IDENTIFYING INDICATIONS OF TOT SURGERY FOR THE TREATMENT OF FEMALE STRESS INCONTINENCE

Mai Trong Hung*, Vu Huy Nung**, Le Anh Tuan**

ABSTRACT

Objectives: We conducted this study with the aim to identify the indications of TOT surgery for the treatment of female stress incontinence. **Subjects and methods**: A cross-sectional study of 74 women with stress urinary incontinence underwent the procedure in Hanoi Obstetrics and Gynecology Hospital. The study was conducted from 1/1/2013 - 5/2018. **Results and conclusions:** Patients with urination (100%) or associated with dysuria (80.7%), or genital prolapse (96.8%). Patients with associated urination have urination disorders such as exertion during urination, urgency (urination) (58.1%), or a combination. Patients with urinary incontinence accompanied by urination disorders such as concomitant bowel movements (19.4%), accompanied by inactive bowel movements (29%). When examining the condition of the muscles of the bladder, urethra: positive Valsalva tests; positive cough test (100%). The amount of residual urine measured by catheterization > 100 ml, and the feeling of wanting to urinate but difficult to urinate. (100%). Maternity status, number of pregnancies, number of births, heaviest birth weight and method of delivery are factors that influence the indications for TOT surgery.

Keywords: *Indication of surgery, TOT surgery, treatment of female stress incontinence.*

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I. BACKGROUND

Uncontrolled urination, as defined by the International Continents Society (ICS) for uncontrolled urination: "Uncontrolled urination or urination is an unexplained urinary outflow will, is a social and sanitary issue related to complaints of quality of life". The disease is mainly found in women, urinary incontinence is a major disease affecting the life, psychophysiology, work, and quality of life around the world. The rate in general of incontinence in the community varies from 25 - 45% [1].

Uncontrolled urination is a psychological burden that reduces the quality of life. Since the first American American Obstetrics & Gynecologist Howard Kelly (1914) first published the technique of exertion of urinary incontinence [2], many studies have been conducted on urinary physiology, pathogenesis, epidemiology and preventive treatments have been implemented to reduce the burden of disease on women. However, incontinence has not been reported and adequate treatment has been addressed in some countries [3].

In Vietnam, due to the current economic conditions and oriental culture, the patient was afraid to go to the hospital, so this condition is rarely mentioned at major hospitals and in the community. Understanding the clinical profile of urination in women and the factors involved as well as evaluating current surgical

treatment for urination will provide useful information for future treatment and prevention.

In Vietnam, female stress incontinence surgery with TOT (trans obturator tape) has only been applied in recent years, although there are many advantages there is no comprehensive research on indications, techniques and effectiveness of this surgery. So, we conducted this study with the goal: determining the indications of TOT surgery for the treatment of female stress incontinence.

II. SUBJECTS AND METHODS

2.1. Subject, location and time

A cross-sectional study of 74 women with stress urinary incontinence underwent the procedure in Hanoi Obstetrics and Gynecology Hospital. The study was conducted from 1/1/2013 - 5/2018.

2.2. Research design

Research design: Apply a cross-sectional descriptive research method.

Sample size: We applied the entire sampling method.

* Inclusion criteria including:

- The patients were diagnosed confirmed
- Patients with urinary disorders come to hospital
- Patients agree to cooperate with conditional research post-surgery monitoring.
- Eligibility of surgery.

*** Exclusion criteria:**

- All patients do not have surgical indications due to general and local conditions without anesthesia or anesthesia conditions.
- Patients do not agree to participate in the study.
- Urinary incontinence patient without a surgical indication.

*** Research outcomes:**

- Questioning and clinical examination of patients coming to examine, exploit medical history and history (according to the sample of the research records), explain to the patients for research cooperation.
- Conduct clinical examination to select patients with urination.

*** Processing and analyzing data**

All information recorded on the patient is entered into the variable table of STATA 12.0 statistical software. Use appropriate statistical algorithms Descriptive statistics: Statistical analysis (Chi-square test, Fisher exact test, t used test, Wilcoxon rank-sum test). Pearson's correlation coefficient is also calculated when considering the correlation between quantitative variables in the study. Tests are considered to be statistically significant (ie with difference or relevance) when the value p is <0.05. When OR is used, the 95% confidence interval of OR passing 1 is considered not statistically significant.

*** Ethical issues**

The research process always ensures compliance with GCP rules - good clinical trials of the Ministry of Health and ICH. During the research process, the researcher always ensures to adhere to the research protocol.

