	0,004 ± 0,714
GG/HbA1c	-0,009 ± 0,099

The mean glycation gap in the study population is 0,004 ± 0,714 %.

Table 3. eGFR using equations based on Scr and Scys

Parameters	GG		p
	< +1	≥ +1	
Cases	45 (90%)	5 (10%)	
Serum creatinine (mg/dl)	1,4 ± 1,0	4,4 ± 5,0	< 0,001
Ccr (mL/min)	56,4 ± 28,9	25,5 ± 17,6	< 0,05
eGFR MDRD (mL/min/1,73m^2)	63,2 ± 32,5	27,0 ± 19,5	< 0,05
eGFR CKD-EPI (mL/min/1,73m^2)	61,2 ± 28,2	26,4 ± 20,0	< 0,05
ACR (mg/g)	427,5 ± 881,8	3272,2 ± 1100,3	< 0,0001
Macroproteinuria (ACR ≥ 300 mg/g)	10/45 (22,2%)	5/5 (100%)	< 0,01
Chronic kidney disease (GFR < 60 mL/min)	21/45 (46,7%)	5/5 (100%)	< 0,05

Serum creatinine, glomerular filtration rate, and albumin-to-creatinine ratio (ACR) have statistically significant differences between the two groups GG < +1 and GG ≥ +1 (P < 0.05).

Table 4. The proportion of patients in different stages of chronic kidney disease categorized according to the level of glycation gap in the study population

eGFR (mL/min/1,73m^2)	GG		Mean GG	p
	< +1 (%)	≥ +1 (%)		
≥ 90	20,0	0,0	-0,880 ± 0, 442	
60 – 89	33,3	0,0	-0,276 ± 0,513	
30 – 59	35,6	40,0	0,120 ± 0, 343	< 0,05
15 – 29	6,7	40,0	0,415 ± 0,479	
< 15	4,4	20,0	0,744 ± 0,503	
Total	45	5	0,004 ± 0,714	

Table 4 shows that when chronic kidney disease processes to its later stages (from stage 1 to stage 5 according to the CKD classification of the American Society of Nephrology, KDIGO 2012), the proportion of patients with glycation gap GG ≥ + 1 increases compared with other individuals in the group with GG <+1, the difference is statistically significant (P < 0.05).

4. DISCUSSION

The relationship between the fructosamine level and the HbA1c level can be present as linear regression analysis: HbA1c = 0.0083 x Fructosamine + 5.17. The results revealed that mean glycation gap was 0.004 ± 0.714%, ranging from -1.8 to 1.4%. These results were similar to those in studies by Emmanuel Cosson [5] and Ananth U. Nayak [6, 7]. We divided patients into groups based on the GG index: the GG <+1 and GG ≥ +1 groups [5].

We noted that there is a statistically significant difference in the degree of proteinuria between two groups of patients with GG <+1 and GG> +1 (P < 0.0001). The ACR mean value of patients with a GG < +1 is 427.5 ± 881.8 mg/g, while the value of patients with a GG gap ≥ +1 is 3272, 2 ± 1100,3 mg/g. This difference revealed a markedly increasing trend for proteinuria in patients with more positive glycation gaps and supporting the theory in which the glomerular filtration barrier is damaged by advanced glycation end products (AGEs). The more positive GG exists, the more AGEs are formed, so the degree of proteinuria in these patients is greater [8,9].

The results showed statistically significant differences between the two groups GG < +1 and GG ≥ +1 in serum creatinine; creatinine clearance calculated by Cockcroft-Gault formula; estimated glomerular filtration rate estimated using the MDRD and CKD-EPI equations. A study by Ananth U. Nayak [6, 7]. also revealed there are differences in serum creatinine and estimated glomerular filtration rate (eGFR) in three different GG groups; the mean eGFR of negative GG group is 80 ± 27 mL/min, the neutral GG group is 76 ± 26 mL/min and the positive GG group is 79 ± 27 mL/min. However, this study reported that there is no a decline in glomerular filtration rate associated with an increase in the GG gap, possibly due to the influence of the variation in health status among racial and ethnic patients [6, 7]. According to our results, the proportion of patients diagnosed with chronic kidney disease (eGFR <60mL/min/1.73m^2) also differed distinctly between two groups of patients with GG <+1 (46.7%) and GG ≥ +1 (100%). At later stages (from stage 1 to stage 5), the more severe chronic kidney disease deteriorates, the higher proportion of patients with a positive GG (GG ≥ +1) compared to the group of GG < +1. We reported that patients in stage 1 of chronic kidney disease has a negative mean GG (-0.880 ± 0.442), which gradually increased in the patients from stage 1 to 5. The stage-5 patients has a positive mean GG (0.744 ±

0.503). These differences showed that GG has a strong association with the development and rate of progression of diabetes.

5. CONCLUSIONS

The results evaluated the correlation of glycation indices with eGFR and ACR. The glycation gap have a significant correlation with ACR on Vietnamese patients. In addition, the study also proved that GG and eGFR have a significant correlation on the patients with CKD stage 1-5. The study suggest that the glycation gap is a potetial value for monitoring glycemic control and prediction of diabetic nephropathy.

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THE RELATIONSHIP BETWEEN SMARTPHONE ADDICTION AND STUDENTS' PERSONALITY IN UNIVERSITIES IN HANOI

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ABSTRACT

***Objective**: To evaluate the relationship between smartphone addiction and the behaviors of university students in Hanoi. **Methods**: A descriptive cross-sectional study was conducted on 1314 students aged 2-4, all of whom were randomly selected from 36 universities in Hanoi. Participants were instructed to answer online questionnaires using Smartphone Addiction Scale - Short Version (SAS-SV) on Google Form. **Results**: Roughly 55.56% of the test participants are addicted to smartphones. The percentage of female smartphone users is higher than that of their male counterparts. There is a correlation between psychological instability – sensitivity/panic and smartphone addiction. ($r = 0.8$; $p < 0.01$). **Conclusion**: Smartphone addiction contributes to the development of psychological instability – sensitivity/panic in university students*

***Keywords**: smartphone, smartphone addiction, personality, student*

1. INTRODUCTION

Smartphones, defined as portable devices bearing both the functions of traditional mobile phones and computers [1], are popular in economically stable countries. While their benefits have been trialed and proven, recent studies suggest that there are more to smartphones than just convenience and functionality – smartphones possess no less harm to users. Specifically, smartphones and social media enable problematic behaviors and attract narcissistic and oversensitive individuals. Similarly, a correlation between the frequency of social media usage and the manifestation of behavioral and cognitive issues has been observed.

Smartphone addiction is a relatively modern phenomenon [2]. Although it has not been recognized by the Diagnostic Manual of Mental Disorders (DMS-5) and the International Classification of Diseases (ICD – 10), many studies express concern regarding its social and economic effects, and a study predicted that it would be one of the biggest addictions of the 21st century [3]. Smartphone addiction is classified as a behavioral addiction [4]. Similar to substance addiction, behavior addiction is marked by compulsive behaviors that are repeated persistently and irrationally despite their impacts on the patients and those around them [5]. Currently, the "Smartphone addiction Scale Short Version" (SAS – SV), is widely used to evaluate smartphone addiction [6]. A recent report predicts that by 2020, the number of global smartphone users would be 3.5 billion, accounting for 45.4% of the world population [7]. In Vietnam, up to 72% of the population owns smartphones and 68% of the population uses phones to search information online [8]. College students are the most frequent smartphone users. Many of whom consider the devices to be an integral part of their lives [10]. Personality is the characteristic patterns of thoughts, feelings, and behaviors that make a person unique. It is believed that personality arises from within the individual and remains fairly consistent throughout life and many researchers believe that there are five core personality traits. Recent studies show that certain personality traits can contribute to smartphone addiction and vice versa [11]. Therefore, it is important to investigate the relationship between college students' personality and smartphone addiction in order to administer early social intervention.

Due to the aforementioned reason, this study was carried out to: *Evaluate the relationship*

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Reviewed date: 25/7/2021
Accepted date: 09/8/2021

between smartphone addiction and the personality of university students in Hanoi.

2. SUBJECTS AND METHODS

2.1. Subjects

1314 students ages 18 through 25 from 36 universities in Hanoi were selected for the study. The test was conducted from June to October 2020.

2.2. Methods

A descriptive cross-sectional study was conducted on test subjects, using an abridged version of SAS-SV that had been translated into Vietnamese. A Likert scale was used to score each question from 1 – 6, with 1 being "Strongly disagree" and 6 being "Strongly agree". Mobile phone addiction is diagnosed in men with scores ≥ 31, and in women with scores ≥ 33 [6].

A personality test was built based on the 5-factor personality model (Factor Model) [12], which consists of 5 personality circuits:

- Extraversion – sociable/strong.
- Conscientiousness – efficiency/setting.
- Agreeableness – friendly/compassionate.
- Openness to experience – creativity/curiosity.
- Neuroticism – sensitivity/panic.

Factor model is a 45-item questionnaire that had been translated into Vietnamese. A Likert scale was used to score each question from 1 – 5, with 1 being "Strongly disagree", 3 being ''neutral'' and 6 being "Strongly agree". For this study, the Cronbach Alpha value was found as 0.86.

All the above-mentioned subjects were presented in the form of a set of questionaries on Google Form and its link was posted on university student forums. Subjects would personally answer the question once through login by link: https://docs.google.com/forms/d/16DoU8B2WK9eMZpBVc7tjo76RihevLkU43flV8rsG3c/closedform

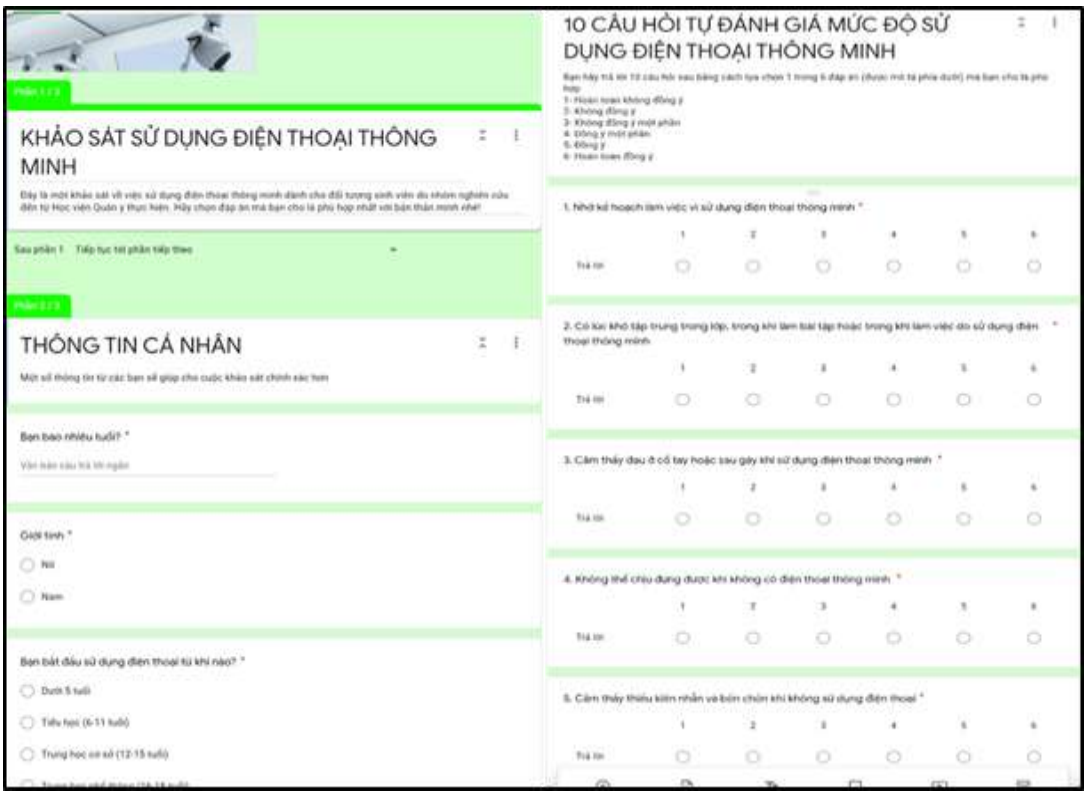

Figure 1. Google Form interface used in the study

2.3. Data analysis

Data obtained from the study were analyzed using SPSS 20 software. Regression analysis was used to analyze the correlation in the study.

3. RESULTS AND DISCUSSION

3.1. General characteristics of the study subjects

Table 1. Gender distribution of study subjects

Subjects	Number (n)	Ratio (%)
Males	373	28,39
Females	941	71,61
Total	**1314**	**100**

Women made up the majority of the study group (71.61%, Table 1). This result is consistent with recent studies [14].

Many previous studies show that women exhibit more attachment to smartphones than men. Women often use smartphones for social purposes, while men often use smartphones for practical purposes and entertainment. Social needs generally require more time than practical needs [13]. Overall, the percentage of women using smartphones is higher than that of men.

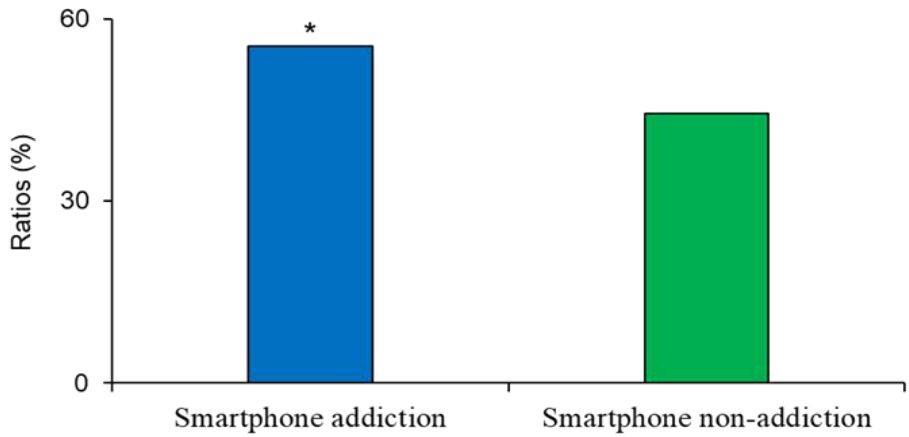

Figure 2. Percentage (%) of students addicted to and not addicted to smartphones

Figure 2 showed that about 55.56% of the students were diagnosed with smartphone addiction. 44.44% of the students were determined non-addicts. In comparison, in the US, 64% of the US population uses smartphones, out of whom 46% are at risk of smartphone addiction [15]. Studies on the prevalence of smartphone addiction in adults in Spain and Belgium reveal that smartphone addicts respectively make up 12.5% and 21.5% of the population [7]. The prevalence of smartphone addiction in this study is bigger than the ones mentioned above possibly due to the relatively recent explosion of information technology in Vietnam [8], and the fact that it only targets students, who are the most likely to be addicted to smartphones.

3.2. The relationship between smartphone addiction and personality of university students in Hanoi

There is a significant correlation between psychological instability – sensitivity/panic and smartphone addiction ($r = 0.82$, $p < 0.01$), (Table 2).

Table 2. Correlation between personality and smartphone addiction

Smartphone addiction	Correlation values (r)
Extraversion – sociable/strong.	0,09
Conscientiousness – efficiency/setting.	0,1
Agreeableness – friendly/compassionate.	0,2
Openness to experience – creativity/curiosity.	0,3
Neuroticism – sensitivity/panic.	0,8**

**p<0,01

The Five-Factor Model is widely used to describe the human personality. It contains five broad dimensions, one of which is neuroticism. Neuroticism is an important personality trait indicating an individual's emotional stability. Moreover, it refers to a tendency to experience such negative feelings such as anxiety, tension, frustration, hostility, impulsivity, depression, and low self-esteem. The less neurotic an individual is, the more meaningful his/her life sounds.

Furthermore, people high in neuroticism experience more stress and are also more over-reacting to adverse experiences which in turns lead to adopt specific coping strategies. Such coping strategies remain constant over time. For example, widely-used coping strategies include alcohol and drug use, web surfing, oversleeping, excessive shopping, denial, daydreaming, and so on [15]. In the study, a large percentage neuroticism students was diagnosed with smartphone addiction. Neuroticism individuals who lack psychological support from their surrounding environments tend to turn to virtual spaces – of which smartphones are effective means. They provide them a relatively safe space and idealized freedom and reduce anxiety. For this reason, these individuals become overly dependent on smartphones. There are recent studies suggesting that people possessing certain personality traits are more likely to be at risk of smartphone addiction [11]. The results of our study enhance this view's credibility. Furthermore, these results also provide evidence for a part of the neuroticism's psychological effect was explained through the mediating role of smartphone addiction in the relationship between neuroticism and quality of life, the mediation was partial. In other words, the relationship between the predictor variable (neuroticism) and the criterion variable (quality of life) was decreased in the presence of the mediator variable but still remained significant. Therefore, there is a great need for more attention to the neurotic students who are prone to addictive behaviours such as smartphone addiction, and this study revealed that it is possible to improve a student's quality of life by managing the mediation paths of this personality trait and other possible characteristics. Educating communicative skills such as courage, polite demands, active listening, etc., helps students feel more satisfied with their interpersonal relationships, and thus less likely to move toward alternative forms of communication – which are likely to be associated with high levels of addiction.

4. CONCLUSIONS

From the results, we draw the following conclusions:

- The percentage of female students at some universities in Hanoi using smartphones (71,61%) are higher than that of male students (28,39%).

- There is a clear correlation between students with psychological instability - sensitivity/panic and smartphone addiction. Individuals with problematic personality are be more likely to be addicted to smartphones.

Limitations

The subjects in this study are a small group who were randomly selected from a number of universities in Hanoi city. Therefore, we need more independent studies with larger sample sizes to evaluate the effects of smartphone addiction on society and mental health. Furthermore, Neuroticism classifies people in a general term and there can be many different components that make overall category as neurotic, so it is important in future studies to discover how these various components interact with smartphone addiction.

Acknowledgements

We would like to express our gratitude to the Department of Physiology, Department of Information and Technology, Vietnam Military Medical University for making this research possible.

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COLD ATMOSPHERIC PLASMA ON TREATMENT CHRONIC SKIN LESSIONS IN DIABETES PATIENT: A CASE REPORT

Nguyen Dinh Minh[2,#], Do Thi Quynh[1,#], Luong Thanh Tu[2], Le Ngoc Thanh[1,2], Do Hoang Tung[3], Vu Thi Thom[1,*]

SUMMARY

Diabetes mellitus has been being a global health problem that leading to increase in diabetes-related complications, including chronic skin lesions. Many studies have shown that atmospheric pressure cold plasma treatment has many positive results in the treatment of acute and chronic wounds. In a case of a patient with Diabetes type 2 with a chronic wound at multiple skin sites and complicated infection. We treated with the PlasmaMed device, a dose of 30 seconds/cm2 of wound area per day. Patient was cared for ulcers according to standard procedures, wound size, infection status and sensation during treatment. After 4 weeks, the wounds reduced inflammation, necrosis and not abnormal sensations at the irradiated area.