III. RESULTS AND DISCUSSION

Table 1: Patient demographics

Indexes	Number (X ± SD)	Percentages (Min-Max)
Age	55,3 ± 10,8	30-83
Maternal age (30-50)	20	27,0%
Out of maternal age (>50)	54	73,0 %
Occupation		
Farmer	21	28,4
Woker	7	9,5
Officer	8	10,8
Other worker	3	4,0
Others	35	47,3
Education		
Not	6	8,1
Degree level 1	22	29,8
Degree level 2	24	32,4
Degree level 3	8	10,8
Collage - University	5	6,7

The results in Table 1 show that the average age of a patient is 55.3 ± 10.8 with the lowest is 30 years old and the highest is 83 years old. Of these, 73% have aged beyond the maternal age. The disease occurs in all professions and education levels.

Our results were higher than those of Ho Nguyen Tien with urinary incontinence treatment when surgery placed Bandelette under the urethra of 51.8 ± 11.9 (the lowest was 39 and the highest was 67 years old) [3] and Nguyen Tan Cuong in the treatment of urinary incontinence in women with urethral augmentation is 49.8 ± 7.2 (the lowest is 33 and the highest is 69) [4]. Uniformly, the incidence of urine incontinence increased with age [6], [7]. Age> 40 has a higher risk of urination (RR = 2, 16, CI = 1, 86 - 2, 57) [7]. Because of its prevalence in the elderly, urination is considered the inevitable normal progression of age. However, urine incontinence should not be considered normal in the elderly, although changes in the bladder and organization in the sub-framework contribute to the occurrence of disease [8].

The proportion of patients with farming occupations accounted for the highest proportion of 29%, followed by workers with 16.1%. Groups of civil servants and hired laborers account for less than 9.7% and 6.5%. Our results are also consistent with the results of Nguyen Thi Tan Sinh, this rate is consistent with the situation of work and the working time of patients who have undergone [9].

Table 2. Distribution of patients by maternity status (n = 74)

Maternity status	Number	Percentage (%)
Used to be pregnant	74	100
Number of births ≤ 2	12	16.2
Number of births 3-4	42	61.3
Number of births> 4	20	27.0
Vaginal discharge	56	75,7
= 1	22	29,7
> 1	34	46,0
Averge time	2,7 ± 1,0	(1 - 6)

The number of people who are pregnant and have given birth is 100% of the cases. The number of people who ever did family planning - abortion was 56 cases, accounting for 75.7%. The average number of births in the study group was 2.7 ± 1.0. The number of people who have experienced 3-4 pregnancies is 42 cases, accounting for 61.3%. The number of people who ever sucked once was 22 cases, accounting for 32.2%.

The results showed that the number of pregnancies and the number of abortions as well as the number of births, the weight of the baby at birth also affect the urination condition of the patient. Our results are also consistent with author Nguyen Tan Cuong (average number of births 2.5 times and the highest to 10 pregnancies) [4] and author Ho Nguyen Tien (average number of children is 3, 4 ± 1,6, the lowest is 1 and the highest is 6) [3]. The number of vaginal births affects the dilatation of the perineum. In our study, the number of birth patients ≥ 2 times accounted for 87.1%. According to Nguyen Tan Cuong, the number of patients with delivery more than 2.5 times accounted for 63% of the total numberofpatientsundergoing TOT surgery [4].

Table 3. Distribution of patients by incontinence status (n = 74)

Incontinence status	Number	Percent (%)
Nocturia	74	100,0
Urine repeatedly / once	27	36,5
Pee hard to push	61	82,4
The urine flows out without a feeling of urination	21	28,4
After finishing, I still want to go but don't come out	47	63,5
Must urinate urgently	44	59,5
Urinary incontinence when exertion	42	56,8
Having suffered from urinary urgency	32	43,2
Urinating during sex	10	13,5

In the sample size of the sample, the number of people who have to urinate at night is 100%, the difficulty of pushing the urine is 82.4%, the urge to urinate but still wants to go but is not 63.5%, urgency is 59.5%, incontinence 56.8% urinary exertion, urinary urgency is 43.2%, frequent urination in one urination accounts for 36.5%, self-exuded urine without feeling urinating 28.4%. Thus, it can be seen that the condition of urination is very diverse and clinical types. This research result is higher than that of Ho Nguyen Tien et al., The rate of accompanying genital prolapse accounts for only 28%, the life with urinary disorders accounts for 30% but the incidence of urinary incontinence. It was also assumed that 100% of the patients were hospitalized on the basis of incontinence, in which level 1 was 12%, level 2 was 52% and level 3 was 36% [3].