Key words: *chronic wound, cold atmospheric plasma, diabetes mellitus*

1. INTROCDUCTION

To date, diabetes mellitus (DM) is considered as one of the global health problems. According to a report by the International Diabetes Federation, the number of people with diabetes worldwide is expected to increase to about 700 million people by 2045 [1]. In Vietnam, it is estimated that in 2020 there will be about 5.76 million people with diabetes. It will be one of the top seven diseases that are likely to cause death and disability in Vietnam by 2030 [2]. According to a study by Harold Brem (2007), foot ulcers accounted for 15% of all patients with diabetes and 84% of amputations due to disorders of healing and opportunistic infections [3]. With the increase of diabetic patients, finding effective wound-healing supportive treatments for diabetic patients is an urgent need to limit the risk of dangerous complications such as infection sepsis or amputation in the patient.

In the recent decades, the use of cold atmospheric plasma (CAP) in medicine has rapidly developed with undeniable results [4]. Many recent in vitro and in vivo studies have shown that CAP has a very good sterilization effect against many different bacterial strains compared to conventional chemical disinfectants [5, 6]. In addition, CAP has been shown to stimulate the growth of fibroblasts and skin epithelial cells in various types of skin lesions or injury [7, 8]. There have been many clinical studies on patients conducted in many countries with different CAP projectors, the treatment results show that CAP is effective in reducing the bacterial load at the wound bed of skin lesions. Accelerated wound healing, reduced treatment time and no undesirable side effects were observed in patients including those with underlying medical conditions such as diabetes mellitus [9]. However, the majority of clinical trial results have reported the efficacy of CAP in acute lesions with good infection control. With a diabetic patient having infected chronic skin lesions, we would like to evaluate the results of CAP treatment in the patient to supporting more evidence of using CAP treatment of chronic lesions with complex pathology status.

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Received date: 20/7/2021
Reviewed date: 28/7/2021
Accepted date: 09/8/2021

2. CASE REPORT

A 72-year-old female patient having type 2 diabetes in 12 years, heart failure, pneumonia. The patient was treated at intensive care unit but started to develop necrosis sores at the big

toe surgery site, the sacral region, an exposed wound of the left instep tendon and get more progression to severe (Figure 1). At that time, patient's HbA1C was 8.9 percent. After that, the patient was transferred to the Department of Plastic, Aesthetic, and Maxillofacial Surgery Collecting to follow up and take care lesions. Discharge on wounds and culturing showed *Acinetobacter Baumannii* and *Klebsiella Pneumoniae* infection, in which *Acinetobacter Baumannii* was resistant to all antibiotic groups. The patient was actively treated with high-dose antibiotics including Merugold 1.V, Linnezolid, Basmicin administered intravenously and controlled blood glucose with 1000mg Glucophase divided 2 times per day. The wounds were cleaned and bandaged 2 times a day with 0.9% saline. In this study, we used a PlasmaMed Machine (a device that the Ministry of Health has licensed for the treatment of skin pathologies) with an input gas source of Argon, a gas flow rate of 8L/min. Surface lesions were cleaned before CAP irradiation with 30 seconds/cm^2 of the wound area, one time per day at E hospital. Wound size was determined with photos of the wound as evidence of wound healing rate during treatment. Patients were asked about sensations at the irradiation site during CAP treatment in third grades (1-no, 2- mild, 3-severe, uncomfortable). If the patient got mild or severe grade of sensation, paint score will be recorded according to the VAS scale (from 0 to 10 points). Progression of the wounds is shown in Figure 1.

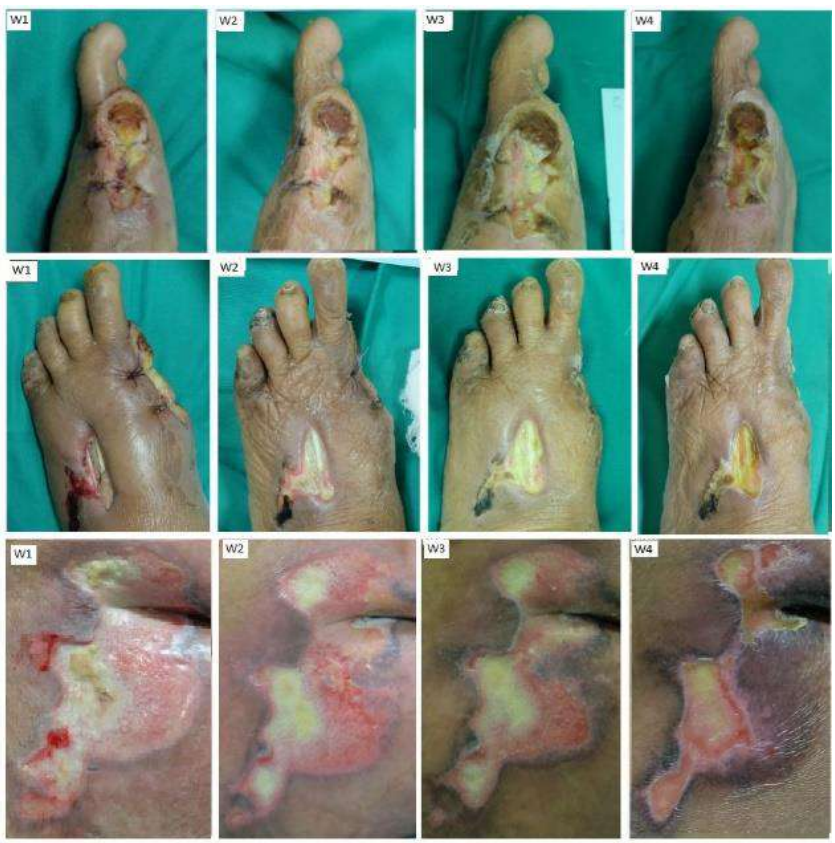

Figure 1: Progression of the maxillofacial wound during cold plasma treatment
(W1, W2, W3, W4 correspond to the CAP treatment time from week 1 to week 4)

When starting plasma treatment, the patient's pressure ulcer wound was relatively severe, the wound border was ulcerated, the percentage of necrotic tissue was high, accounting for about 30% of the wound area, and the amount of pus was quite large.

Progression results of the wound showed that the inflammation, the amount of exudate at the wound improved. No more pus, especially since the necrotic tissue was completely removed in the first week of treatment. After four weeks of treatment, the patient's wound area narrowed to about 10% to 40% depending on the location of the lesion. During the treatment, the patient did not notice any abnormal sensations in the irradiation area.

This study was approved by the ethic committee of Hanoi Medical University IRB 00003121 with decision number 488/GCN-HDDDDDDNCYSSH-DHYHN and Scientific committee of 0E hospital with the volunteer of patient to join the study.

3. DISCUSSION

3.1. CAP's effect on wound healing

The present study is the first case report of plasma adjuvant therapy for complex skin lesions with nosocomial infection in patients with diabetes mellitus. After CAP treatment, patients had improved epithelialization without any local effect noted in this case. To interpret this result, several CAP studies showed that the growth and migration of the cells of the wound-healing complex and the promotion of angiogenesis in the injured tissue [4]. In contrast to the physiological healing process, in diabetic patients, a healing disorder is often attributed to the high levels of inflammatory cytokines such as Interleukin-1β and tumor necrosis factor-α TNF-α. These cytokines are not only present during the period initial acute repair phase, but also maintains high concentrations for a long time, leading to the risk of maintaining a prolonged inflammatory response in the tissue, thereby causing wound healing [9]. In addition, in patients with diabetes, a decrease in the production of insulin-like growth factors IGF-1 and transforming growth factor-β was observed in patients with diabetes, which play an important role in the production of insulin, granulomatous tissue, re-epithelialization, angiogenesis, and ECM formation [10]. Therefore, diabetic patients often have many different skin lesions that tend to slow healing, leading to opportunistic infections, especially dangerous sepsis, which significantly affects health as well as limbs, treatment and care costs. Recent studies in animals and humans have also shown the efficacy of CAP on wound healing in diabetes. Fathollah's study in diabetic rats (2016) showed wound healing rates in diabetic rats compared to non-diabetic mice. In addition, plasma treatment promoted a reduction in blood glucose levels, improving wound healing rate in diseased mice [11]. Mirpour et al. performed patients with type II diabetes and a history of previous antibiotic use, the results showed that the rate of wound reduction in the two groups of patients and the control group was similar, but the rate of decrease in CAP treatment group was higher than antibiotic treatment group. In addition, the bacterial load in CAP-treated wounds was significantly reduced compared with the time of initiation of treatment [12]. The study of Chuangsuwanich et al. (2016) performed on a group of randomly selected patients with pressure ulcers showed an average reduction in wound size of 19.2% after the first week and 46.2 % after second week of treatment with CAP [13]. In our study, the wound size reduction rate was lower. This can be explained by the difference in the study subjects. Specifically, our case had diabetes for many years, extensive wounds accompanied by soft tissue injuries after an accident. This cause can be factors that delay the healing process.

3.2. Inflammation in the wound

During the treatment, we noted that the local inflammation in the patient decreased significantly after one week of treatment. This result may contribute to the explanation of the patient's wound healing efficiency. Many clinical studies also showed a reduction in the frequency as well as the load of bacterial strains in the CAP-treated group [3]. Notably, the patient is an elderly patient having diabetes mellitus heart failure, pneumonia with a complicated chronic wound and an opportunistic infection in the wound tissue. Due

to drug-resistant bacterial infections on the background of severe disease at elder patient, it is difficult to predict effectively on using antibiotics. In fact, during the treatment in the intensive care unit, the patient was also given antibiotics but the ulcer got more severe. Therefore, additional treatment to support healing such as CAP in the ulcers area is very important to limit and prevent the risk of infection, excessive inflammation that damages the intrinsic tissue structures, especially sepsis.

3.3. Feelings of patients during CAP treatment

We did not record any side effects related to the treatment of CAP for the patient, did not record any other sensations during and after the treatment. In the study of Julia Heinlin et al. (2013), there was no difference in pain sensations (according to the VAS scale) before and after each CAP treatment, most patients responded positively to the treatment [14]. Mentelmann et al. (2013) aimed to evaluate the treatment effect of CAP on 20 laser lesions with the Argon CAP device showed that lesions treated with CAP were more effective in avoiding the following skin disorders trauma, aesthetic enhancement and no precancerous skin features occurred within 12 months after treatment [15]. Therefore, the patient was not asked the VAS pain score. Thus, we had no evidence that CAP improves patient pain scores at the end of treatment.

In summary, the patient did not have side effects and sensations at the site of irradiation is a positive signal for applying CAP in clinical treatment for chronic wounds at diabetic patients.

4. CONCLUSION

Despite the background of diabetes, which is an adverse risk factor for the patient's treatment, with complex and extensive trauma, the results of CAP treatment show that the wound healing process is favorable beneficial, no side effects were recorded during the treatment.

Acknowledgements

We would like to thank the Vietnam National University, Hanoi for the project QG.20.06, University of Medicine and Pharmacy and Hospital E for supporting and creating conditions to conduct this work.

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OLEANOIC ACID ALLEVIATES BONE DAMAGE IN A MEDAKA OSTEOPOROSIS MODEL

To Thanh Thuy[1]*, Mai Duy Hung[1], Phuong Thien Thuong[2], Tran Duc Long[1]

ABSTRACT

Objective: *To evaluate bone antiresorptive effect of oleanoic acid (OA) in the rankl:HSE:CFP transgenic medaka (Oryzias latipes) fish model for osteoporosis.* ***Method:*** *Transgenic larvae were treated with OA at different concentrations from 7 days post fertiliztion (dpf) onwards, heatshocked at 9 dpf to induce osteoporosis-like phenotype and fixed at 11 dpf to stained for mineralized bone matrix. Length of the first 15 neural arches were measured and indexes of bone mineralization (I_M) and indexes of mineralization protection (I_P) were calculated to evaluate bone protective effect of OA. Results: Mean I_M of larvae treated with OA at concentration of 7.5 µM, 12.5 µM, 15 µM or 20 µM was significantly higher than that of the DMSO control larvae and similar to that of alendronate-treated larvae. Moreover, 15 µM or 20 µM OA has higher I_P than 25 µg/ml alendronate, a common anti-osteoporosis drug.* ***Conclusion:*** *Oleanoic acid has bone protective effect in the Rankl-induced osteoporosis medaka and this bone protective effect is comparable to alendronate.*

Keywords: *Index of bone mineralization, medaka, mineralized bone matrix, oleanoic acid, osteoporosis, Rankl fish.*

1. INTRODUCTION

Bone is a dynamic tissue which is continuously being remodeled by osteoclasts (bone resorbing/ “eating” cells) and osteoblasts (bone forming cells). Osteoporosis is resulted when bone resorption exceeds bone formation [1]. It is one of the most common skeletal disorders that mainly affects postmenopausal women and aged people. The disease is manifested by a decrease in bone density and structure that leads to bone fragility and high risk of bone fracture [1].

Osteoporosis can be treated by antiosteoporosis drugs, which can be classified as antiresorptive or anabolic. Antiresorptive drugs inhibit bone resorption and bone loss while anabolic drugs promote bone formation and increase bone mass [2]. The most common antiresorptive drugs are bisphosphonates (alendronate, etidronate, ibandronate, risedronate and zoledronate), and to a lesser extent, estrogen and calcitonin. These drugs can inhibit or kill osteoclasts to reduce bone resorption. However, all current medications for osteoporosis still show limitation in their efficacy and safety [3]; thus research and development of new drugs are of high demand, especially when number of affected people is increasing worldwide.

RANKL (receptor activator of nuclear factor kappa-B ligand) is a key stimulator for osteoclastogenesis, the process of formation and activation of osteoclasts. Increased RANKL therefore increase bone resorption, reduce bone mass and bone density, resulting in osteoporosis [7]. A rankl:HSE:CFP transgenic medaka *Oryzias latipes* was generated containing a Rankl-encoding gene *rankl* and a cyan fluorescent protein (CFP)-encoding gen *cfp* under control of a heat-inducible promoter [8]. When being heat-shocked at 39^{o}C, the transgenic fish expresses both CFP, which serves as a visual reporter, and Rankl, which leads to formation and function of osteoclasts. Rankl-induced osteoclasts result in damaged mineralized bone matrix, an osteoporosis-like phenotype

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Received date: 20/7/2021
Reviewed date: 28/7/2021
Accepted date: 09/8/2021

that is mainly observed in the vertebrae, even when fish are still in their larval stages (To et al., 2012). The original Rankl fish was crossed and segregated into sublines with different degrees of bone damage, mainly in neural arch of vertebrae [9]. Using these sublines, we have recently established a method to quantify level of bone damage through the index of bone mineralization (I_M) [10]. I_M value is the total length of mineralized neural arches of the first 15 vertebrae of the larvae. The level of bone damage of a fish is therefore inversely corelated with the I_M, meaning the higher the I_M value, the lower the level of bone damage of the fish [10].

Oleanolic acid (OA) is one of the most common pentacyclic triterpenoid compounds found in many plant species, such as olives, bilberry and pears. It has biological effects potential for treatments of many diseases [4]. A number of *in vivo* studies have showed bone protective effects of OA on animal model of osteoporosis [5, 6]. However, mechanisms underlying bone protective effects of OA still need to be further investigated. In this study, bone-protective effects of OA was evaluated on the *rankl*:HSE:CFP transgenic medaka model for osteoporosis [8] using the I_M method [10].

2. MATERIALS AND METHODS

2.1. Fish lines and fish maintenance

Wildtype fish and c8 *rankl*:HSE:CFP subline transgenic fish whose osteoporosis-like phenotype only observed in neural arches were used [9]. The c8 rankl:HSE:CFP transgenic fish/embryos is hereafter referred to as **Rankl fish/embryos**. Hemizygous Rankl embryos were obtained by crossing homozygous Rankl fish with wild type fish. All fish experiments were performed in accordance with the animal welfare laws and guidelines from Dinh Tien Hoang Institute of Medicine, Hanoi, Vietnam (Approval number: IRB-AR.002).

Fish were raised and maintained according to established procedures at temperature of 28–30^{o}C with light cycles of 14-hour light and 10-hour dark [10, 11]. Transgenic embryos were screened for by fluorescent reporter CFP at 11 days post fertilization (dpf), 2 days after heat-shock.

2.2. OA treatment

Oleanolic acid (OA) was provided by the National institute of Medicinal Materials. OA treatment was performed as described previously [10]. An OA stock solution of 100 mM was prepared in anhydrous DMSO and diluted in E3 solution to final concentrations of 1.25 µM, 7.5 µM, 12.5 µM, 15 µM, and 20 µM (final DMSO concentration was 0.1%). Seven days post fertilization (dpf) Rankl hemizygous larvae were divided randomly into groups and raised in medium containing OA at a tested dose (+Rankl +OA groups), or containing 0.1% DMSO (DMSO control or +Rankl-OA group), or containing alendronate at the dose of 25 µg/ml (positive control or + Rankl + Alen group) in a well of a 24-well cell culture plate. Larvae were heat-shocked at 9 dpf at 39^{o}C for 90 minutes. After a 2-hour recovery period at 30^{o}C, the drug-containing medium was changed, and embryos were raised until 11 dpf when they were screened to confirm CFP expression before being fixed with 4% paraformaldehyde (PFA) for bone staining. A group of heat-shocked wild type embryos (Wt group) were also included as control for bone development without drug treatments.

2.3. Staining of mineralized bone structures

Fish larvae at 11 dpf were fixed and stained with alizarin red (Sigma A5533) to visualize mineralized matrix as previously described [8, 10].

2.4. Quantification of level of bone mineralization and bone mineralization damage

Level of bone mineralization and bone mineralization damage of the fish larvae were determined via Index of bone mineralization (I_M) and Index of mineralization damage (I_D), respectively using the I_M method published previously [10]. Mineralized neural arches of the 11 dpf fish larvae were chosen as representative bone structures to be analyzed as bone damage occurred mostly in these

structures of the Rankl fish larvae. I_M was defined as the sum of lengths of the first 15 mineralized neural arches that is calculated by the formular: $I_M = \sum_{k=1}^{15} L$, where k is the ordinal number of neural arch and L is the length of each arch. Based on the I_M of Rankl fish and of WT fish, Index of mineralization damage I_D of Rank fish was calculated by the formular: $I_D = [I_{M\ (WT)} - I_{M\ (Rankl)}]/I_{M\ (WT)}$ x 100 %, where I_D is the percentage of mineralization damage of neural arches of a larva, $I_{M\ (WT)}$ is the Index of bone mineralization of wild-type fish, and $I_{M\ (Rankl)}$ is Index of bone mineralization of the corresponding Rankl fish. I_M is inversely correlated to I_D.

2.5. Statistical analysis

Student t-tests (two-tailed, unequal variance) or one-way ANOVA followed by Tukey's multiple comparison test were used to compare different experimental groups and to determine significance with Prism 5 software (GraphPad Software Inc., San Diego, CA). Differences were considered statistically significant when $p<0.05$ (marked with one asterisk *); or $p<0.001$ (***), or $p<0.0001$ (****). Results are presented as mean ± S.E.M.