Evaluation of the incidence of urination in our study showed that among the patients with urination, 100% of the patients had urination on exertion, 58.1% had urgent and coordinated urination. There were 22.6% of patients had urinary incontinence during intercourse, 19.4% urinary incontinence associated with feces and 29% urinary incontinence accompanied by inactive feces. Our research is consistent with local authors such as Nguyen Thi Tan Sinh [9], Nguyen Thi Thanh Tam [6] and Nguyen Thi Ngoc Phuong [8] but higher than those of Nguyen Tan Cuong [4]

Table 4. Distribution of patients by reason of admission (n = 74)

Reason for admission	Number	Percentage (%)
Urination	74	100,0
Genital prolapse	71	96,0
Genital prolapse 1	56	78,9
Genital prolapse 2	5	7,0
Genital prolapse 3	10	14,1
Rectal prolapse	0	0,0
Cervical prolapse	19	25,7
Vaginal prolapse	66	89,2

Number of people admitted to the hospital with the reason of urination accounted for 100.0%, genital prolapse accounted for 96.0% (78.9% suffered from grade 1, 7% of grade 2 and 14.1% of grade 3), prolapse cervix accounts for 25.7%, prolapse into the vagina accounts for 89.2%. There are no cases of rectal prolapse. Our study is higher than that of author Ho Nguyen Tien, the rate of attached sex drive is 28% [3] and that of author Daher N. The rate associated with genital prolapse is 30% [5].

Table 5. Patient distribution by associated disease and some risk factors (n = 74)

Diseases	No	Per (%)
Urinary tract infections	50	67,6
Cystitis	21	28,4
Trauma to the genital area (cesarean section)	7	9,5
Constipation	36	48,7
Hemorrhoids	10	13,5
Menopause	20	27,0
Heavy work	60	81,1
Stress	60	81,1

The number of people who ever had urinary tract infection accounted for 67.6%, cystitis accounted for 28.4%, genital trauma accounted for 9.5%, constipation accounted for 48.7%, combined hemorrhoids accounted for 13.5 %, hard work accounted for 81.1%, stress in life 81.1%.

Table 6. Distribution of patients by degree of incontinence upon examination (n = 74)

Urinary incidence	Number	Percentage
Urinary incidence during examination		
Wet panties (grade 1)	67	90,5
Wet outer pants (grade 2)	7	9,5
Time to urinate		
Several times a year	15	20.2
Several times/month	54	73
Several times a week	5	6.8

100% of the patients had urination, including 67 cases of wet urine incontinence accounted for 90.5% and wet pants with 7 cases accounted for 9.5%. There are 20.2% of patients have urination annually, 73% have several times a month to urinate and 6.8% urinate several times a week.

Table 7. Distribution of study subjects according to BMI and urination status (n = 74)

BMI	Exertion		Urgent		Total	
	n	%	n	%	n	%
< 18,5	4	9,5	4	12,5	8	10,8
18,5 - 22,9	26	61,9	21	65,6	47	63,5
≥ 23	12	28,6	7	21,9	19	25,7

Among patients with incontinence, 10.8% were considered thin, 63.5% normal for BMI, and 25.7% were obese. In terms of exertion and urgency of urinary incontinence at BMI, levels were not statistically significant with p> 0.05. Compared to the results of Nguyen Thi Tan Sinh [9], there is a relationship between body mass index ≥22 and urination status. The risk of having urinary incontinence in people with BMI ≥ 22 is higher than those with BMI <22 with OR = 1.77, 95% CI: 1.31 - 2.4). Our results also showed a gradual increase in urination incidence with an increase in BMI. Our results are also consistent with the authors Ho Nguyen Tien with a BMI ≥ 23 of 32% [3].

Table 8. Evaluation of patients through diagnostic tests (n = 74)

Diagnostic test	Number	Per (%)
Valsava test (positive)	73	98,7
Cough test (positive)	73	98,7
Test Bonney (negative)	2	2,7
The amount of urine remaining after urinating	132,6 ± 19,5	(100 - 170)
≥ 100 - 150 ml	65	87,8
> 150 ml	9	12,2
The amount of urine remaining after urinating		
General	132.6 ± 19.4	
Minor incontinence	132.4 ± 19.5	P-value = 0,808
Moderate incontinence	134.3 ± 19.9	

98.7% of the patients who tested Valsalva and cough tests were positive and 2.7% of Bonney tests were negative. Our results are also consistent with studies of domestic authors [6], [9], [10]. primary) before surgery was> 100 ml of which 65 cases accounted for 87.8% and urine residues> 150 ml had 9 cases accounting for 12.2%. The average residual urine volume of all patients was 132.6 ± 19.5 ml of which the lowest was 100 ml and the largest was 170 ml. There was no difference in the average amount of residual urine by urinary incidence. Our findings are also consistent with authors such as Nguyen Tan Cuong [4] and Le Si Trung [10].