3. RESULTS

3.1. Osteoporosis-like phenotype of Rankl larvae

As expression and function of transgene may change overtime in transgenic animals, Rankl fish were checked to ensure that they still retain suitable osteoporosis-like phenotype for OA experiments. Offspring of c8 Rankl homozygous parents and wild type fish were collected and raised in E3 medium until 7 dpf when they were dechorionated and transferred to a 24-well culture plate. A group of wild-type embryos of the same age were also included for control of a non-transgenic bone phenotype. By 9 dpf, fish larvae were heat-shocked at 39°C for 90 minutes and raised until 11dpf when they were fixed and stained with alizarin red and photographed. Images of mineralized bone structures of the first 15 vertebrae of representative fish of the two groups are presented in Figure 1.

Eleven days post fertilization wild-type larvae had intact mineralized bone structures in the head, the tail (data not shown) and intact vertebral columns of 28 vertebrae, of which the first 15 were shown in Fig.1A-Wt, each having a vertebral body (white asterisk in Fig.1A-Wt), and an intact neural arch (black arrowhead in Fig.1A-Wt), while all c8 Rankl fish showed damaged neural arches (white arrowheads in Fig.1A-Rankl #1-3).Levels of mineralization of fish larvae of the two groups (n=10 for each group) were then determined by mean value of Indexes of mineralization IM as 1283.69 (Wt larvae) and 383.21 (Rankl larvae) (Fig.1B). From these data, the Index of bone mineralization damage ID of the Rankl fish (see formula for ID index in 2.1.4) was determined as 70.5%, meaning these fish had a loss of about 70% of their mineralized neural arches, a damage level suitable for this study. Thus, these Rankl fish were used for OA experiments.

3.2. Oleanolic acid reduces bone loss in Rankl larvae in a dose-dependent manner

OA was tested at five different doses of 1.25, 7.5, 12.5, 15, and 20 µM. Effect of OA at these doses on level of bone damage in the Rankl larvae was demonstrated in Figure 3. Wild type larvae showed intact mineralized bone structures of neural arches (Fig.3A-Wt, black arrowhead) and have significantly higher mean I_M (1267.9) than other experimental groups. Rankl larvae treated with alendronate as positive controls (+Rankl+Alen+DMSO), DMSO control (+Rankl+DMSO), and OA (+Rankl+OA+DMSO) manifested different levels of damage in neural arches (Fig.3A, white arrowheads). ANOVA statistical analysis for mean I_M of these larvae groups revealed that mean I_M of alendronate-treated Rankl larvae (766.1, n=34) is significantly higher than that of the DMSO control group (390.5, n=33, $p<0.001$), illustrating antiresorptive effect of alendronate. Mean I_M of each of four OA-treated groups are significantly higher than that of the DMSO control group (OA 7.5 µM: 653.3, n=30, $p<0.05$; OA 12.5 µM: 653.7, n=30, $p<0.05$; OA 15 µM: 862.2, n=35, $p<0.001$; and

OA 20 µM: 808.6, n=35, p<0.001), indicating that OA at those concentrations reduce bone loss in Rankl larvae. Among OA-treated groups, larvae treated with 15 µM OA have highest mean I_M, followed by larvae treated with 20 µM OA, 12.5 µM OA or 7.5 µM OA. Although mean I_M of the 1.25 µM OA-treated larvae (602.9, n= 33) is higher than that of the DMSO group, the difference is not statistically significant (p>0.05). Thus, OA at concentration of 1.25 µM show no significant bone protective effect in Rankl larvae while OA at higher concentration do protect Rankl larvae form bone loss.

3.3. The indexes of mineralization protection of four effective doses of OA

OA at four doses of 7.5, 12.5, 15 and 20 µM significantly reduce bone loss in Rankl larvae. Their indexes of mineralization protection I_p were calculated as 20.73, 20.75, 37.2 and 32.97, respectively (Figure 3). OA at dose of 15 or 20 µM has stronger bone protective effect than that of alendronate at 25 µg/ml with I_p=29.62. Interestingly, 15 µM OA has stronger protective effect than OA at higher concentration of 20 µM or OA at lower concentration of 7.5 or 12.5 µM.

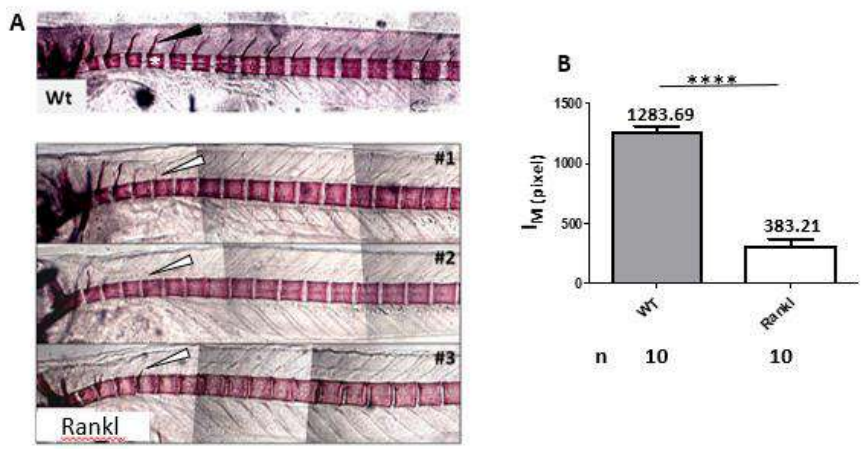

Figure 1. Osteoporosis-like phenotype and quantification of Index of bone mineralization in Rankl larvae used for the study

A. Images of alizarin red-stained the first 15 vertebrae of a normal wild type (Wt) larva and of 3 representative larvae of c8 Rankl fish (Rankl, #1-3). Black arrowhead indicates intact neural arch, white arrowheads indicate damaged neural arches, asterisk notes a normal vertebral body. B. Mean mineralization index I_M of wild-type (Wt) and c8 larvae. n: number of embryos in corresponding fish larvae. (****) p <0.0001.

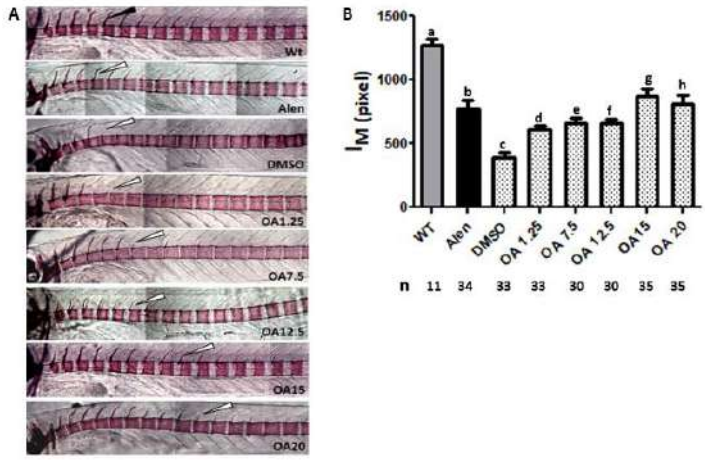

Figure 3. Oleanolic acid (OA) reduces bone damage in Rankl larvae a dose dependent manner.

A. Representative images of alizarin red-stained the first 15 vertebrae of wild-type (Wt) control, DMSO control (+Rankl+DMSO), alendronate treated (+Rankl+DMSO+Alen) groups and five OA-treated groups of 1.25, 7.5; 12.5; 15, and 20 μM as indicated. Black arrowhead indicates an intact mineralized neural arch of wild-type larva; white arrowheads mark damaged mineralized neural arches at different extents. B. Mean mineralization index (I_M) for wildtype (Wt), alendronate-treated (Alen, 25 μg/ml) and OA-treated groups (in μM). a–h: I_M values of corresponding fish group (1267.9, 766.1, 390.5, 602.9, *653.3, 653.7, 862.2, 808.6 respectively). a ‡ b,c,d,e,f ($p < 0.001$) a ‡ g,h ($p<0.01$); b‡c ($p< 0.001$); c vs. d ($p>0.05$) c‡ e,f ($p < 0.05$); c‡g,h ($p < 0.001$) (‡ indicates statistical differences between presented values that were identified by one-way ANOVA with Turkey post hoc test). n: number of embryos in corresponding group. Bars indicate S.E.M. (c) Index of mineralization protection (I_P) of OA at effective doses of 7.5, 12.5, 15, and 20 μM) and alendronate.*

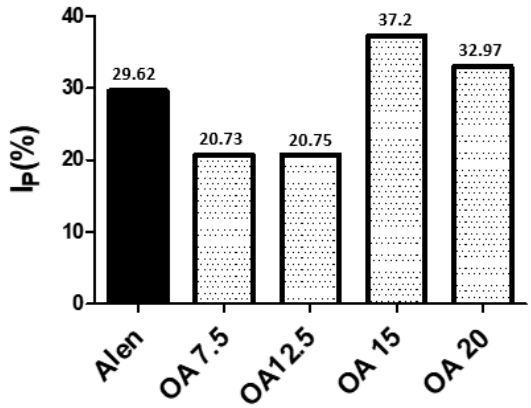

Figure 3. Larvae treated with OA at dose of 15 or 20 μM have higher indexes of mineralization protection (I_P) than those treated with 25 μg/ml alendronate

4. DISCUSSION

In this study, we have shown that OA at concentrations of 7.5, 12.5, 15 or 20 μM reduced bone damage in the *rankl*:HSE:CFP medaka osteoporosis model whose osteoporosis-like phenotype was induced by overexpression of Rankl, a stimulator for osteoclastogenesis and bone resorption. OA at dose of 15 or 20 μM was even more effective than 25 μg/ml alendronate, a commonly used antiresorptive drug.

Previous studies have shown that OA is a highly bioactive substance with anti-inflammatory, anti-cancer, anti-oxidant, anti-hyperlipidemia, and liver protective properties [4]. However, OA activity on bone has not been well studied. Some mouse and cell studies have demonstrated that OA has the ability to prevent osteoporosis by inhibiting the formation of osteoclasts and activating the differentiation of pre-osteoblasts into osteoblasts [5, 6]. Our data, for the first time obtained on a fish model, supports previous studies. We have shown that OA could reduce mineralized bone loss in a Rankl-induced osteoporosis model. Further study is certainly needed to understand mechanisms underlying bone protective effects of OA. However, it is likely that any substance that can reduce bone loss in Rankl larvae should have antiresorptive effect, as the osteoporosis-like phenotype was induced by increased bone resorption in this model. These results also further confirm the value of our medaka osteoporosis model. This fish model, with many experimental

advantages of a fish, together with different bone reporter fish lines available in our laboratory, is valuable for investigation of bone mechanisms of active substances, especially at the cellular levels using live fluorescent imaging techniques.

Our study also provided data on dosage of OA treatment. OA was showed to have dose dependent effects on bone protection in the range of 7.5 to 15 µM; dose of 15 µM OA seems to have highest protective effect, suggesting application of this dose in further study on OA. More importantly, OA was shown to have an equivalent effect with alendronate, a commonly used antiresorptive synthetic drug, in protecting bone from being resorbed. Together with the fact that OA was shown as a safe substance in other studies [4], our data support and encourage further studies to develop this potential natural compound into supplement and drugs for osteoporosis.

5. CONCLUSION

Oleanoic acid has antiresorptive effect on bone in the Rankl-induced osteoporosis medaka and this bone protective effect is comparable to alendronate, a common anti-osteoporosis drug.

Acknowledgements

We thank Pham Van Cuong and Lai Thi Thuy for the help in guiding students to perform experiments, Nguyen Thi Phuong and Nguyen Thi Ha Ly for the help in isolating OA. We are also grateful to Assoc. Prof. Nguyen Lai Thanh, Dr. Vu Thi Thu and CELIFE and staff, Faculty of Biology, VNU University of Science for assistance in microscopic techniques and suggestion in experiment design.

This research was funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under the Grant number 106-YS.06-2014-15.

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ELECTROPHYSIOLOGICAL AND NEUROMUSCULAR CHARACTERISTICS IN MAN PATIENTS WITH CHRONIC GOUT

Tang Thi Hai[1,2], Le Dinh Tung[1,*], Tang Thi Hao[2]

ABSTRACT

Background: *Electrophysiological neuromuscular exploration can be used to diagnose and evaluate the degree of nerve damage in patients with chronic gout.* ***Objectives****: The study was carried out (1) to assess the characterization of peripheral nerve conduction in male patients with chronic gout aged over 30 and (2) to determine the relationship between some clinical and subclinical features of chronic gout with peripheral nerve conduction in the gout's patients.* ***Methods:*** *A cross-sectional descriptive study was conducted on 2 groups: a group of 60 male patients with chronic gout and a control group of 60 normal people who visited Hanoi Medical University Hospital.* ***Results and discussion:*** *The mean age of the study group was 55,45 and that of the control group was 53,25. Peripheral nerve damage in patients with chronic gout occurred in 41/60 patients (68,33%), in which mainly axonal damage.* ***Conclusion:*** *The results have provided useful information on the changes of nerve conduction parameters in chronic gout patients in Vietnam. These parameters should be considered to support in the early diagnosis and prognosis of complications in patients with chronic gout.*

Keywords: *nerve conduction study, chronic gout, peripheral neuropathy.*

1. INTRODUCTION

Gout is a metabolic disorder caused by the deposition of uric acid in tissues due to increased uric acid levels in the blood. Gout occurs mainly in men, middle age, in women, it is common after menopause and now tends to be younger [2], [6], [7].

The onset of gout by acute gout attacks when turning into chronic gout has the main clinical symptoms are gouty arthritis, tophi, gouty kidney disease [2]. Tophi along with osteoarthritis will cause joint deformity, compression of blood vessels, nerves causing vascular and peripheral neuropathy, typically in carpal tunnel syndrome secondary to nerve compression by tophi or ulnar nerve damage at the elbow [7]. Clinically, many patients have neurological symptoms such as numbness, pain, and paresthesias, but examination does not show tophi and is difficult to distinguish from symptoms caused by gouty arthritis and routine clinical examination. The neurologic etiology of these symptoms and manifestations is often overlooked. At that time, neuromuscular electrophysiological exploration can be a method of providing evidence, contributing to the diagnosis, classification and assessment of the correct degree of peripheral nerve damage in patients with chronic gout. Therefore, we carry out this research with two objectives to describe the characteristics of peripheral nerve conduction in a man over 30 with chronic gout who came for examination and treatment at Hanoi Medical University Hospital and to determine the relationship between some clinical and subclinical features of chronic gout with peripheral nerve conduction characteristics in the examined patients.

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Received date: 20/7/2021
Reviewed date: 28/7/2021
Accepted date: 09/8/2021

2. SUBJECTS AND METHODS

2.1. Research subjects

Selection criteria: ***Disease group:*** Men, aged over 30 years, diagnosed with gout according to Bennet and Wood 1968 criteria

[2]. Chronic gout is defined as having at least one of the following conditions: tophi, evidence of chronic gouty bone and joint damage, and kidney damage due to gout. *Control group:* Male, over 30 years old, healthy.

2.2. Methods

Study Design

- A cross-sectional study

Research process: according to the guidelines of the American Society of Electrodiagnostic and Neuromuscular Medicine - AANEM.

Research indicators

- Alcohol use (quantity, time: number of ml/day/year):

- According to the World Health Organization (WHO), alcohol abuse is when men drink more than 3 units of alcohol/day and women drink more than 2 units of alcohol/day, lasting for more than 5 years. One unit of alcohol is equivalent to: ¾ bottle/can of 330ml beer (5%), 1 glass of 100 ml wine (13,5%), 1 glass of draft beer 330ml or 1 cup of spirits 30ml (40%).

- Smoking habits: if the patient smokes ≥ 20 cigarettes/day, the duration is ≥ 5 years.

- History of corticosteroid use: Abuse of corticosteroids when using prednisolone-equivalent preparations at a dose of ≥ 5 mg/day for 3 consecutive months.

- Measure height, weight, calculate BMI according to the formula and evaluate BMI according to WHO standards and exclusively for Asians:

BMI (kg/m^2) = weight (kg)/height^2 (m^2)

- Assess overweight: BMI ≥ 23 kg/m^2

- Distal motor latency: DML (ms), Distal sensory latency: DSL (m/s)

- Motor response amplitude: CMAP (mV), sensory response amplitude: SNAP (mV)

- Motor conduction velocity: MCV (m/s), Sensory conduction velocity: SCV (m/s)

Neuromuscular electrophysiological measurement procedure: recording electrode and reference electrode are placed on the skin/muscle area of the dominant nerve; the stimulation electrode is placed on the path of the nerve (Figure 1).

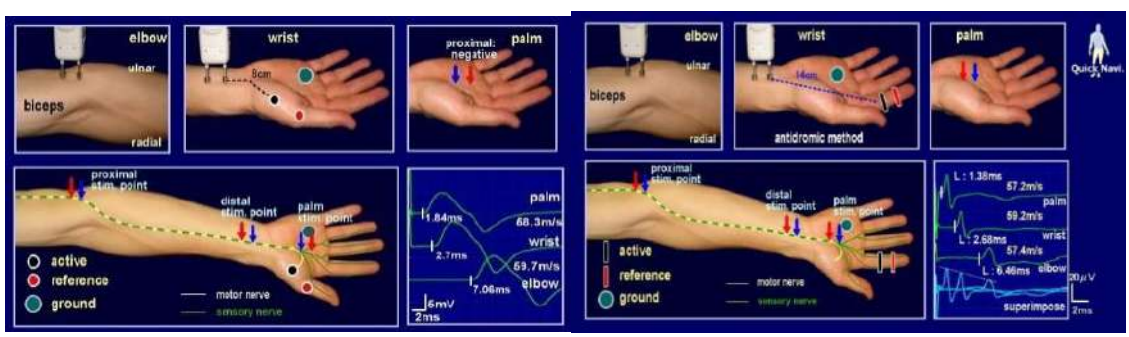

Figure 1. Diagram of measuring motor and sensory conduction velocity of the median nerve

2.3. Data processing

Data were processed using SPSS 16.0 software, described as a percentage (%) if it is a qualitative variable, $\bar{X}$ ± SD if it is a quantitative variable. Use the Student T test when comparing two means for a normally distributed variable and the Wilcoxon signed ranks test when comparing two means for a non-normally distributed variable. The difference was statistically significant when p < 0,05.

2.4. Research ethics: The study complies with ethical regulations in scientific research and has the consent of research participants.

3. RESULTS

3.1. Characteristics of research subjects

In our study, the patient group had an average age of 55,45 ± 11,684 years old, the control group: 53,25 ± 11,696 years old. The percentage of BMI of 23 or more in the disease and control group was 58,3% and 10%,

respectively. Regarding the disease duration, mainly over 10 years, accounting for 60%, only 1 patient in the study had a disease duration of less than 5 years, accounting for 1,67%. 100% of patients in our study have clinical tophi, this is enough to diagnose chronic gout, in which the proportion of patients with 2-4 joints with tophi granules accounts for the largest proportion (41,67%). In the group of chronic gout patients studied, the proportion of risk factors and comorbidities accounted for a high proportion. Among the risk factors, dyslipidemia accounted for 83,33%; followed by alcohol abuse accounting for 73,33%; smoking habit accounted for 41,67%. Among the diseases associated with gout, hypertension accounted for the highest rate at 43,3%; followed by kidney failure accounting for 6,7%.