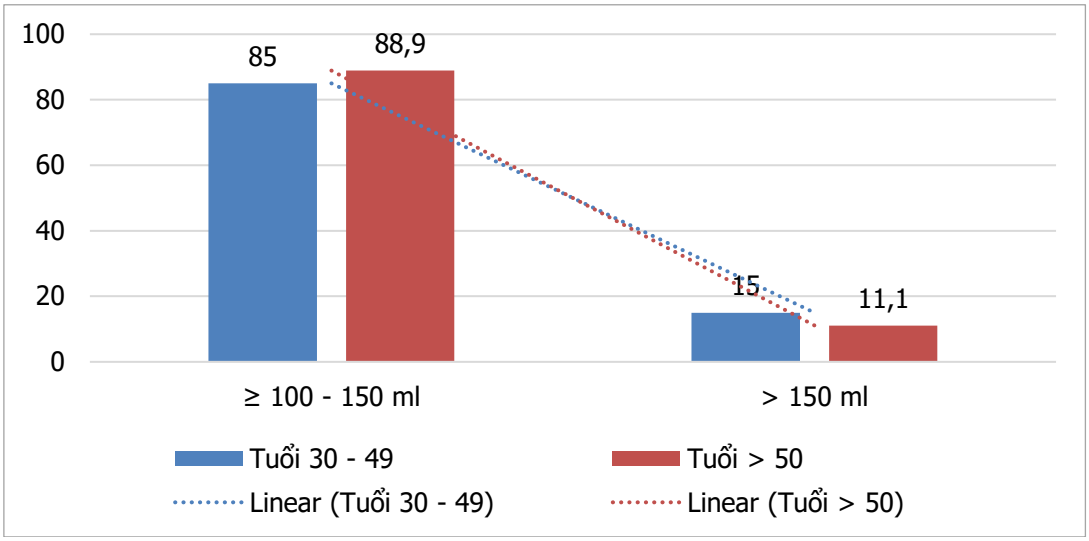

Figure 1. Relationship between age and residual urine (n = 74)

There was no statistically significant difference in the proportion of urine residues by level between the age groups of 30 - 49 years old and over 50 years old) and p> 0.05 (Fisher's exact = 0.696). Specifically: in the group of 30 - 49 years old, 85% of patients have urine output <150 ml while in the age group> 50 is 88.9%. Similar to the assessment of the amount of residual urine> 150ml, in the age group of 30-49, there is 15% while in the age group> 50, it is 11.1%.

Table 9. Some biochemical indicators by degree of urination

Indicators	Grade 1	Grade 2	p-values
RBC	4,5 ± 0,4	4,5 ± 0,2	0,61[a]
WBC	7,9 ± 6,5	6,7 ± 1,4	0,64[b]
PLT	272.3 ± 51.3	268.1 ± 86.9	0.85[b]
Hemoglobin	130.5 ± 7.7	130.6 ± 7.5	0.98[a]
Ure	4.6 ± 1.3	5. ± 1.5	0.4[b]
Creatinin	66.2 ± 13.3	73.7 ± 9.2	0.11[b]
SGOT	25 ± 9.6	23.9 ± 4.8	0.83[b]
SGPT	24.2 ± 14.1	26.1 ± 4.9	0.48[b]

a. T-student testb.

b. Mann - Whitney U test

The table above shows that there is no difference in biochemical indices according to the level of urination with p> 0.05.

IV. CONCLUSION

Patients with urination (100%) or associated with dysuria (80.7%), or genital prolapse (96.8%). Patients with associated urination have urination disorders such as exertion during urination, urgency (urination) (58.1%), or a combination. Patients with urinary incontinence accompanied by urination disorders such as concomitant bowel movements (19.4%), accompanied by inactive bowel movements (29%). When examining the condition of the muscles of the

bladder, urethra: positive Valsalva tests; positive cough test (100%). The amount of residual urine measured by catheterization > 100 ml, and the feeling of wanting to urinate but difficult to urinate. (100%). Maternity status, number of pregnancies, number of births, heaviest birth weight and method of delivery are factors that influence the indications for TOT surgery.

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HUE BIOSTIMULATOR INDUCE DIFFERENTIATION OF UMBILICAL CORD MESENCHYMAL STEM CELLS INTO CARDIOMYOCYTE-LIKE CELLS

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ABSTRACT

Background: We considered setting up a cost-effective and non-risky technique for differentiation umbilical cord mesenchymal stem cell (UC-MSCs) into cardiomyocyte-like cells. This is rooted in Mesenchymal stem cells (MSCs) that were announced can differentiate into functional cardiomyocyte or cardiomyocyte like-cells but the afore-agent which caused differentiation often is a hazardous chemical like 5-azacytidine (5-aza). Also, most of the previous publish focus on bone marrow mesenchymal stem cells (BMSCs) while umbilical cord mesenchymal stem cells (UC-MSCs) is a source of MSCs have more strength than BMSCs, thus we emphasis to affect of our electrical stimulation device (Hue Biostimulator) on UC-MSCs. ***Methods***: UC-MSCs were isolated by the standard procedure of Stem cells Laboratory, Hue University of Sciences; Following by characterization through flow cytometry, colony-forming unit-fibroblast assay and evidence for their potential to transform into different cell types and cell lineages with StemMACS™ Trilineage Differentiation Kit, human(Miltenyi Biotec). Besides, Hue Biostimulator was build up to perform electrical stimulation in cultured UC-MSCs. After stimulation with monophasic current, morphologic assay analyzed the morphology and orientation change of UC-MSCs by using ImageJ. Finally, RT-PCR and immunocytochemistry were used to verify the upregulation of cardiac-specific genes and the expression of heart-specific protein.Analysis of cardiomyocytes derived from UC-MSCs. ***Results***: UC-MSCs after isolation were positive for CD90, CD73, CD105 ,and negative for CD45. After 15 days stimulation, UC-MSCs exhibit the morphology change through shape index (0.25) and clear orientation. The re-inspection of RT-PCR revealed the upregulation of Desmin, GATA-4, Nkx-2.5, α-MHC (Myosin heavy chain), cTNT (cardiac troponin T). At the same time, immunocytochemistry indicated the exhibit of Desmin which is a specific protein of the heart. ***Conclusion***: A low-cost device was successfully set up which name Hue BioStimulator. This device was demonstrated that can cause pre-differentiation from UC-MSCs into cardiomyocyte like-cells. This thing promises a helpful method for cell therapy now and in the future. The cardiomyocyte like-cells can be more play on heart attack in particular and in general on cardiovascular disease. Hue Biostimulator can be expected to have more and more missions on clinical and regenerative medicine.

Keywords: *Mesenchymal stem cell; Cardiomyocyte; Electrical stimulation; Differentiation.*

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I. INTRODUCTION

Nowadays, cardiovascular diseases are one of the top dead-reason on the planet. This outcome was from the cutoff in the

proliferative and re-newal ability of cardiomyocytes in vivo. In line with this, fibroblastic replace and non-contractile scar tissue formation is prevalent events exhibit in heart muscle which can result in contractile dysfunction and congestive the heart failure. When that, patients face treating with the last option which is allogeneic heart transplantation, but the donor organ resource always limits. The promising alternative method is cell therapy, especially stem cell therapy aims to replace disease dysfunction cells with contractile cells. This treatment is demonstrated to improve the function of the heart through scar reduction, support to the resident cardiac progenitor cells(1).

The capability of human pluripotent stem cells (hPSCs) to differentiate into any cell type has revolutionized medical research.MSCs which are been the focus of modern stem cell research, also were reported that differentiation ability to cardiac-linage. Cardiomyocyte-like contracting cells were differentiated from MSCs that were performed by Peter Szaraz (2); Potapova et al alsoreported that differentiated cells havean electrophysiological function similar to cells originated from cardiac tissue (3), although most of afore researches on cardiomyocyte differentiation from embryonic stem cells or pluripotent stem cells.Besides, UC-MSCs is an emerged source because they can avoid the ethical issue and eliminate weakness of BMMSCs .Thus, the entirety of that helps the assumption regarding UC-MSCs to deal with this issue.

Various methods for differentiation into cardiomyocyte strategies were published such as co-culture with cardiomyocyte, using demethylating agents, oxytocic hormone (4), several growth factors like transforming-growth-factor-β1 (TGF-β1), platelet-derived-growth factor (PDGF), basic-fibroblast-growth-factor (bFGF), using molecular technics or using pool of different methods. A common controversial procedure is a treatment with 5-azacytidine(5,6) and although it can induce successfully to cardiomyocyte, it is demonstrated that has random demethylation and non- specific epigenetic activity. These things make 5-azacytidine can not be used for clinical applications.

Our research aims to build up a cost-efficient method distinct above methods and can apply for the clinical. This method is electrical stimulation on the stem cells.In this work, we choose UC-MSCs which is an attractive source of MSCs to eliminate the disadvantage of bone marrow mesenchymal stem cells that have limited differentiation abilities. This research investigates the effect of monophasic electrical pulse on cardiac-linage differentiation from UC-MSCs instead of the effect on pluripotent stem cells were before demonstrated by numerous researches. This things can be promised to reduce the distance of research and clinical on treatment for heat failure in particilar and in general for cardiovascular diseases.

II. METHOD AND MATERIAL

Isolation and in vitro culture of UC-MSCs:

Umbilical cords were collected under sterile conditions at Hue Central Hospital and transported to the laboratory in 0.9% normal saline containing 100 U/ml penicillin and 100 mg/ml streptomycin at 4°C from the informed consent donors, who were negative for hepatitis virus markers, syphilis, and HIV. They are full-term birth donors and their ages ranged from 22 to 30 years.