3.2. Peripheral nerve conduction characteristics in patients with chronic gout

3.2.1. Features of motor and sensory conduction in upper extremities

Table 1. Results of upper extremity nerve conduction in the control group and the disease group

Parameter		Left			Right		
		Disease group ($\bar{x} \pm SD$)	Control group ($\bar{x} \pm SD$)	p	Disease group ($\bar{x} \pm SD$)	Control group ($\bar{x} \pm SD$)	p
Median nerve	DML (ms)	4,01 ± 0,99	3,52 ± 0,36	0,000	4,26 ± 1,59	3,56 ± 0,35	0,001
	AMP (mV)	6,82 ± 2,42	8,45 ± 2,15	0,000	6,79 ± 2,52	8,68 ± 2,16	0,000
	MCV (m/s)	54,18 ± 7,95	55,39 ± 4,07	0,300	54,10 ± 5,33	55,45 ± 3,31	0,097
	DSL (ms)	3,69 ± 1,04	3,32 ± 0,21	0,007	4,17 ± 1,15	3,27 ± 0,22	0,000
	SNAP (µV)	23,05 ± 12,68	33,30 ± 12,58	0,000	18,88 ± 11,78	29,14 ± 10,85	0,000
	SCV (m/s)	48,11 ± 9,99	55,23 ± 4,19	0,000	47,88 ± 8,56	55,55 ± 4,35	0,000
Ulnar nerve	DML (ms)	2,78 ± 0,30	2,70 ± 0,28	0,155	2,74 ± 0,36	2,71 ± 0,27	0,655
	AMP (mV)	7,51 ± 0,77	8,92 ± 1,91	0,000	7,64 ± 1,69	9,40 ± 2,62	0,000
	MCV1 (m/s)	56,92 ± 0,59	56,77 ± 3,96	0,854	56,78 ± 6,84	57,30 ± 4,41	0,619
	MCV2 (m/s)	55,03 ± 6,13	59,30 ± 9,08	0,003	56,13 ± 8,12	59,19 ± 3,78	0,012
	DSL (ms)	3,33 ± 0,25	3,37 ± 0,28	0,508	3,30 ± 0,25	3,28 ± 0,28	0,631
	SNAP (µV)	21,31 ± 10,45	25,89 ± 8,05	0,008	21,28 ± 11,08	25,54 ± 9,08	0,023
	SCV (m/s)	53,51 ± 4,22	54,92 ± 3,74	0,058	54,58 ± 4,95	55,85 ± 4,04	0,129
Radial nerve	DML (ms)	2,84 ± 0,27	2,78±0,42	0,411	2,85 ± 0,25	2,82 ± 0,27	0,578

	AMP (mV)	6,71 ± 1,40	6,72 ± 1,11	0,960	6,58 ± 1,48	6,60 ± 1,35	0,918
	MCV (m/s)	56,06 ± 3,30	57,57 ± 6,34	0,104	56,93 ± 6,70	56,93 ± 4,19	0,919
	DSL (ms)	2,93 ± 0,31	2,88 ± 0,35	0,437	2,86 ±0,32	2,91 ± 0,34	0,444
	SNAP (µV)	18,06 ± 5,15	22,13 ± 6,01	0,000	17,19 ± 5,48	21,93 ± 5,94	0,000
	SCV (m/s)	58,55 ± 4,70	59,72 ± 5,90	0,231	61,26 ± 5,29	61,59 ± 5,38	0,734

Conduction parameters such as distal sensory latency, sensory conduction velocity, motor and sensory amplitude of the median nerve on both sides; amplitude of motor and sensory conduction, motor conduction velocity through the elbow of the ulnar nerve on both sides, sensory conduction amplitude of the radial nerve on both sides of the forearm in the chronic gout group was significantly reduced compared with the control group (p<0,05)

3.2.2. Features of motor conduction, nerve sensation in the lower extremities

Table 2. Lower extremity nerve conduction results in control and disease group

Parameter		**Left**			**Right**		
		Disease group ($\bar{x} \pm SD$)	**Control group** ($\bar{x} \pm SD$)	**p**	**Disease group** ($\bar{x} \pm SD$)	**Control group** ($\bar{x} \pm SD$)	**P**
Peroneal nerve	**DML (ms)**	3,60 ± 0,54	3,56 ± 0,56	0,692	3,71 ± 0,64	3,54 ± 0,56	0,109
	AMP (mV)	3,69 ± 1,52	5,32 ± 1,71	0,000	3,85 ± 1,76	4,89 ± 1,07	0,000
	MCV1 (m/s)	46,72 ± 4,26	47,45 ± 5,38	0,410	47,84 ± 4,94	46,89 ± 7,41	0,411
	MCV2 (m/s)	51,11 ± 8,50	50,40 ± 7,36	0,625	50,01 ± 9,80	51,76 ± 9,65	0,327
	DSL (ms)	3,68 ± 0,47	3,54 ± 0,34	0,067	3,64 ± 0,42	3,61 ± 0,38	0,702
	SNAP (µV)	6,24 ± 4,44	9,69 ± 3,90	0,000	7,50 ± 3,91	9,34 ± 3,98	0,017
	SCV (m/s)	35,70 ± 16,58	44,27 ± 6,75	0,000	42,29 ± 4,63	44,38 ± 4,07	0,015
Tibial nerve	**DML (ms)**	4,01 ± 0,86	3,92 ± 0 ,71	0,546	4,13 ± 0,86	3,98 ± 0,76	0,293
	AMP (mV)	10,83 ± 4,33	14,74 ± 4,90	0,000	11,09 ± 3,93	13,71 ± 5,21	0,002
	MCV1 (m/s)	45,71 ± 3,96	46,40 ± 4,14	0,352	45,38 ± 3,54	46,06 ± 4,76	0,367
	DSL (ms)	3,71 ± 0,23	3,65 ± 0,43	0,354	3,52 ± 0,49	3,49 ± 0,41	0,739
	SNAP (µV)	12,02 ± 5,84	15,65 ± 5,71	0,001	12,14 ± 5,18	15,77 ± 5,73	0,001
	SCV (m/s)	50,30 ± 9,46	50,27 ± 6,11	0,906	52,32 ± 7,57	52,31 ± 5,10	0,957

The results of lower extremity nerve conduction show the amplitude of motor and sensory response of the tibial and peroneal nerves on both sides of the lower leg, and the sensory conduction speed of the superficial peroneal nerve on both sides in gout patients

decreased compared to the control group with statistical significance (p<0,05) (Table 2).

3.2.3. Types of peripheral nerve damage in patients with chronic gout

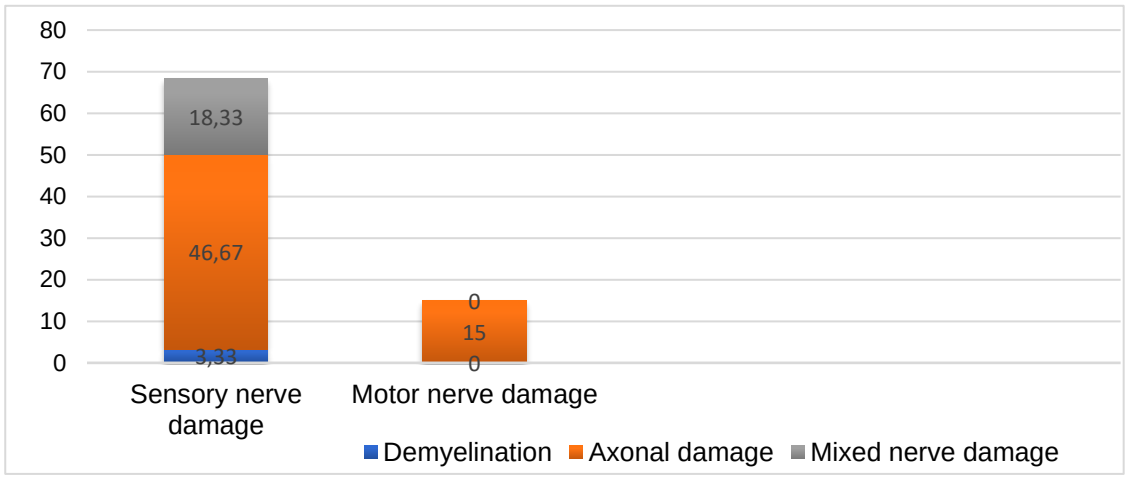

Figure 2. Types of peripheral nerve damage in patients with chronic gout

The results show that peripheral nerve damage is common in patients with chronic gout, with many sensory axonal lesions (46,67%), mixed damage of both demyelinating and sensory axons (8,33%), myelin damage is uncommon. Motor lesions were all axonal lesions (15%, Figure 2).

3.3. Relationship between some clinical and laboratory characteristics of patients with chronic gout and peripheral nerve conduction

In our study, the risk of peripheral nerve damage in chronic gout patients with alcohol abuse, smoking, hypertension, disease duration over 10 years, BMI ≥ 23 were all higher statistically significant compared with the control group. Specifically, the group that abuse alcohol has a 4,371 times higher risk of peripheral nerve damage than the group that does not abuse alcohol. The group with a habit of smoking had a 6,175 times higher risk of peripheral nerve damage than the group without the habit of smoking. This result was similar in the hypertensive group. The group with a disease duration of more than 10 years had a 16,333 times higher risk of peripheral nerve damage than the group with a disease duration of less than 10 years. The group of patients with BMI ≥ 23 has a higher risk of peripheral nerve damage than the group with BMI < 23 (Table 3).

Table 3. Relationship between nerve damage and some factors in chronic gout patients

Peripheral nerve damage	OR	CI
Abuse of alcohol	4,731	1,299- 14,712
Smoking habit	6,175	1,558- 24,484
Time of illness (≥ 10 years)	16,333	4,281- 62,31
Hypertension	6,175	1,558- 24,484
BMI (≥ 23)	4,263	1,341- 13,549
Corticosteroid abuse	4,407	0,889- 21,853

4. DISCUSSION

4.1. Research object characteristics

The group of male patients with chronic gout that we studied had an average age of 55,45 ± 11,68, a mean duration of 9,87 years. This result is similar to the results of many previous studies in Vietnam and around the world [4, 8]. 100% of patients in our study had clinical tophi, in which the proportion of patients with 2-4 joints with tophi granules accounted for the most (41,67%).

Anthropometric indices such as height between the patient group and the control group had no difference, but the index of weight and BMI in the patient group was significantly higher than that of the control group with $p < 0,05$. Among the patient's risk factors, dyslipidemia accounted for 83,33%; followed by alcohol abuse accounting for 73,77%; smoking habit accounted for 41,67%. Among the diseases associated with gout, hypertension accounted for the highest rate at 43,3%. This can be explained by gout or associated with other metabolic disorders that have been shown in domestic and foreign studies [2, 5].

4.2. Features of conduction of peripheral nerves

Peripheral nerve damage in patients with chronic gout is quite high: 68,33% (41/60 patients), this result is similar to some studies in the world [9, 10], in which mainly median nerve damage (39/41), followed by ulnar nerve injury in 9 patients. In our study, sensory damage was seen in 41 patients (68,3%), motor damage was found in only 9 patients. Among these types of lesions are mainly axonal lesions: 46,67% for sensory damage and for motor axonal damage is 15%; and simple myelin damage is rare. The conduction parameters of the median nerve in the two groups showed significant differences in the latency time, amplitude of motor and sensory conduction and sensory conduction velocity on both sides. For ulnar nerve conduction, the amplitude of sensory and motor response and the motor conduction velocity through the elbows on both sides between the patient group and the control group were statistically significant. With radial nerve conduction, the amplitude of sensory response on both sides in the disease group decreased significantly compared with the control group, while other parameters were not different.

The results of peroneal and tibial nerve conduction in the two groups showed that the group of chronic gout patients changed mainly in sensation: the amplitude of sensory response of the superficial peroneal nerve and the sural on both sides as well as the sensory conduction velocity of superficial peroneal nerve decreased significantly compared with the control group. The motor conduction parameters had a marked reduction in amplitude in the tibial and peroneal nerves on both sides.

4.3. Identify some factors related to nerve damage in patients with chronic gout

The results of our study show that the rate of peripheral nerve damage in chronic gout patients with alcohol abuse is 4,371 times higher than the group of patients who do not abuse alcohol; in the group with a habit of smoking is 6,175 times higher than in the group without the habit of smoking, in the group with hypertension is 6,175 times higher than in the group of patients without hypertension; in the group with a disease duration of 10 years or more, it was 16,333 times higher than in the group with a disease duration of less than 10 years, and 4,263 times higher in the group with BMI ≥ 23 than in the group with BMI < 23.

However, when running multivariable correlation between one of the parameters: latency, amplitude and speed of motor conduction, sensation of nerves with some clinical and subclinical factors of the disease. Individual, we found a moderate inverse relationship between median and ulnar nerve sensory amplitudes with the amount of alcohol consumed by the patient; besides, we also found this relationship between the amplitude of sensory response of the ulnar, radial, superficial peroneal and sural with age, while

some other parameters we investigated did not find any correlation.

Thus, the nerve damage in patients with chronic gout, we see most clearly, is the axonal damage that causes a decrease in the amplitude of the sensory response, and is negatively related to the average amount of alcohol in the patient used.

5. CONCLUSION

- Peripheral nerve damage in patients with chronic gout is common (68,33%), in which mainly sensory function damage, motor nerve damage occurs with a low rate.
- Research results show that the main damage in chronic gout is damage to motor and sensory axons, but mainly sensory damage, characterized by reduced amplitude of sensory and motor responses.
- The risk of peripheral nerve damage in chronic gout patients with alcohol abuse, smoking, hypertension, disease duration over 10 years, BMI ≥ 23 were all higher statistically significant compared with the control group. However, when running multivariate correlation, we found most clearly that axonal damage caused a decrease in sensory response amplitude, and was moderately related to the amount of alcohol the patient consumed.

Acknowledgements

The study was carried out at the Electromyography Department, Functional Exploration Unit of Medical Center No. 1, Hanoi Medical University Hospital. The authors would like to thank the teachers, colleagues and research participants for facilitating us to carry out this study.

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THE RELATIONSHIP BETWEEN PLASMA PCSK9 PROTEIN LEVELS AND SOME RISK FACTORS OF DYSLIPIDEMIA AMONG AIR-FORCE PERSONNELS

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ABSTRACT

Objective: *Determine the relationship between plasma PCSK9 protein levels and some risk factors of dyslipidemia among air-force personnels.* ***Methods:*** *This was designed as cross-sectional study in 129 soldiers working on Air Defense - Air Force. Their lipid indexes and PCSK9 levels were measured and some risk factors of dyslipidemia of them were investigated.* ***Results:*** *Among air-force personnels, the average plasma PCSK9 protein levels of smokers was higher than that of non-smokers (225.62 ± 69.25 and 197.62 ± 66.16 ng/ml) and the average plasma PCSK9 protein levels of the drinking alcohol group was higher than that of the non-drinking group (249.65 ± 74.82 and 193.04 ± 60.31 ng/ml).* ***Conclusion:*** *There were a relationship between plasma PCSK9 protein levels,smoking and drinking among air-force personnels.*

Keywords: *Dyslipidemia, plasma PCSK9 protein, air-force personnels.*

1. INTRODUCTION

Dyslipidemia is one of the main risk factors of cardiovascular diseases. In 2013, a survey on the disease structure of military pilots was carried out and showed that, up to 49.6% of military pilots had dyslipidemia [1], and the number of prevalence of dyslipidemia among air-force personnels in a reseach by Do Thanh Tuan (2018) was 64.5% [2]. It means that, the prevalence of dyslipidemia is increasing. It leads to an increase in the risk of dangerous diseases such as atherosclerosis, myocardial infarction, hypertension, stroke [3], thereby causing losses on forces engaged in combat.

Several risk factors are associated with dyslipidemia in air-force personnels such as adverse working conditions, imbalance between a nutrient-rich diet and physical activity, and stress during flight. The study of the cause, the degree of dyslipidemia and prediction of the risk of dyslipidemia in military pilots which is always interested in research to propose the most effective prevention and treatment methods.

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Received date: 21/7/2021
Reviewed date: 28/7/2021
Accepted date: 09/8/2021

Currently, many studies have discovered the role of PCSK9 protein that is strongly associated with levels of low-density lipoprotein cholesterol in the blood plasma and, thereby, occurrence or resistance to atherosclerosis and coronary heart disease [4]. According to Shapiro (2018), it is possible to base on the PCSK9 protein levels to evaluate and predict dyslipidemia and cardiovascular diseases [5]. However, in Vietnam, there has not been any research on the relationship between plasma PCSK9 protein and dyslipidemia in air-force personnels. From the mentioned issues, we would like to initially learn about the relationship between plasma PCSK9 protein levels and some risk factors of dyslipidemia among air-force personnels.

2. METHODS

2.1. Subjects

Including 129 soldiers working on Air Defense - Air Force.

Inclusion criteria:

+ Male

+ Get a health assessment in 2020 according to the process specified in the Air Force Medical Assessment Regulation

Exclusion criteria:

+ Subjects are suffering from acute and chronic conditions affecting health and research indicators.

+ Subjects do not agree to participate in the study.

2.2. Methods

Study design: a prospective, cross-sectional

Research duration: 4 months (from June 2020 to October 2020).

Research indicators:

- Smoking habit was investigated following the standards of the COMMIT (Community Intervention Trial).

- Drinking habit was investigated According to the World Health Organization (1996)

- BMI was assessed according to WHO standards (2002) for Asian-Pacific Islanders: Normal: from 19 to 22.9 (kg/m^2); overweight: from 23 to 24.9 (kg/m^2); obesity: from 25 (kg/m^2).

- Subjects were collected 2ml venous blood in the morning, before breakfast for the laboratory test of blood lipid and plasma PCSK9 protein levels.

- Indexes of blood lipid (Cholesterol, Triglycerides, HDL-C, LDL-C) were conducted on the automated biochemical test machine Chemwell 2902.

- Dyslipidemia was assessed according to the recommendations of the Vietnam Heart Association (2008), when one of the following factors is present: Triglyceride increased > 1.7 mmol/l; Increased cholesterol > 5.2 mmol/l; HDL-C decreased < 1 mmol/l; LDL-C elevation > 3.4 mmol/l.

- Plasma PCSK9 protein levels PCSK9 was quantified according to the ELISA method using KIT Dueset ELISA PCSK9 (R&D manufacturer, USA).

2.3. Data processing

The research data was processed using SPSS 22.0 software based on biomedical statistics method. The results were described in the following form: Mean ($\overline{X}$), standard deviation (SD), percentage (%). Comparing the means by testing quantitative variables on independent samples (Independent-Sample T-Test, Mann-Whitney U). The difference was statistically significant with $p < 0.05$.

2.4. Research ethics

- The research was conducted after obtaining approval from the Air Defense - Air Force Medical Institute and Vietnam Military Medical University. The study protocol was approved by the Ethics Committee of Vietnam Military Medical University in Decision No. 1754/QD-HVQY on April 16th, 2020.

- Subjects voluntarily agreed to participate in the study.

- The research process did not affect the operation of the unit and the health of research subjects.

3. RESULTS AND DISCUSSION

3.1. General characteristics of subjects

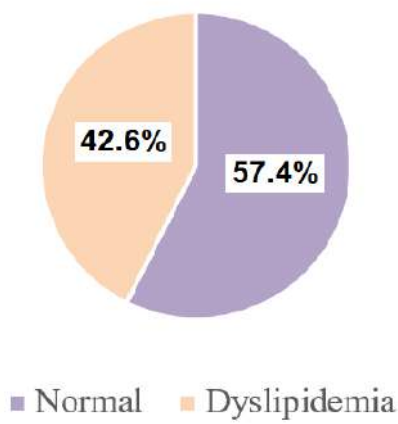

Figure 1. Rate of dyslipidemia

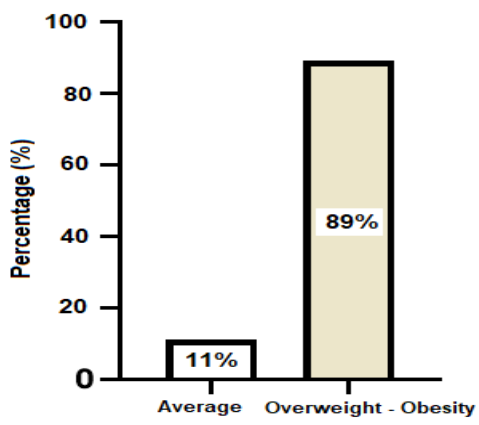

Figure 2. BMI classification of subjects

The majority of the group subjects aged ≥ 35 years old accounted for 75% and aged < 35 years old accounted for 25%. According to the results of many studies showed that the age

from 35 to 40 years of the human body has many changes in physiology and psychology [6]. Therefore, in this study, we also divided into two main groups: group <35 years old and group ≥ 35 years old.

The rate of dyslipidemia was 57.4% higher than the group without dyslipidemia (42.6%) Figure 1.). This result is similar to some results of other authors who are also concerned about dyslipidemia in the Air force [7, 8].

The rate of overweight - obesity accounted for 89% (Figure 2). These characteristics of our research subjects are similar to previous studies [2, 7].

Of the 129 study subjects, 97 soldiers were non-smokers or had quit, accounting for the majority with 75%, 32 non-smokers accounting for 25%. There were 103 people who do not drink alcohol or rarely drink alcohol, accounting for 80%. There were 26 people drink alcohol more than 3 times/week, accounting for 20%.

3.2. The relationship between plasma PCSK9 protein levels and dyslipidemia

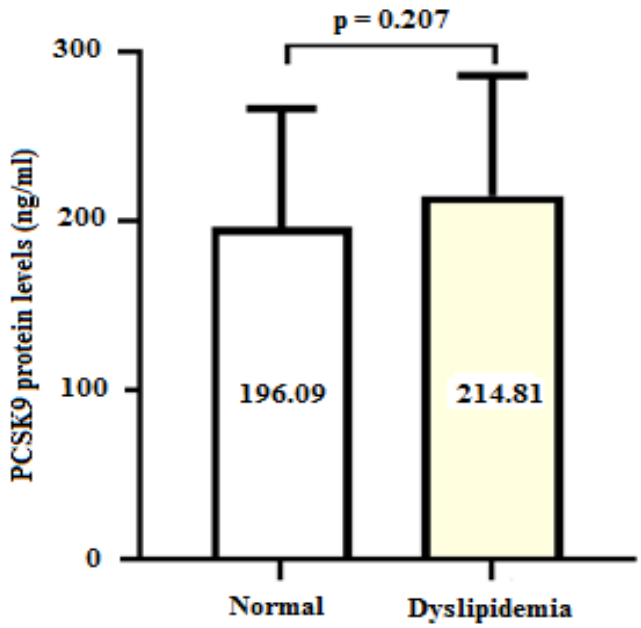

Figure 3. The relationship between dyslipidemia and plasma PCSK9 protein levels

The levels of plasma PCSK9 of the normal group were lower than that of the dyslipidemia group (196.09 ± 70.29 and 214.81 ± 71.18 ng/ml) but the difference was not statistically significant (p>0,, Figure 3). Our results are different from those of Huaying Shen (2020), the author investigated the relationship between plasma PCSK9 protein levels and dyslipidemia in patients with nephrotic syndrome. The results showed that the average PCSK9 levels in the healthy group were lower than that in the disease group (255.57 ng/ml and 310.86 ng/ml). There was a significant difference between the two groups with p = 0.0002 [9].

The difference could be explained by our subject is military force, although they have dyslipidemia, they do not have comorbidities and have different working and living modes from the subjects of the author's research by Huaying Shen.

3.3. The relationship between plasma PCSK9 protein levels and some risk factors of dyslipidemia

Table 1. The relationship between plasma PCSK9 protein levels, age and BMI

Characteristics	Group	PCSK9 levels (ng/ml)		
		$\overline{X}$ ± SD	Min-Max	p
Age	< 35 years old (n=32)	197.52 ± 61.56	71.2 – 378.15	0.312
	≥ 35 years old (n=97)	207.72 ± 68.91	118.25 – 382.0	
BMI	Normal (n=14)	188.21 ± 55.97	111.4 – 319.9	0.372
	Overweight - Obesity (n=115)	206.43 ± 68.36	71.2 – 382.0	

According to Table 1, the group of subjects with aged under 35 years old had average plasma PCSK9 protein levels of 197.52 ± 61.56 ng/ml, while the group of subjects with aged above 35 years old had higher average plasma PCSK9 protein level with a value of 207.72 ± 68.91 ng/ml. However, this difference was not statistically significant with p>0.05. The two-way ANOVA test also was applied to analyze the interaction effect of age factor and dyslipidemia on plasma PCSK9 protein levels (data not show). We found that, there was no interaction effect of the age group and dyslipidemia factors on plasma PCSK9 protein levels among air-forces. Our study is different from the study by Susan (2009), the author investigated the relationship between the anthropometric body indexes and PCSK9 levels in 3138 people. The author has shown a weak positive correlation between age and PCSK9 content with r = 0.18 and p<0.0001. For males, the cut-point is 50 years old, and for women, the time before and after menopause ranges from 45-50 years old. The cut-point is defined as the time period when the body begins to change physiologically most markedly [10]. In our study, due to the age of 35 years, this increasing trend is not really clear.

Table 2. The relationship between plasma PCSK9 protein levels and smoking and drinking habits

Characteristics	Group	PCSK9 levels (ng/ml)		
		$\overline{X}$ ± SD	Min-Max	p
Cigarette	Non-smokers (n=97)	197.62 ± 66.16	71.2 – 382.0	0.048
	Smokers (n=32)	225.14 ± 66.98	118.25 – 357.65	
Alcohol	Non-drinking (n =103)	193.04 ± 60.31	71.2 – 382.2	0.001
	Drinking (n= 26)	249.65 ± 74.82	112.0 – 372.3	

The average plasma PCSK9 protein levels in the smoker group were 225.14 ± 66.98 ng/ml, higher than the average PCSK9 protein levels in the non-smoking group: 197.62 ±

66.16 ng/ml. This difference is statistically significant with p<0.05. Currently, in the world and in Vietnam, there are no studies on the relationship between PCSK9 levels and smokers and non-smokers.

The group of alcohol drinkers had an average PCSK9 protein levels of 249.65 ± 74.82 ng/ml, which was higher than the average PCSK9 protein levels of the non-drinking group: 193.04 ± 60.31 ng/ml. This difference is statistically significant with p<0.01 (Table 2). Our results are similar to the research by Lee (2019) [11]. The author studied 42 alcohol abusers and 25 healthy subjects as a control group. Take samples of cerebrospinal fluid and plasma fluid to quantify PCSK9 levels. The results showed that the PCSK9 levels in the cerebrospinal fluid of the alcohol abuse group was higher than that of the control group, the difference was statistically significant (p<0.001), and there was a positive correlation between the PCSK9 levels in the fluid, cerebrospinal fluid and in plasma. In this case alcohol use, might lead to changes in which genes which turned on or off the function of PCSK9 protein are expressed when alcohol is consumed. Thus, PCSK9 levels in people who abuse alcohol are higher than in the control group.

4. CONCLUSION

Among air-force personnels, plasma PCSK9 protein levels were higher in smokers and drinkers than that in non-smokers and non-drinkers, and no association was found between plasma PCSK9 protein levels and age and overweight - obesity.

Acknowledgements

The authors also thank all the staff at Department of Occupational Military Medicine, Vietnam Military Medical University and Air Defense - Air Force Medical Institute for their cooperation.

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ASSESSMENT THE CLINICAL CHARACTERISTICS OF DIABETIC PATIENTS HAVING RECOMMENDATION ON PHYSICAL ACTIVITY FOR HEALTH PUBLISHED BY WHO

Nguyen Thi Tam[1,*], Pham Thang[2], Vu Thi Thanh Huyen[2,3]

ABSTRACT

Objective: *To assess the clinical characteristics of diabetic patients who having global recommendation on physical activity for health published by World Health Organization (WHO).* ***Subjects and methods:*** *A cross-sectional study was conducted at the Outpatient Department, Dong Anh General Hospital from January 2016 to October 2016. The level of physical activity was determined by the GPAQ (Global Physical Activity Questionnaire) questionnaire. Reaching the recommendations of WHO was defined as ≥ 600 METs-minute/week. Clinical and laboratory features were determined including age, sex, anthropometric indices, blood pressure and fasting blood glucose.* ***Results:*** *The study was conducted on 295 diabetic patients with the level of physical activity as recommended by WHO with the proportion of men being 42.0%. The average age of the study population is 62.8 ± 9.2 years. The main physical activity of patients with diabetes was at working time and leisure time. Daily sedentary time was 223 minutes/day for women and 186 minutes/day for men. In subjects with a physical activity level that met WHO recommendations, the mean BMI and Waist Hip Ratio (WHR) were within normal range. The fasting blood glucose was 7.0 mmol/l in men and 6.9 mmol/l in women.* ***Conclusion:*** *Physical activity in patients with diabetes that meets WHO recommendations is mainly at work and at leisure. Blood glucose and some cardiovascular risk factors such as BMI, WHR and blood pressure were well controlled in this patient population.*

Keywords: *physical activity, diabetic, cardiovascular risk factors.*

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia, caused by absolute and/or relative insulin deficiency (insulin resistance). The prevalence of diabetic is increasing, according to the World Health Organization (WHO), the prevalence of diabetes in 2025 is estimated to 5.4%, i.e. 300 million patients. This is a big problem of global health. In Vietnam, the prevalence of diabetes was 2.7% in 2002 and increased to 5.7% in 2008 [1]. This rapid increasing is closely related to lifestyle changes such as unhealthy diet, reduced physical activity.

The chronic condition has many serious complications in cardiovascular, renal, ocular, neurological diseases. Diabetes reduces quality of life and the patient's life expectancy. According to WHO, about 4 million deaths each year are related to hyperglycemia. This is followed by an increase in direct and indirect costs for diabetic patietns by themselves, their family member, the health sector as well as the whole society [2].

The diabetic managment is aimed to achieve the target glycemic index, delay the occurrence of complications, improve the patient's quality of life and reduce the burden caused by this disease. To gain that goal, treatment requires a combination of pharmacological and non-pharmacological measures. In Vietnam, the proportion of diabetes with a level of physical activity that meets WHO recommendations is relatively high (79%) [3]. Clinical and subclinical data are needed for better therapeutic intervention and control of diabetes in patients with good adherence to physical activity. However, there have been limited publications on this issue in Vietnam. Therefore, we conducted this study

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Received date: 21/7/2021
Reviewed date: 02/8/2021
Accepted date: 09/8/2021

with the goal of describing the clinical and subclinical characteristics of diabetic patients whose physical activity level met the recommendations of the WHO.

2. SUBJECTS AND METHODS

2.1. Subjects

Diabetic patients who had been diagnosed as type 2 diabetes are being treated as outpatient clinics, Dong Anh General Hospital who meet the following criteria:

Inclusion criteria: - Have a level of physical activity that meets WHO recommendations:

Assessment by GPAQ (Global Physical Activity questionnaire) [4]: The questionnaire includes 16 questions about frequency (day/week), time (hour or minute/day) used for physical activity severe or moderate level in 3 areas: 1) Activity at work (P1- P6). 2) Walking activities (P7- P9). 3) Recreational activities (P10- P15), the question on sedentary (P16). When using GPAQ, 4 METs were assigned to the time spent in moderate-intensity activities and 8 METs in vigorous-intensity activities Total METs-minutes/week calculated by the summary of all domains. Physical activity level: The level of physical activity is considered to meet WHO recommendations when it reaches 600 METs-minute/week.

- Ability to answer interviews and conduct exploratory tests

Exclusion criteria:

- Have severe acute diseases or acute complications of diabetes
- The patient did not agree to participate in the study

2.2. Methods

Design study: Cross-sectional study

Time and location: The study was conducted from January 2016 to October 2016 at the outpatient department, Dong Anh General Hospital.

***Sample size*:** Convinience sampling

Variables

- Physical activity characteristics

Characteristics of physical activity on three parts, including activities at work, walking and leisure activities were conducted using the GPAQ questionnaire.

Sedentary time includes the sitting time, lying down (excluding sleep time) of the patient (minutes/day)

-Clinical, subclinical and general characteristics:

+ Age, gender, education level (low: not graduated from primary school), ethnicity

+ History: high blood pressure, dyslipidemia, family history of diabetes

+ Duration of diabetes, current diabetes medications (lifestyle changes, pills, insulin)

+ Anthropometric indices: weight (kg), BMI (kg/m2), waist circumference (cm), hip circumference (cm), Waist Hip Ratio. Body weight was measured in light clothing and without shoes. Waist circumference was measured mid-way between the lower rib margin and the iliac crest. Hip circumference was measured at the broadest circumference around the buttock.

+ Systolic blood pressure and diastolic blood pressure (mmHg) were measured after the patient rested for at least 5 minutes

+ Fasting blood glucose (mmol/l)

Data analysis: Using the software SPSS 16.0. Continuous variables are presented as mean and standard deviation, and categorical variables as frequency and percentage. Chi-square test was used to compare two groups.

2.3. Ethical

The study adheres to the ethical issue of biomedical research. All patient information is kept completely confidential and used for research purposes only.

3. RESULTS

The study was conducted on 295 diabetic patients with a recommended level of physical activity. The proportion of men is 42.0%. The mean age of the study population was 62.8 ± 9.2 years.

Table 1. Physical activity characteristics of diabetic patients

Characteristics (n=295) *	Men (n = 124)	Women (n=171)	p
General physical activity (METs- minutes/week)	3577 (4132)	3279 (3653)	> 0.05
Physical activity at work (METs- minutes/week)	1820 (4014)	1379 (3646)	> 0.05
Physical activity when walking (METs-minutes/week)	644 (420)	508 (0)	> 0.05
Physical activity at leisure time (METs- minutes/week)	1112 (919)	1392 (1198)	< 0.05
Sedentary time (minutes/day)	186 (101)	223 (128)	< 0.01

* Values are described by Mean (median)

The main physical activity of diabetic patients was physical activity at work and at leisure. Daily sedentary time is 223 minutes/day for women and 186 minutes/day for men (Table 1).

Table 2. Demographic characteristics of diabetic patients with standard physical activity level

Characteristic	All	Men (n = 124)	Women (n = 171)
Age (years)	62.8 ± 9.2	63.0 ± 9.5	62.6 ± 9.1
Kinh ethnicity	288 (97.6)	121 (97.6)	167 (97.7)
Low education (not graduated primary school)	25 (8.4)	15 (12.1)	10 (5.8)
Family history of diabetes	79 (26.8)	37 (29.8)	42 (24.6)
Diabetic duration (years)	5.0 ± 4.7	4.95 ± 4.9	4.97 ± 4.5
History of hypertension	159 (53.9)	62 (50.0)	97 (56.7)
History of dyslipidemia	125 (42.4)	57 (46.0)	68 (39.8)
Treatment			
Lifestyle	15 (5.0)	5 (4.8)	10 (5.9)
Medication only	234 (79.3)	106 (85.5)	128 (74.9)
Insulin	45 (15.3)	12 (9.7)	33 (19.2)

The mean duration diabetes was 5.0 ± 4.7 years, equivalent in men and women. The most common treatment for diabetes was using medication with 79%. The prevalence of hypertension or dyslipidemia was about 50% (Table 2).

Table 3. Anthropometric and laboratory characteristics of diabetic patients with standard physical activity level

Characteristics	All (n=295)	Men (n = 124)	Women (n = 171)
Weight (kg)	56.6 ± 8.4	52.6 ± 7.4	60.0 ± 7.8
BMI (kg/m^2)	22.3 ± 2.6	22.1 ± 2.8	22.4 ± 2.5
Waist (cm)	84.9 ± 7.8	83.2 ± 7.5	86.1 ± 7.8
Hip (cm)	92.1 ± 6.0	90.7 ± 6.4	93.2 ± 5.3
WHR	0.921 ± 0.054	0.917 ± 0.055	0.923 ± 0.054
Systolic (mmHg)	121.3 ± 14.9	121.0 ± 14.5	121.6 ± 15.2
Diastolic (mmHg)	76.7± 7.8	76.5 ± 8.7	76.9 ± 7.1
Fasting blood glucose (mmol/l)	6.96 ± 1.83	7.00 ± 1.67	6.93 ± 1.94

Patients with standard physical activity meeting WHO recommendations, the mean BMI and WHR in men and women were within normal limits. The systolic blood pressure was not different between men and women; the mean was within the normal range (Table 3).

The fasting blood glucose was 7.00 mmol/l in men and 6.93 mmol/l in women, the difference was not statistically significant.

4. DISCUSION

The results of this study are similar to the published studies. The time of physical activity at work and at leisure time mainly contributes to the total time for physical activity. The physical activity model in our study is similar to the model in some developed countries such as France and US [5, 6]. The results of this study are different from previous published by Trinh T.H. Oanh (2005) in Ho Chi Minh City [7] and Hoang Thu Trang (2013) that conducted in rural communes of Hanoi city [8] showed that the physical activity in working and walking were high. This difference shows the changing trend of physical activity model in urban areas in Vietnam after 10 years and the difference between urban and rural areas of Hanoi today. With the main means of transportation being motorbikes, it has completely changed the model of physical activity, the walking time is decreased, and instead, the amount of physical activity in leisure tends to increase. The prevalence of physical activity during leisure time in our study was 40.0%, higher than the study in Ho Chi Minh City which was 13.5% [7]. The popularity of beauty-fitness clubs, swimming pools, yoga classes contribute to increasing the level of physical activity of people.

Sedentary time includes sitting or lying down time (working, watching television, reading books) but not sleeping time. Our research results are similar to Au Bich Thuy in Can Tho, 4.1 hours/day and lower than Nguyen Do Nguyen (2009), 5.7 ± 3.7 hours/day [9, 10]. According to Tatiana et al (2011), higher sedentary time 3.29 hours/day was a predictor of 64% higher risk of death from cardiovascular causes compared with the group with sedentary time <1.57 hours/day [11]. Thus, although the sedentary time according to studies in our country is different, it is higher than 3.29 hours/day. The above results show that it is necessary to propagate more widely and have an appropriate intervention program to increase the level of physical activity and reduce the sedentary time of the day.