UC-MSCs were isolated by thestandard protocol of Stem cells Laboratory, Department of Biology, Hue University of Sciences. The blood vessels surrounding the umbilical cord were removed in 0.9% normal saline. The umbilical cord was cleaned by PBS and mechanically fragmented into 1-2 cm^2 sections with ophthalmic scissors. Following the fractions were rinsed with PBS and centrifuged at 2500rpm for 5 min two times. Those sections were suspended in StemMACS™ MSC Expansion Media Kit XF (Miltenyi Biotec, Germany) and were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Afterward, the medium was replaced every 3 days, and tissue block cells were passaged upon reaching 80% confluence. The morphology of UC-MSCs was observed under an inverted optical microscope (Olympus Corporation)

When the attached cells cultured reached around 80% confluence, the cells harvested by trypsin digestion, counted and re-seeded into culture dishes at a density ofaround 1000 cells/cm^2. The cells at passage 2-3 were also collected for phenotype analysis and in vitro differentiation assay.

Colony-forming unit-fibroblast (CFU-F) assay:

For CFU-F culturing at passage 2-3, the cells were seeded at density 1000 cells per well. Colony-forming unit-fibroblast (CFU-F) was stained with Giemsa solution and counted by usingImageJsoftware.

Flow cytometry

The attached cells from thepassage culture were detached by trypsin digestion, washed in PBS, and reacted with FITC -conjugated or PE-conjugated monoclonal antibodies (BD, United States) against human CD34, CD45, CD73, CD90, and CD105 for 30 min in dark at room temperature. The cells were washed twice in PBS and at least 10000 events were collected with FACSCanto (BD, United States) at Haematology and Blood Transfusion Center, Hue Central Hospital, Vietnam. The data were analyzed with FlowJo software after the targeted events were gated which basing on isotype control.

Assessment of differentiation potential of UC-MSCs

When cultured in vitro, MSCs exhibit strong proliferation and can be induced to differentiate into bone, cartilage, adipogenic, and myogenic tissue

Build electrical stimulator:

We built and developed a novel cost-effective electrostimulation system for culture. This system has three-part (Fig.1A): Mainboard control, cell culture wells, and a personal laptop. Cell culture wells were modified by using commercial 6-well plates (Eppendorf Asia Pacific Sdn. Bhd., Selangor Darul Ehsan, Malaysia). Carbon rods were carried out from AAA battery (Toshiba lifestyle products & services corporation, Japan) were used to be electrode. Two electrodes per well were put pass-throughlid of the plate, avoid reach adherence cell in the bottom of the wells, and distance with each other is 30 mm.

The second part of the system is mainboard control, is designed base on low-cost and open source I/O board (Arduino UNO, Smart Projects, Strambino, TO, Italy). The code is sketched in Arduino IDE software and loaded to theboard. This part is connected with a personal laptop which is third part.

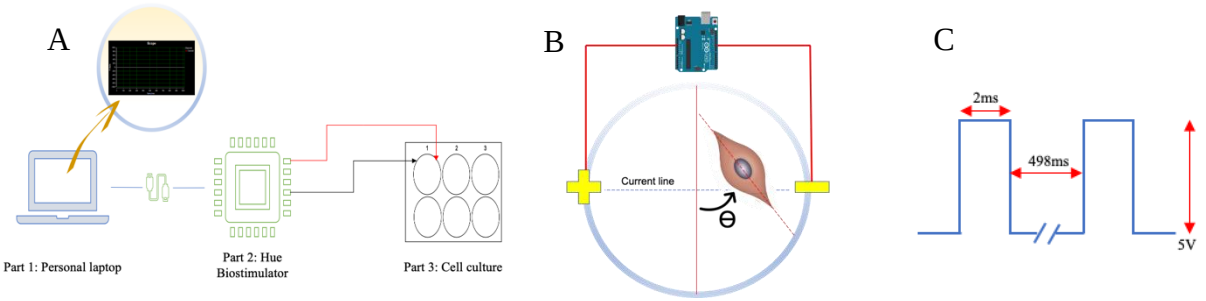


Figure 1. Electrical stimulator and used current
A. System of Hue Biostimulator. B. Θ angle C. Monophasic current

Cell viability and electrical stimulation:

Cell viability assay figures out the wellness of UC-MSCs after electrical stimulation. UC-MSCs at first passage were treated base on data of afore-research(7,8) (Electrical field strength: 5V/cm, cycle duration: 0.5 s, pulse width: 5 ms, monophasic pulse) (Fig.1C). The duration of stimulations was ranged from 5 mins to 50 mins (5 mins, 10 mins, 15 mins, 20 mins, 25 mins, 30 mins, 35 mins, 40 mins, 45 mins, 50 mins). The cells after treatment were stain by before procedure (9). For thisstaining, cells were washed three times thenincubated with trypan blue 0.4% for 10 minutesat room temperature (RT), following by washing again. Cells were fixed with 4% PFA solution and incubate for ½ hour at RT. Finally, cells were visualized in 1X PBS and counted manually from microscopic photos.