The clinical and subclinical characteristic in diabetic patients with the recommended level of physical activity such as BMI, WHR, systolic blood pressure and diastolic blood pressure were all within the normal range. The research results of author Nguyen Ngoc Tam (2014) also showed that patients with adequate levels of functional activity as recommended by WHO, BMI, waist circumference, and WHR tend to lower than people with inadequate physical activity [12]. Thus, the problem of adherence with non-pharmacological method such as physical activity contributes to the control of associated cardiovascular risk factors in addition to blood glucose values.

5. CONCLUSION

Physical activity in diabetic patients that meets WHO recommendations is mainly at work and at leisure time. Blood glucose and some cardiovascular risk factors such as BMI, WHR and blood pressure were well controlled in this patient population.

Acknowledgements

We would like to thank to all healthcare workers in Outpatient Department, Dong Anh General Hospital for helping me complete the study. Support with inputting of the data from Dr Nguyen T. T. Huong is also gratefully acknowledged. We are deeply grateful to all patients who gave their time to participate in this study.

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PREVALENCE OF SARCOPENIA IN OSTEOPOROSIS PATIENTS

Nguyen The Hoang[1,*], Nguyen Ngoc Tam[1,2], Vu Thi Thanh Huyen[1,2], Nguyen Trung Anh[1,2]

ABSTRACT

***Objective**: To determine the prevalence of sarcopenia in patients with osteoporosis. **Method**: A cross-sectional study was conducted on 253 patients aged 60 years or above admitted to National Geriatric Hospital. Osteoporosis was evaluated by using WHO 2001 criteria and sarcopenia was evaluated by using ASGW 2019 criteria. **Results**: The average age was 72.79 ± 8.71 years old. The prevalence of sarcopenia and severe sarcopenia in osteoporosis patients were 31.5% and 25.9%, respectively. The prevalence of sarcopenia and severe sarcopenia was significantly higher in the osteoporosis group than in the non-osteoporosis group (31.5% versus 25.9%, p< 0.05; and 31.8% versus 8.2%, p< 0.05, respectively). The prevalence of sarcopenia increased as age advanced and male gender. **Conclusion**: The prevalence of sarcopenia in osteoporosis patients was high (57.4%). In both the osteoporosis and non- osteoporosis groups, the prevalence of sarcopenia increased as age advanced and male gender.*

***Keywords:** Sarcopenia, osteoporosis, older patients*

1. INTRODUCTION

Sarcopenia is a decreased muscle mass, muscle strength and physical activity condition, mainly in the older people [4]. Worldwide, there have been studies showed that higher muscle mass is closely associated with increased bone mass and reduced fracture risk [4]. Along with the aging population, it will refer to decrease muscle mass, muscle strength and increase osteoporosis [2].

Sarcopenia and osteoporosis are important problems affecting older people health, increasing dependence, hospital admissions and mortality. Besides it affects the economy and society [4]. The changes in muscle and bone that occur continuously can interact and harmonize with each other to create balance [4]. The decrease in absorption or the lack of calcium and vitamin D supply to the body from an early age or a long period of nutritional imbalance in the daily diet is one of the risk factors for sarcopenia and osteoporosis [2, 5].

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Received date: 21/7/2021
Reviewed date: 03/8/2021
Accepted date: 09/8/2021

Results from J. Reiss et al (2019) study on 141 older people in Austria showed that the prevalence of sarcopenia was higher in the osteoporosis group than in the non-osteoporosis group [6]. However, in Vietnam, there are no published studies on the prevalence of sarcopenia in osteoporosis patients. Therefore, we conducted this study with the aim to determine the prevalence of sarcopenia in osteoporosis patients.

2. SUBJECTS AND METHODS

2.1. Subjects

Patients aged 60 years older or above admitted to National Geriatric Hospital.

Inclusion criteria: Subjects were measured bone density by dual X-ray absorptiometry scan (DXA Medic DR C12, Mauguio, France) method to identify and classify into osteoporosis and non-osteoporosis group, according to WHO 2001 criteria.

Exclusion criteria:

+ Having serious acute diseases such as: sepsis, coma due to hypoglycaemia, increased osmotic pressure, metabolic acidosis, severe liver failure, heart failure, acute stroke, mental disorder or delirium

+ Patient or patient's family refused to participate in the study.

2.2. Study design

A cross-sectional study

2.3. Sample size

The sample size was determined using a single population proportion formula:

$n=Z^2_{1-\alpha/2} * [p*(1-p)/d^2]$

n=the required sample size

$Z_{1-\alpha/2}$ = 1.96 (with α = 0.05 and 95% confidence interval)

p=0.361 (prevalence of sarcopenia among patients with osteoporosis [4])

d=precision (assumed as 0.05)

The sample size for our study was calculated to be at least 183 participants.

2.4. Variables

All research subjects were conducted data through pre-designed research medical records. Research variables include: age, gender, living area

Bone density was measured by DXA. It took 10 minutes for each person to do this examination. Osteoporosis was diagnosis by using WHO criteria: T score < 2.5.

Sarcopenia was determined and classified by ASGW 2019 with 3 criteria:

(1) Low muscle strength was defined as handgrip strength <28 kg for men and <18 kg for women (using Jamar 5030J1 hand dynamometer)

(2) Low physical performance was 6-m walk <1.0 m/s

(3) Low muscle mass: Measure by bioimpedance, <7.0 kg/m2 in men and <5.4 kg/m^2 in women (using Inbody 770 machine)

Diagnosis sarcopenia was confirmed by (3) + (2) or (1)

If Criteria 1, 2 and 3 were all met, sarcopenia was considered severe

2.5. Data analysis

Data was entered using Redcap software and data were processed using SPSS 20.0 software. Continuous variables are presented as mean (± standard deviation), and categorical variables as frequency and percentage Comparison of the prevalence of sarcopenia between 2 groups with osteoporosis and without osteoporosis was assessed using Chi-square tests. Two-tailed P values < 0.05 were considered statistically significant.

2.6 Ethical issue

Research ensures all ethical issues in biomedical research.

3. RESULTS

3.1. General characteristics

Our study recruited 253 participants. The general characteristics of the participants were shown in Table 1.

Table 1. General characteristics (n=253)

Characteristics (n=253)	n	%
Age group		
60-69	106	41.9
70-79	86	34.0
≥ 80	61	24.1
Age (Mean ± SD)	**72.8 ± 8.7**	
Gender		
Male	36	14.2
Female	217	85.8
Living area		
Urban	60	23.7
Rural	179	70.8
Mountainous	14	5.5
Osteoporosis		
Yes	143	56.5
No	110	43.5

The mean of age was 72.79 ± 8.71 years old, the age group 60 - 69 was the highest (41.9%), the age group ≥ 80 was lowest with 24.1%. Percentage of female was higher than male (female: 85,8% and male: 14,2%). The proportion of patients living in urban area was highest with 70.8%, followed by rural and mountainous area, were 23,7% and 5,5%. The rate of osteoporosis and non-osteoporosis of our study population was 56.5% (n=143) and 43.5% (n=110), respectively.

3.2. Prevalence of sarcopenia in osteoporosis patients

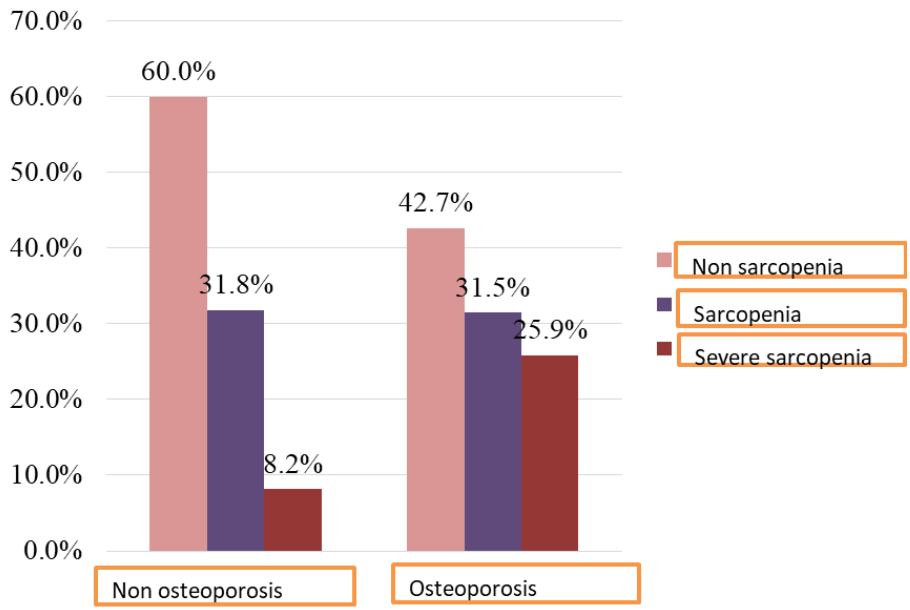

Figure 1. Prevalence of sarcopenia according to osteoporosis status (n=253)

The prevalence of sarcopenia and severe sarcopenia in 143 osteoporosis patients were 31.5% (n=45) and 25.9% (n=37), respectively (Figure 1). The prevalence of sarcopenia and severe sarcopenia in 110 non-osteoporosis patients were 31 % (n=35) and 8.2% (n=9) respectively. The prevalence of severe sarcopenia in osteoporosis group (25.9%) was higher than the non-osteoporosis group (8.2%) with statistical significance.

3.3. The proportion of each component of sarcopenia according to osteoporosis status

The prevalence of decreased walking speed in osteoporosis patients was 32.2%, higher than in non-osteoporosis patients (16.4%). The prevalence of low muscle mass in osteoporosis patients was 56.6%, higher than non- osteoporosis patients (39.1%, Figure 2).

3.4. Prevalence of sarcopenia in osteoporosis patients by age and gender

The proportion of sarcopenia or severe sarcopenia in older patients with osteoporosis was 71.4% in male and was 55.9 % in female

With increasing age, the prevalence of severe sarcopenia also increased. In the osteoporosis group, the prevalence of severe sarcopenia increased gradually by age groups, respectively 11.9%, 23.4% and 51.4%. This is similar in the non-osteoporotic group, the prevalences were 4.3%, 10.3% and 13.0%, respectively (Table 2).

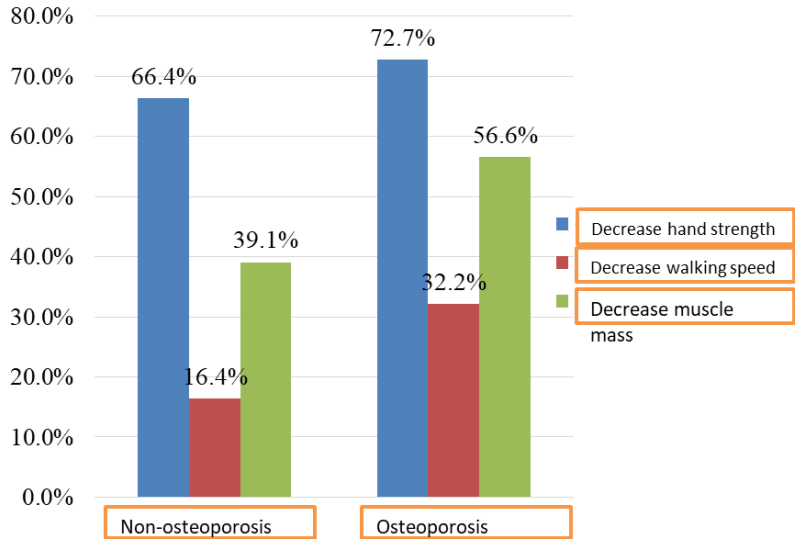

Figure 2: The rate of three components of sarcopenia according to osteoporosis status (n=253)

Table 2. Prevalence of sarcopenia in osteoporosis patients by age and gender (n=253)

			Non-osteoporosis			Osteoporosis		
			Non-Sarcopenia	Sarcopenia	Severe sarcopenia	Non-Sarcopenia	Sarcopenia	Severe sarcopenia
Gender	Male	n	10	11	1	4	7	3
		%	45.5%	50.0%	4.5%	28.6%	50.0%	21.4%
	Female	n	56	24	8	57	38	34
		%	63.6%	27.3%	9.1%	44.2%	29.5%	26.4%
Age group	60-69	n	36	9	2	37	15	7
		%	76.6%	19.1%	4.3%	62.7%	25.4%	11.9%
	70-79	n	20	15	4	18	18	11
		%	51.3%	38.5%	10.3%	38.3%	38.3%	23.4%
	≥ 80	n	10	11	3	6	12	19
		%	42.0%	46.0%	13.0%	16.2%	32.4%	51.4%

4. DISCUSSION

Our study was conducted in total 253 older patients with the mean age of 72.79 ± 8.71. The results showed that the prevalence of sarcopenia in the osteoporosis group was 1.43 times higher than the prevalence of sarcopenia in the non- osteoporosis group. This result is higher than the result in the study of J. Reiss et al [6]. Besides, we also found that the prevalence of severe sarcopenia in osteoporosis patients (25.9%) was 3 times higher than prevalence of severe sarcopenia in non-osteoporotic patients (8.2%). This is consistent with the physiology of muscle mass development and bone bile according to the

study of Greco EA et al., when bone density is reduced, muscle mass will decrease by different mechanisms including malnutrition, life style and genes regulating size [2, 4, 7]. And as the results, patients were at higher risk of falls, trauma, functional disability, hospitalization, mortality and decrease quality of life [6].

Patients with osteoporosis had a 2.4 times higher prevalence of decreased walking speed than that non-osteoporosis. In addition, patients with osteoporosis had a 2-fold higher prevalence of decreased muscle mass than that non- osteoporosis. The difference was statistically significant with $p < 0.05$.

In the results of our study, the prevalence of sarcopenia in general was higher in male than in female, it is similar to the results of a study in Japan [8]. This may be due to a significant decrease in the amount of testosterone hormone in elderly men both in osteoporotic and non-osteoporotic patients. In both of osteoporosis and non-osteoporosis patients, the proportion of sarcopenia had a statistically significant difference by age with $p<0.05$. Specifically, osteoporosis patients aged ≥80 years had more than twice the prevalence of severe sarcopenia than patients aged 70-79 years and more than four times higher than patients aged 60-69 years. This may suggest advanced age as one of the risk factors for sarcopenia. Research by Hirschfeld HP et al (2017) also supports our hypothesis [4]. The strength of the study was the use of DXA as the gold standard to measure bone density. The main weakness was its cross-sectional design in one hospital which may limit the external validity of the study results.

5. CONCLUSION

The prevalence of sarcopenia in osteoporosis patients was high (57.4%). In both the osteoporosis and non- osteoporosis groups, the prevalence of sarcopenia increased as age advanced and male gender. The results showed the role of diagnosis and treatment of sarcopenia and osteoporosis in clinical practice, especially for older people.

Acknowledgements

We would like to thank to doctors and nurses in National Geriatric Hospital for helping me complete the study. We are deeply grateful to all patients who gave their time to participate in our study. Dr Nguyen T. H. Thu is also gratefully acknowledged with controlling the data.

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DETECTION AND QUANTIFICATION OF GROWTH FACTORS IN UMBILICAL CORD MESENCHYMAL STEM CELL-DERIVED EXOSOMES AND UMBILICAL CORD BLOOD-ORIGNATED PLATELET RICH PLASMA

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SUMMARY

Objective: *Describing the expression of several growth factors in exosomes released by umbilical cord-derived mesenchymal stem cells (UCMSCs) and platelet rich plasma (PRP) derived from umbilical cord blood (UCB).* ***Methods:*** *05 UCMSC samples were cultured for the release of exosomes into conditioned media. 05 UCB-dereived plasma samples were used to prepare PRP. Conditioned media was harvested from UCMSCs at passage four (P4) was differentially centrifuged for exosomes. Isolated exosomes and PRP were then investigated for the expression of growth factors. The growth factors which are from exosomes and PRP include: hepatocellular growth factor (HGF), platelet-derived growth factor BB (PDGF-BB), vascular endothelial growth factor A (VEGF-A), and fibroblast growth factor 2 (FGF-2).* ***Results:*** *Both UCMSC-derived exosomes and UCB-derived PRP carried growth factors with different amount. The expression of HGF (2176.82 pg), PDGF-BB (1765.82 pg) and VEGF-A (2336.43 pg) is much greater than FGF-2 (39.64 pg) and HGF (2176.82 pg) is larger than PDGF-BB (1765.82 pg) in the UCB-derived PRP. Regarding UCMSC-derived exosomes, HFG (312.62 pg) and PDGF-BB (674.87 pg) were expressed larger than VEGF-A (72.50 pg) and FGF-2 (34.51 pg).* ***Conclusion:*** *Both UCMSC-derived exosomes and UCB-derived PRP contained abundant amount of growth factors, including HGF, PDGF-BB, VEGF-A, and FGF-2. Expression level of each factor is different and the FGF-2 is the lowest expression in both exosomes and PRP.*

Key words: *umbilical cord blood-derived PRP, platelet rich plasma, UCMSC-derived exosomes, growth factors.*

1. INTRODUCTION

Platelet rich plasma (PRP) is an autologous serum containing a high concentration of platelets and many growth factors. That the high enrichment of growth factors, such as platelet-derived growth factors, epithelial growth factor, insulin-like growth factor, vascular endothelial growth factor and transforming growth factor beta, enables PRP to play an important role in tissue regeneration [1]. Medical applications of PRP are very popular, including cardiac surgery, oral surgery, orthopedics, osteoarthritis, facial plastic surgery, hair restoration, and skin rejuvenation [1]. The further potential of PRP is continuing to develop as a versatile therapy in dermatology [2]. Mechanism under the PRP roles is many growth factors are also known to induce cell proliferation, angiogenesis, and chemotaxis, as well as contain serotonin, dopamine, histamine, adenosine, and calcium, which increase membrane permeability [1]. Additionally, these factors stimulate the stem cell development to compensate the lost cells due to many reasons. Previously, peripheral blood is a source of autologous PRP for treatment. However, according to development of umbilical cord blood banks both in quantity and quality, plasma separated from umbilical cord blood have been investigating as an alternative source for autologous PRP [3].

Extracellular membrane vesicles (EVs) is nano-scale size and enclosed by a lipid bilayer and have spherical or cup-shaped. They are

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Reviewed date: 26/7/2021
Accepted date: 09/8/2021

released by majority of cell types into the extracellular microenvironment and found in culture media or body fluids, such as saliva, plasma, breast milk, amniotic fluid, and urine. EVs can be classified into three population: exosomes, microvesicles or ectosomes, and apoptotic bodies [4]. Among them, exosome population is the most interested by scientists up-to-now, as they are believed as the most potential for application. Many studies have illustrated that exosomes carry variety of functional molecules, from lipids and proteins to genetic materials that are involved in a variety of biological processes. For example, exosomes can enhance osteoclastogenesis, alleviate liver fibrosis, and promote angiogenesis. In addition, exosomes protected cells from oxidative stress induced cell apoptosis, promoted cutaneous wound healing, and human skin rejuvenation [5, 6].

Due to both PRP and exosomes are very promising in the field of tissue regeneration, we would like to investigate the differences between them, especially the expression levels of several growth factors. Thus, this initial study is to compare some growth factors present in umbilical cord blood-derived PRP and UCMSC-derived exosomes. Data generated from this study is important as the first comparison of the PRP and exosomes. Additionally, this will reveal and suggest the further application of both exosomes and allogeneic PRP for treatment, especially in dermatology.