By the result of this assay and previous researches, UC-MSCs were stimulated in 10 minutes every day for 15 days with the purpose of differentiation.

Analysis of morphometric:

The images in these assayswere taken by theLWD-20X objective lens with theOlympus CKX31SF inverted microscope (Olympus Corporation, Tokyo, Japan). TheUC-MSCs morphology wasevaluated by using ImageJ software like previous.The morphology of cells was followed everyday before and after in three different treatments. Microscopic images were taken at three experiment-points: 0,5,10,15 days after treatment. Every experiment was triplicated

The cellular morphology can become preliminary reflections on types of cells and recently the cardiac cells inside the myocardium were reported that had an elongated morphology (10). Besides, in cardiomyocyte- predifferentiation from MSCs strategies, before researches also demonstrated that appeared to elongate and became more spindle-like (11). On the other hand, the shape index can measure cellular morphology (12).The shape index was calculated in ImageJ, using Eq. 2.

$$\text{Shape index} = 4\pi \times \frac{Area}{(Perimeter)\text{^}2}$$

The direction of electrical stimulation to cellular behavior was represented through θ angles(13,14). This angle is made between the width vector of cells (Fig.1B, red line) and vertical line (Fig.1B, blue line). The width vector per cell was set to pass the nucleus and connects the very far ends of the cells (Fig.1B). The θ angles were analyzed by ImageJ software. The average amount of θ angle range from 0° to 90°. The levels of

alignment and orientation were represented as percentages of total cells.

Semi-quantitative RT-PCR:

Total RNA was carried out by theusing InviTrap® Spin Universal RNA Mini Kit (STRATEC Biomedical AG, Berlin), according to the manufacturer's instructions. First-Strand cDNA was synthesized with Promega GoScript(TM) Reverse Transcriptase (Promega Corporation) and random hexamer primers. cDNA was diluted using Nuclease free water. PCR was performed with PHUSA Taq_500 and specific primer (Table 1). Primers were designed online using primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized by PHUSA Biochem Company, Viet Nam. All reagents and primers were purchased from PHUSA Biochem and reactions were performed with the recommended procedure of the manufacturer.

Table 1. Primers for RT-PCR

	Primer	Sequence (5'-3')
1	GAPDH_F	CAGGGCTGCTTTTAACTCTGG
2	GAPDH_R	AGGGATCTCGCTCCTGG
3	cTNT_F	ATGAAGATCAGCTGAGGGAGAA
4	cTNT_R	GTCGAACTTCTCTGCCTCCAAG
5	Desmin_F	TGCCCTCAACTTCCGAGAAAC
6	Desmin_R	ACTTCATGCTGCTGCTGTGT
7	Nkx-2.5_F	GAGCCGAAAAGAAAGCCTGAAA
8	Nkx-2.5_R	TCCCTACCAGGCTCGGATAC
9	GATA-4_F	CCGTGTCCCAGACGTTCTC
10	GATA-4_R	GCATAGCCTTGTGGGGAGAG
11	α-MHC _F	TCCTGCGGCCCAGATTCTTC
12	α-MHC _R	TCCGGACAGTCTTGGCATTG

Immunohistochemistry

Untreated, treated UC-MSCs were clung to chamber slides and fixed with methanol for 10 min at - 20°C. In the wake of washing multiple times with PBS, cells were hatched at 4°C. Polyclonal essential antibodies against desmin were diluted (1:100) and incubated with the cells for 1.5 h at 37°C, following by 30 min incubation with secondary antibody. Slides were taken photo utilizing diaminobenzidine substrate and counterstaining with hematoxylin for microscopic assessment.

Statistical analysis

All data is shown as mean ± standard deviation (SD) and differences between samples were determined by Student's t-test. One-way ANOVA, Turkey's HSD post-test among selected pairs of groups were also analyzed and performed with R software for MacOS. Values with a $p < 0.05$ or $p < 0.01$ were considered as statistically significant.

III. RESULTS

Human mesenchymal stem cell characteristics:

Characteristics of UC-MSCs were evaluated through morphology, surface markers (Figure 2).The UC-MSCs isolated from theumbilical exhibit specific shape of MSCs from primary

culture to passage culture (Figure 2A-D). The confluence of hUM-MSCs in culture in the second passage, displaying a typically homogeneous fibroblast-like morphology (Figure 2D).These specimens could be placed in 4% paraformaldehyde for short-term preservation. Three independent flow cytometric experiments were performed.

Base on flow cytometry analysis, the UC-MSCs were determined to be negative for CD45, and positive for CD73, CD90, CD105 (Figure 2E-H).