2. MATERIALS AND METHOD

2.1. Materials and subjective

Materials: Umbilical cord blood (UCB) and umbilical cord-derived mescenchymal stem cells (UCMSCs) from healthy donors at Hitech Center - Vinmec General International Hospital.

Subjective: Conditioned media collected from UCMSC cultures PRP activated from UCB plasma.

Research sites: Hitech Center - Vinmec Institute of Applied Science and Regenerative Medicine and Vinmec General International Hospital.

2.2. Methods

UCMSC-derived exosome isolation

Umbilical cord-derived mesenchymal stem cells (UCMSCs) were received from the EV groups at the passage two. The cells were thawed and seeded as passage three into a cell culture flask T75 cell culture flasks (Nunc, Thermo Scientific, Massachusetts, United States) containing StemMACS culture medium (Miltenyi Biotec, Bergisch Gladbach, Germany) with density of 375 x 10^3 cells /cm^2. The flask was surface-coated with CTS™ CELLstart™ substrate (Gibco, Massachusetts, USA) diluted in PBS at the rate of 1: 300 before cell seeding. Cells then were cultured at the condition of 5% CO_2, 37°C to reach 80% confluency. Next, cells were split using CTS™ TrypLE™ Select Enzyme (Thermo Fisher Scientific, USA) for the next passage. At the culture of P4, conditioned media containing exosomes was collected when the cells reach 80% confluency for further exosome isolation. The UCMSCs were harvested for marker analysis.

Conditioned media that contain exosomes was firstly centrifuged at 300 x g 10 min 4°C and then at 2,000 × g for 10 min to remove cell debris and apoptotic bodies. Supernatant was followed by a centrifuge at 16,500 × g for 30 min at 4°C to remove microvesicles. Finally, exosomes (EXs) were collected by a centrifuge at 100,000 × g for 90 min at 4°C (Optima XPN-100 Ultracentrifuge, Beckman Coulter, California, USA). The EX pellets were resuspended in 100 µL PBS and stored at −80°C for further uses.

Umbilical cord blood-derived platelet rich plasma preparation

Human umbilical cord blood (UCB) samples were processed routinely at Hitech Center (Vinmec General International Hospital) to obtain umbilical cord blood plasma (UCBP) fraction and mononuclear cell population. Briefly, the red blood cells was separated by centrifugation at 1400 rpm for 20 min. Next, the buffy coats (mononuclear cell) and plasma

fraction are separated by a centrifugation at 80 rpm for 10 min. The mononuclear cell population were stored in liquid nitrogen. UCBP were used to prepare PRP by a centrifugation at 3600 rpm for 10 min [7]. UCBP-PRP was activated by using calcium chloride and incubated at 37°C for 30 - 45 min. The cell types presented in the UCBP and PRP platelet concentration in the UCB-PRP was calculated by automated hematology analyzer.The platelet recovery efficiency in the PRP samples was calculated using:

$$\text{Recovery (\%)} = \frac{V_{PRP} x [PLT]_{PRP}}{V_{UCBP} + [PLT]_{UCBP}} \text{ x } 100$$

- V_{UCBP}: volume of the umbilical cord blood plasma sample
- V_{PRP}: volume of the platelet rich plasma
- $[PLT]_{UCBP}$: platelet concentration in the umbilical cord blood plasma sample
- $[PLT]_{PRP}$: platelet concentration in the PRP sample

Growth factor analysis

Growth factors concentrations such as fibroblast growth factor 2 (FGF-2), hepatocyte growth factor (HGF), platelet-derived growth factor-BB (PDGF-BB), and vascular endothelial growth factor A (VEGF-A) (pg/ml) were measured by Luminex assay using ProcartaPlexTM Multiplex Immunoassays (Human Custom ProcartaPlex 4-Plex Kit, ThermoFisher, Massachusetts, US). Frozen exosome suspension was thawed and kept on ice for sample preparation following the manufacturer's instruction. The luminescent signal was detected using Luminex™ 100/200™ system equipped with xPONENT 3.1 software.

Data analysis

Data were analysed using Excel and presented as mean ±SD. One tail T-tests were used to evaluate the difference between two groups. Bar charts were generated using the GraphPad Prism (Version 8.4.3). P-value < 0.05 was considered as statistically significant difference.

3. RESULTS

3.1. Cell culture and exosome isolation

In order to determine the physiology of UCMSCs at the time of exosome collection, we perform the morphology. At the time point of conditioned harvestmen, cells were imaged for morphology and. Results indicated that UCMSCs have a similar typical fibroblast-like morphology (Figure 1). There were a uniform of cells observed.

To evaluate the isolated exosomes, we observed the EV pellet under transmission electron microscope. Data showed that isolated EV have cup-shaped morphology (yellow arrow, Figure 2). This is typical morphology of exosomes isolated from many cell types.

3.2. PRP preparation from umbilical cord blood

Umbilical cord blood units were centrifuged for total nuclear cells for banking and plasma for PRP preparation. The plasma was evaluated for cell phenotypes, including red blood cells white blood cells and platelets. Recovery rate of platelet is considered as PRP preparation efficacy, that is 74.66 %. Data also indicated that there was different cell type remained in the pre-activated PRP.

3.3. Growth factor expression

In order to understand the expression of growth factors in UCB-PRP and UCMSC-derived exosomes, we measured the amount of growth factor expression, including HGF, PDGF-BB, VEGF-A, and FGF-2, using multiplex immunoassays. Results are displayed in the table three and four. A large amount of growth factors detected from UCB-PRP, which is 2176.82 pg HGF, 1765.82 pg PDGF-BB, 2336.43 pg VEGF-A, and 39.64 pg FGF (Table 1, Figure 3A). However, less amount of growth factors was measured in the UCMSC-derived exosome samples, which are 312.62 pg HGF, 674.87 pg PDGF-BB, 72.50 pg VEGF-A, and 34.51 pg FGF (Table 1, Figure 3B). The difference of growth factors does not reflect the fact as the different nature of samples and the input is not equivalent.

Regarding UCB-derived PRP, while the FGF-2 expressed with smallest amount ($p<0.05$) compared to others, the HFG

expressed with greatest amount compared to the PDGF-BB ($p<0.001$) (Figure 3A). In terms of UCMSC-derived exosomes, the HGF and PDGF-BB were the highest expression compared to VEGF-A ($p<0.05$) and FGF-2 ($p<0.05$) (Figure 3B). There was not different between HGF and PDGF-BB and between VEGF-AA and FGF-2.

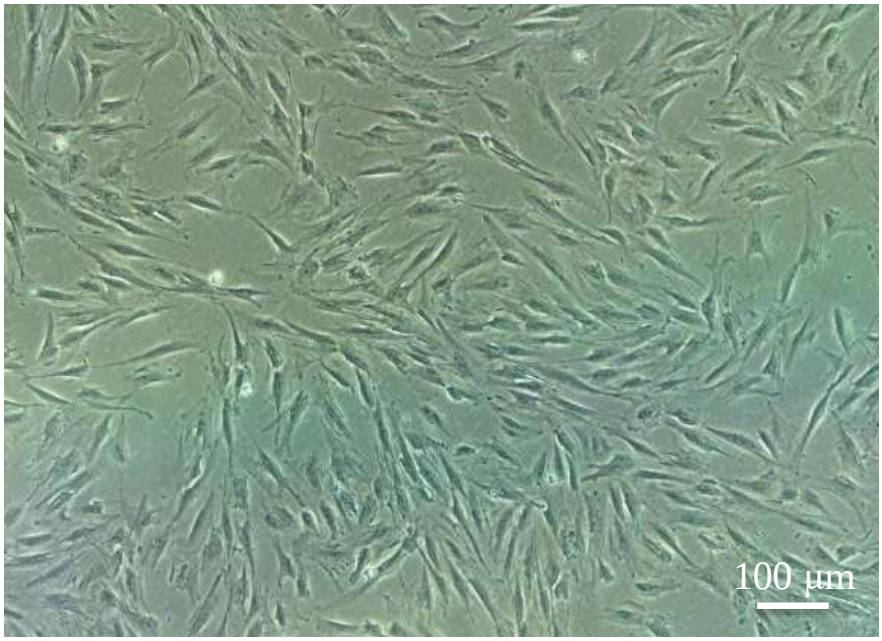

Figure 1. Morphology of umbilical cord-derived mesenchymal stem cells at the passage four (P4)

Table 1. Expression of growth factors in PRP prepared from umbilical cord blood.

Sample	Original source		Growth factor quantity (pg/ml) (Mean ±SD)			
			HGF	PDGF-BB	VEGF-A	FGF-2
UCB-PRP	50 ml UCB plasma	1 ml PRP	2176.82 ±659.94	1765.82 ±829.13	2336.43 ±686.45	39.64 ±4.54
UCMSC-EX	30 x10^6 cells	1 ml EX	312.62 ±225.92	674.87 ±64.78	72.50 ±90.37	34.51 ±5.68

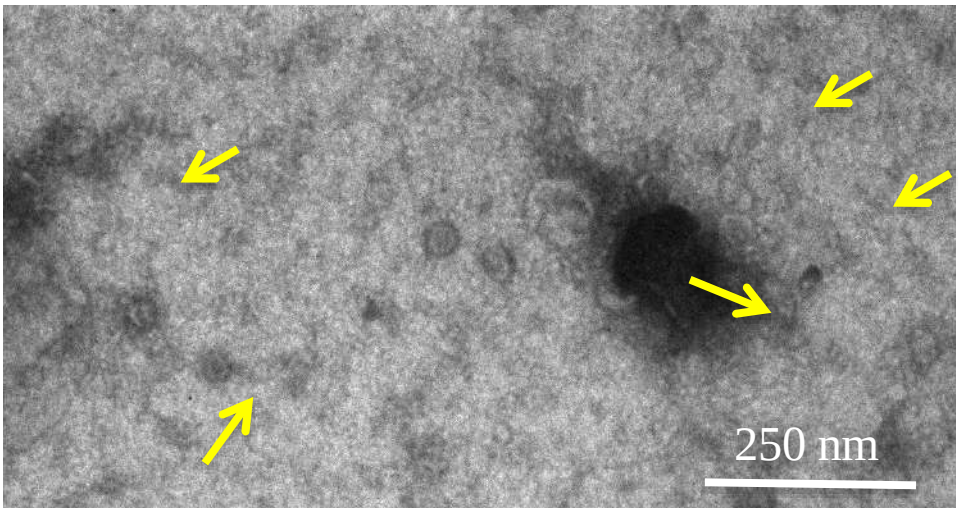

Figure 2. Exosome morphology isolated from conditioned media originated from UCMSC culture at passage four

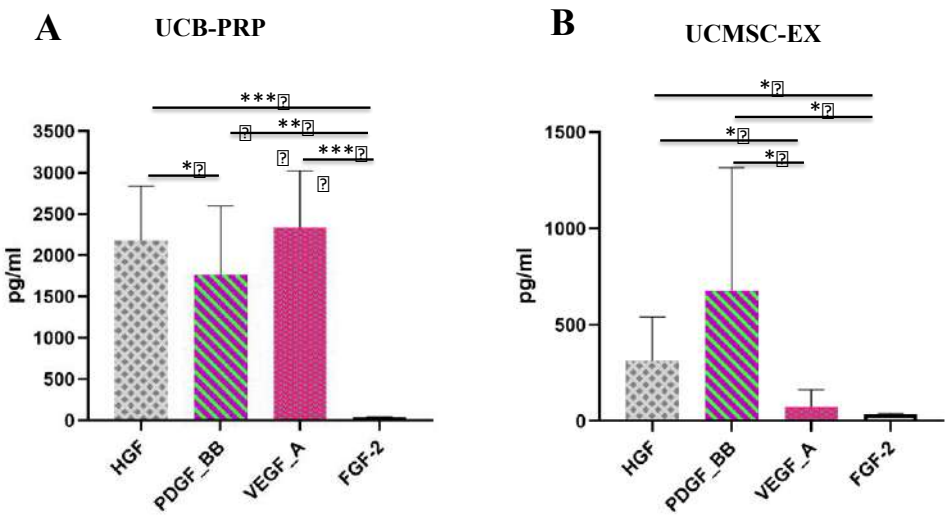

Figure 3. Different expression of growth factors from UCB-PRP and UCMSC-EX

UCB-PRP: Umbilical cord blood-derived platelet rich plasma, UCMSC-EX: Umbilical cord mesenchymal stem cell-derived exosomes

4. DISCUSSION

Growth factors play an important role in various biological processes such as morphogenesis, embryonic development, adult stem cell differentiation, immune regulation, wound healing, angiogenesis, inflammation and cancer [8]. Due to their crucial roles, many growth factors and cytokines have been utilized for clinical applications. Evidently, sorting of these factors into exosomes depends many factors, such as secreting cell sources or culture conditions [9]. In this current study, we expanded UCMSCs using the commercial StemMACS culture medium. Under this culture condition, UCMSCs maintain the typical of cell morphology (Figure 1) and expressed their marker as recommended by the International Society for Cell & Gene Therapy (ISCT®) [10]. And the UCMSCs under this culture condition released exosomes that have typical cup-shaped morphology (Figure 2). This means that UCMSCs under the culture in this study could secrete exosomes into the conditioned media.

The PRP can be prepared from peripheral blood as a routine for many applications at clinics. However, the use of umbilical cord blood-derived PRP is less popular due to the collection cost and accessibility. Todays, according to the development of UCB bank, plasma derived UCBs is a biological waste after total nuclear cell harvestmen. This current study collected UCB-derived plasma from routine processing of UCB for total nuclear cells for banking. With the PRP recovery rate was quite high (74.66 %), we detected the high enrichment of HGF, PDGF-BB, VEGF-A and FGF-2 in UCB-derived PRP (Table 1, Figure 3A). Interestingly, those growth factors were also detected in exosomes originated from UCMSCs (Table 1, Figure 3B). Despite the quantity of these exosomal growth factors were less than PRP's, we do not state the less expression of growth factors in UCMSC-derived exosomes compared to UCB-derived PRP as the different inputs.

Previously, several studies have reported that exosomes originated from different tissues, for examples bone marrow, adipose and umbilical cord, carried growth factors [11]. Those exosomes including derived from umbilical cord-derived mesenchymal stem cells have showed their functions in regulating many biological processes [5, 6]. Mechanism under this is exosome's cargoes, including these growth factors. Results from this study indicated that some growth factors, such as HGF and PDGF-BB, was more enriched in the UCMSC-derived exosomes. This means UCBMSC-derived exosomes may have greater modulation to downstream targets of these factors compared to VEGF-AA and FGF. Data from this study is consistent with a previous investigation that FGF-2 expressed less than other growth factors such as VEGF-A and TGF-β [11]. The exosomal FGF-2 secreted from UCMSCs was even lower than secreted from other sources, such as bone marrow-derived MSCs and adipose-derived MSCs [11]. That different profile of exosomes associated with secreting cell origins as well as stimuli will define the further application of exosomes as innovative medicines.

In summary, data from this current study indicates that both UCB-derived PRP and UCMSC-derived exosomes can be utilized for the real application. The UCB-derived PRP could be an alternative to peripheral blood-derived PRP and UCMSC-derived exosomes can be serves as a substitute of UCMSC for treatment.

5. CONCLUSION

UCMSCs secreted exosomes into culture media and the rate of PRP recovery is high. Both UCMSC-derived exosomes and UCB-derived PRP carrying four growth factors, including HGF, PDGF-BB, VEGF-A and FGF-2, with different amount. The FGF-2 was the smallest expression while the HGF was the largest expression in the UCB-derived PRP. However, there are two groups that HGF and PDGF-BB were larger expressed in UCMSC-derived exosomes. Level of growth factors detected in both sample types is important to indicate their potential roles in regenerative medicine.

Acknowledgement

We would like to acknowledge Prof. Nguyen Thanh Liem (Director of Vinmec Research Institute of Stem Cell and Gene Technology) and Dr. Nguyen Xuan Hung (Director of Vinmec Institute of Applied Science and Regenerative Medicine) have supported authors to conduct this study. And, we would like to thank Ms. Do Thi Xuan Phuong helped to perform the Luminex assay.

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EXPANSION OF $CD3^+CD8^+$ T LYMPHOCYTES FROM HUMAN CORD BLOOD

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ABSTRACT

Human cord blood (CB) has been used as a source of allogeneic hematopoietic stem cells for patients of hematologic malignancies as it lowers the risk of graft versus host effect while delivering more graft versus tumor effect. T cell immunotherapy can be developed from CB, however, with the limited volume of cord blood collected and stored in CB banks, procedures to expand T lymphocytes to a sufficient number are urgently required. Hence, we conduct the study with an aim of establishing an optimal procedure for isolation and expansion of T lymphocytes from human cord blood that meets the requirement for cell therapy in cancer treatment. Three CB mononuclear cell (MNC) samples were collected by Ficoll and then the T cells were expanded by using BINKIT kit from Biotherapy Institute of Japan. After 20 days of culture, the average number of $CD3^+CD8^+$ T cells reached $890.3\pm150.3\times10^6$ cells, increased 403.8±240 times compared to the number of these cells at seeding. The purity of cell population was not high, with the average percentage of $CD3^+CD8^+$ T cells (%) in the total cell was 49.4±10%, meanwhile this value of $CD3^+$ T cells was 74.5±14.4%. Of note, 2/3 samples had the decrease in the number of $CD3^+CD8^+$ T cells at day 20 compared to day 13 of culture. In conclusion, we were successful in expansion of $CD3^+CD8^+$ T cells from human cord bloods, with the adequate number for the cell therapy. However, the length of cell culture should be considered for each individual sample and the selection of $CD3^+CD8^+$ T cells at seeding should be carried out in order to increase the purity of cell population after expansion.

Key words: *$CD3^+CD8^+$ T lymphocyte, immune cell expansion, cord blood.*

1. INTRODUCTION

Cancer has always been a leading cause of mortality among non-communicable diseases in the world. Conventional therapies for cancer treatment such as chemotherapy and radiation result in many side effects because these treatments can destroy healthy cells. To offset these limitations in clinical context, immunotherapy offers a more therapeutically advantageous option for patients suffering from malignancies for its target cancerous cells in a more selective way [1].

Transfer of autologous or allogenic immune effector cells such as natural killer cells or T lymphocytes, more specifically, cytotoxic T lymphocytes (CTLs), can direct the destruction at transformed cells which express tumour-specific antigens [2]. Cytotoxic T cells are CD8-postive T cells that are specialized for the surveillance for all the cells of the body, ready to kill any that is deemed as a threat to the

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Received date: 21/7/2021
Reviewed date: 03/8/2021
Accepted date: 09/8/2021

integrity of the host. For example, CTLs kill virally infected cells, cancerous or damaged cells. When a $CD8^+$ T cell recognizes its antigen and becomes activated, it has three major mechanisms to kill infected or malignant cells: (1) secretion of cytokines, primarily TNF-α and IFN-γ, which deliver anti-tumour and anti-microbial effects; (2) production and release of cytotoxic granules; and (3) destruction of infected/cancerous cells is via Fas/FasL interactions [3].

To deliver to desired therapeutic effects, there need to be a sufficiently-expanded pool of immune effector cells to transfer into the patients' body. Human cord blood (CB) has been used as a source of allogeneic hematopoietic stem cells for patients of hematologic malignancies as it lowers the risk of graft versus host effect while delivering more graft versus tumor effect [4]. The cord blood is composed of all the elements found in whole blood: red blood cells, white blood cells, plasma, platelets and is also rich in hematopoietic stem cells [5]. Advantages for the use of CB include low risk of viral transmission from donor to recipient, rapid availability of CB units serving as an immediate "off-the-shelf" product, less stringent requirements for HLA matching, and lower risk of graft versus host disease (GvHD) [4, 5]. T cell immunotherapy can be developed from CB, which is interesting in clinical context as the patient can receive the source of T cells of the same graft as the hematopoietic cells transferred to them after radio-therapy [6].

However, with the limited volume of cord blood collected and stored in CB banks, procedures to expand T lymphocytes to sufficient number are urgently required. Hence, we conduct the study with aim of establishing an optimal procedure for isolation and expansion of $CD3^+CD8^+$ T lymphoctyes from human cord blood that meet the requirement for cell therapy in cancer treatment.

2. MATERIALS AND METHODS

2.1. Subject of the research

In this research, the main subject is human cord blood which is collected directly from the umbilical cord of the newborn baby at Vinmec International Hospital. Before collection, the mother was diagnosed healthy and does not carry any of the following viruses: HIV, HBV, HPV, and HCV. The total volume of collected cord blood is about 80 mL and the isolation process would be conducted as soon as possible or preferably within 24 hours. The mother was also informed completely about the research purposes and agreed to donate cord blood sample. Three cord blood units were collected in this study, and named as: CB1, CB2, and CB3.

2.2. Isolation and expansion of $CD3^+CD8^+$ T lymphocytes from cord blood

Cord blood mononuclear cells (CBMNCs) were obtained by density gradient centrifugation using Ficoll-Paque media (GE Healthcare Life Sciences, Uppsala, Sweden) following the manufacturer's instructions. Subsequently, CBMNCs were cultured using BINKIT (Biotherapy Institute of Japan, Japan). Briefly, the CBMNCs were seeded at a density of 1×10^6 cells/ml in the cell initial medium. For $CD3^+CD8^+$ T cells expansion, CBMNCs were cultured in cell initial medium containing 700 IU/ml rhIL-2 in an anti-CD3 monoclonal antibody-immobilized flask. The cells were incubated at 37°C in an atmosphere with 5% CO2 for 3 days. After 3 days, the culture medium was changed and subcultured every 2–3 days in cell subculture medium (provided in the kit) containing 350 IU/ml rhIL-2 supplemented with 5% heat-inactivated autologous plasma to maintain a concentration of $0.8–1.0\times10^6$ cells/ml, without discarding the old medium. When the number of cell increased logarithmically, the cultured cells were transferred into culture bags (Nipro, Osaka, Japan) until day 20 of culture.

2.2. Phenotypic analysis

The phenotype of expanded cells and CBMNCs at baseline (day 0), day 13 and at the end of the culture (day 20) was analyzed by flow cytometry. Monoclonal antibodies specific for CD3, CD8, CD56 and CD4 that were conjugated with Pacific Blue, fluorescein isothiocyanate, R-phycoerythrin and

Allophycocyanin-Alexa Flour 750, respectively (Beckman Coulter, Inc.), and the corresponding isotype were used for the characterization of cell population. Cells were analyzed by Navios flow cytometer (Beckman Coulter, Inc.), and data were acquired by Navios software (version 3.2) according to the manufacturer's instructions.

2.3. Quality control testing

Quality control testing was examined by assessing samples obtained during the culture period and at the final product. For sterility examination, the BacT/ALERT Plus microbiological detection system (bioMérieux, Marcy-l'Étoile, France) was used, while a MycoAlert Mycoplasma Detection kit (Lonza Group, Ltd., Basel, Switzerland) was applied for mycoplasma contamination testing. The viability of expanded cells was measured by trypan blue exclusion assay and tested for endotoxin by a kinetic colorimetric LAL assay using the Endosafe-PTS portable test system (Charles River Laboratories, Inc., Wilmington, MA, USA).

2.4. Statistical analysis

Statistical analyses were performed with STATA software (version 12.0; StataCorp LLC, College Station, TX, USA) to determine the P-value. A value of $P<0.05$ was considered to indicate a difference that was statistically significant.

3. RESULTS AND DISCUSSION

3.1. Mononuclear cell isolation from cord blood

Cord blood after collection were diluted with phosphate buffer saline (PBS), followed by dropping down to Ficoll and then centrifuged. The Ficoll solution plays a role as a tool to separate the whole blood into many layers follow the density gradient. As a result, the following layers will be visible in the conical tube after centrifugation, from top to bottom: plasma and other constituents, a layer of mono-nuclear cells called buffy coat (MNC), Ficoll, and erythrocytes & granulocytes.

The mononuclear cells after isolation and resuspension were stained with Turk solution. Turk solution is a composed of a stain (Gentian violet) and 1-2% acetic acid. This solution destroys red blood cells and stains the nuclei of the white blood cells, making them easier to see and count (Figure 1A).

We have conducted the research in 3 CB units. The immunophenotype of cells was determined by flow cytometry system (Figure 1B). As the result, total isolated-MNCs were $23.4\pm3.1\times10^6$ cells. The average number of $CD3^+$ T cells in three samples was $15.9\pm3.5\times10^6$, accounting for 67.7±7.7 % in MNC population. The average number of $CD3^+CD8^+$ T cells in three samples was $2.6\pm1.1\times10^6$, accounting for 11.3±5.6 %, and 16.5±7.2 % in MNC and T cell populations, respectively (Table 1).

3.2. Immune cell expansion

We observed that our method of expansion has yielded a good fold of expansion of cells, in terms of both total mononuclear cells and the population of T cells and subpopulation of $CD3^+CD8^+$ T cells.

Regarding the course of expansion of cells, there are differences between 3 cases. As regards the whole population of mononuclear cells, case 1 shows steady increase throughout the whole period of culture in 20 days, peaking at the final day while the other 2 cases show their peak of cell proliferation at day 17, then gradually decreases (Figure 2).

With respect to the population of T cells and CTLs, the three CB units continued to show variances. CB 1 showed continuous increase of T and CD3+CD8+ T lymphocyte population until the last day of experiment. Meanwhile, for CB 1 and CB 2, the peaks of T and CD3+CD8+ T cell proliferations were at day 13 (Figure 3).

At the final day of culture (day 20), the total number mononuclear cells were 1857.7±448 ×106 cells (Table 2), increased by 82±29.3 times, with the highest in CB3 (99.95 times), followed by CB2 (97.9 times), and CB3 (50.27 times) (Figure 4).

At the final day of culture (day 20), the total number mononuclear cells were 1857.7±448 ×106 cells (Table 2), increased by 82±29.3 times, with the highest in CB3 (99.95 times), followed by CB2 (97.9 times), and CB3 (50.27 times).

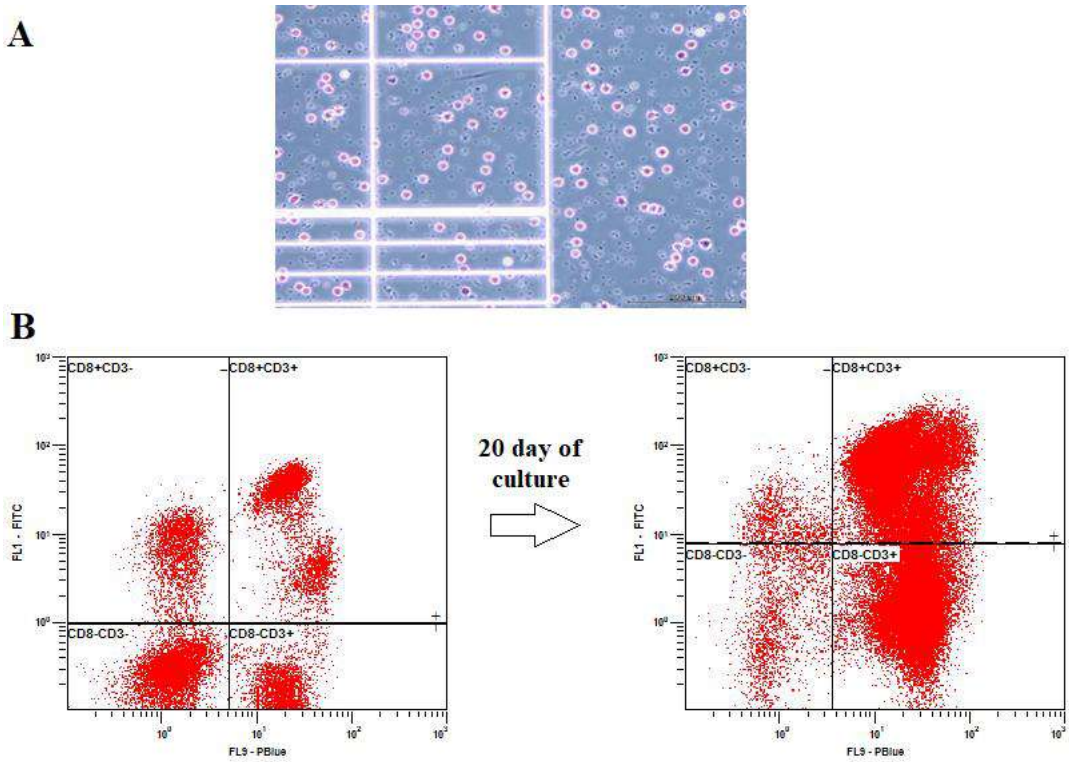

Figure 1. Characterization of immune cells

(A) Mononuclear cells after isolation (Day 0). The cells were stained with Turk dye. The violet cells are mononuclear cells black arrows). The non-color and black dots are hemolyzed red blood cells (red arrows). (Objective 20x). (B) Immune phenotyping of whole blood cell and expanded cells at day 20 of culture.

Table 1. Characterization of three CBMNCs at collection

	CB 1	CB 2	CB 3	Average±SD
Total number of MNC (×10^6)	27	21.6	21.6	23.4±3.1
Number of T cells (×10^6)	19.7	15.43	12.71	15.9±3.5
Number of CD3$^+$CD8$^+$ T cells (×10^6)	2.44	3.82	1.57	2.6±1.1
CD3$^+$CD8$^+$ T cells in T cell population (%)	12.4	24.8	12.4	16.5±7.2
CD3$^+$CD8$^+$ T cells in MNC population (%)	9.0	17.7	7.3	11.3±5.6
T cells in MNC population (%)	73.0	71.4	58.8	67.7±7.7

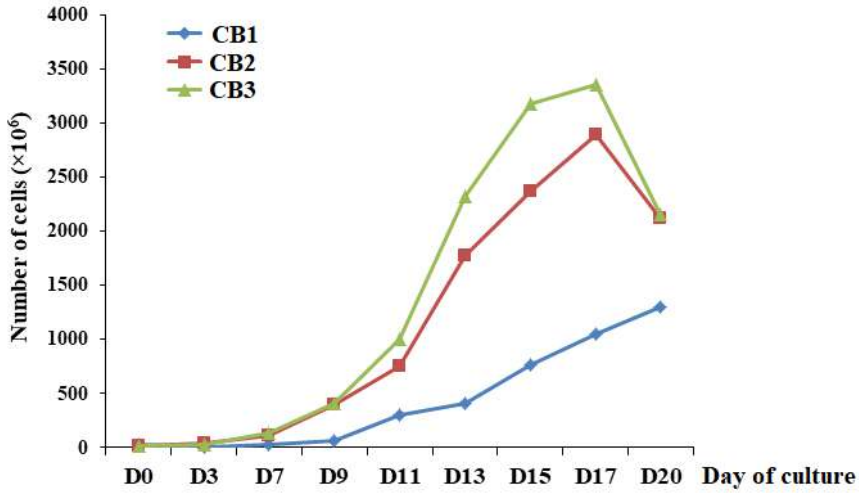

Figure 2. Total cell growth curve in three CB units in 20 days of culture

D, day after the start of culture; CB, cord blood unit.

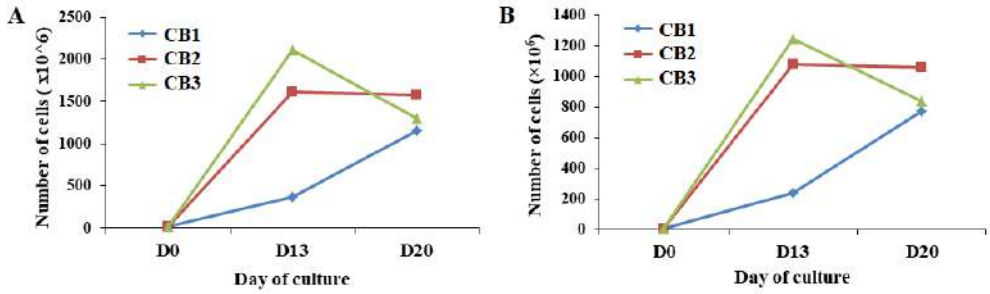

Figure 3. Cell growth curves at three time points of (A) T cell population and (B) $CD3^+CD8^+$ T cell sub-population

D, day after the start of culture; CB, cord blood unit

Table 2. Characterization of expanded-cell population

	Day 13 of culture			Day 20 of culture		
	CB 1	**CB 2**	**CB 3**	**CB 1**	**CB 2**	**CB 3**
Total number of MNC ($\times10^6$)	409.17	1770.38	2316.56	1299.33	2114.73	2158.89
Number of T cells ($\times10^6$)	367.19	1608.21	2110.62	1152.39	1582.88	1293.39
Number of $CD3^+CD8^+$ T cells ($\times10^6$)	235.47	**1080.82**	**1243.53**	773.6	**837.43**	**1059.9**
$CD3^+CD8^+$ T cells in T cell population (%)	64.1	67.2	58.9	67.1	52.9	81.9
$CD3^+CD8^+$ T cells in MNC population (%)	57.5	61.1	53.7	59.5	39.6	49.1
T cells in MNC population (%)	89.7	90.8	91.1	88.7	74.9	59.9

Interestingly, our method of expansion was well selective towards population of T cells and $CD3^+CD8^+$ T lymphocytes, whose fold of expansion was significantly higher than that of T lymphocytes and total mononuclear cells. The average number of T cells at day 20 was 1342.9±219.5 $\times 10^6$, accounting for 74.5% of total cells, meanwhile the number of $CD3^+CD8^+$ T cells was 890.3±150.3$\times 10^6$ cells, accounting for 49.4±10 % and 67.3±14.5% of total cell and T cell populations, respectively (Table 2). The expansion fold of T lymphocytes population ranged from 58.5 to 102.6 while that in $CD3^+CD8^+$ T cells was even more significant, ranging from 219.2 to 675.1 (Figure 4). Interestingly, the peak number of T cells and $CD3^+CD8^+$ T cells in samples CB2 and CB3 were reached at day 13 but not day 20 (Table 2), with the fold increase of $CD3^+CD8^+$ T cells in CB3 was 792.1, and that in CB2 was 282.9. Then cell numbers continued to decrease at the following days in these two samples (Figure 4).

In a experiment by Okas and colleagues in 2010 [7], sufficient pool of $CD3^+CD8^+$ T cells for adoptive T cells transfer in clinical applications had been expanded from HCB in 8-11 days span of time. Our experiment was expanded in a time span of 20 days and at the end of culture procedure, the expansion fold of T cells as well as T CD8 population was significantly higher than that of Okas experiment. However, we observed that, T cells and $CD3^+CD8^+$ T cells in 2 samples CB2 and CB3 showed proliferation until day 13 while the other case CB1 showed steady expansion until the end of the experiment at day 20. Generally, the time span of 2 weeks has yielded sufficient amount of T lymphocytes and CTLs for clinical application [6,7]. There is considerable variation between 3 cases, which is consistent with reports from other experiments. This variation is not clearly understood and may be due to the variation in cell disposition of CB units at the time of collection. Of note, the purity of $CD3^+CD8^+$ T cells was not very high in our study. To resolve this problem, the pre-selection of these cells form peripheral blood should be taken prior the expansion. The selection of $CD3^+CD8^+$ T lymphocytes could be done using commercial kits, such as $CD8^+$ T cell isolation kit (Mitenyl), or perform the isolation of $CD3^+CD8^+$ T cells using Fluorescence-activated Cell Sorting technique.

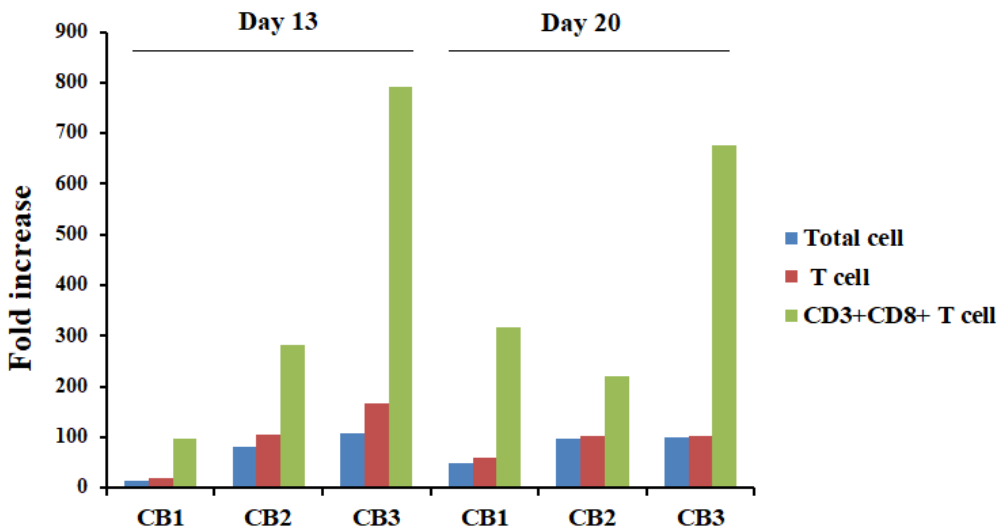

Figure 4. The fold increase of cell number after a large-scale expansion at day 13 and 20 of culture

4. CONCLUSION

From the results as we achieved and discussed before, it can conclude that we were success in expansion of $CD3^+CD8^+$ T cells from human umbilical cord blood to relevant number for clinical use. However, the length of cell culture should be considered for each individual sample and the pre-selection of $CD4^+CD8^+$ T cells at seeding should be carried out in order to increase the purity of cell population after expansion.

Acknowledgments

This project was funded by Vingroup Inc, Hanoi. Project code: DT.01.

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VIETNAM JOURNAL OF PHYSIOLOGY

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√ 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 nghiên cứu, các 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.

HỘI SINH LÝ HỌC VIỆT NAM
TẠP CHÍ SINH LÝ HỌC VIỆT NAM

Tạp chí

SINH LÝ HỌC VIỆT NAM

Tập 25, Số 3
9/2021

Tạp chí Sinh lý học Việt Nam
Tập 25, Số 3, Tháng 9/2021