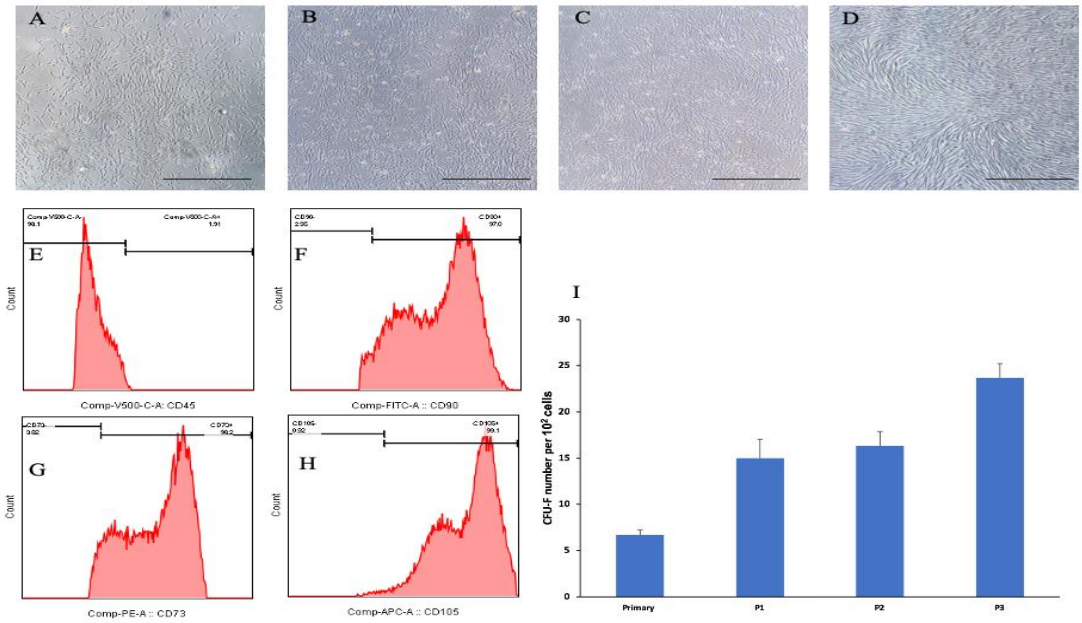


Figure 2. Characterization of human umbilical cord mesenchymal stem cells.
(A-D) Fibroblast-like morphology of UC-MSCs at primary culture, passage 1 to 3, respectively.
(E-F) Histograms of FACs were analyzed by FlowJo revealed that the UC-MSCs be negative for CD45 and positive for CD90, CD73, CD105 (Respectively). Scale bar = 1000 μm
(I) Result of CFU-F assay.

Assessment of differentiation potential of human pluripotent stem cells.

Adipogenic and osteogenic differentiation of mesenchymal stem cells. To evaluate MSC abilities, adipogenic and osteogenic differentiation assays were performed on isolated cells. Osteogenesis differentiation medium (Miltenyi Biotec) or adipogenesis differentiation medium (Miltenyi Biotec) was added into a culture when the fusion rate reached approximately 80%. The cells were cultured for 3 to 4 weeks before collection.The media were changed every 3 days. The cells were cultured at 37°C, 5% CO_2, in 95% humidified air. Adipogenic, osteogenic and chondrogenic differentiation assays were conducted three times. Alizarin Red S staining was used to analyze osteogenic lineages, whereas Oil Red O was used to analyze lipid droplets.

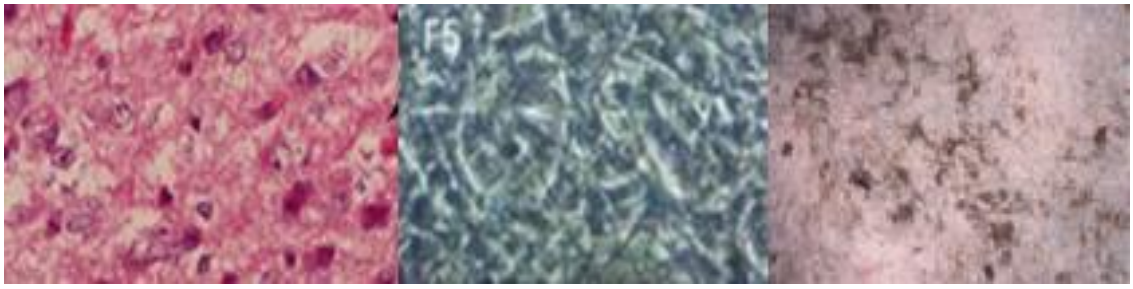


Figure 3. Differentiation along adipogenic, osteogenic, and chondrogenic lineages.

Cell viability assay

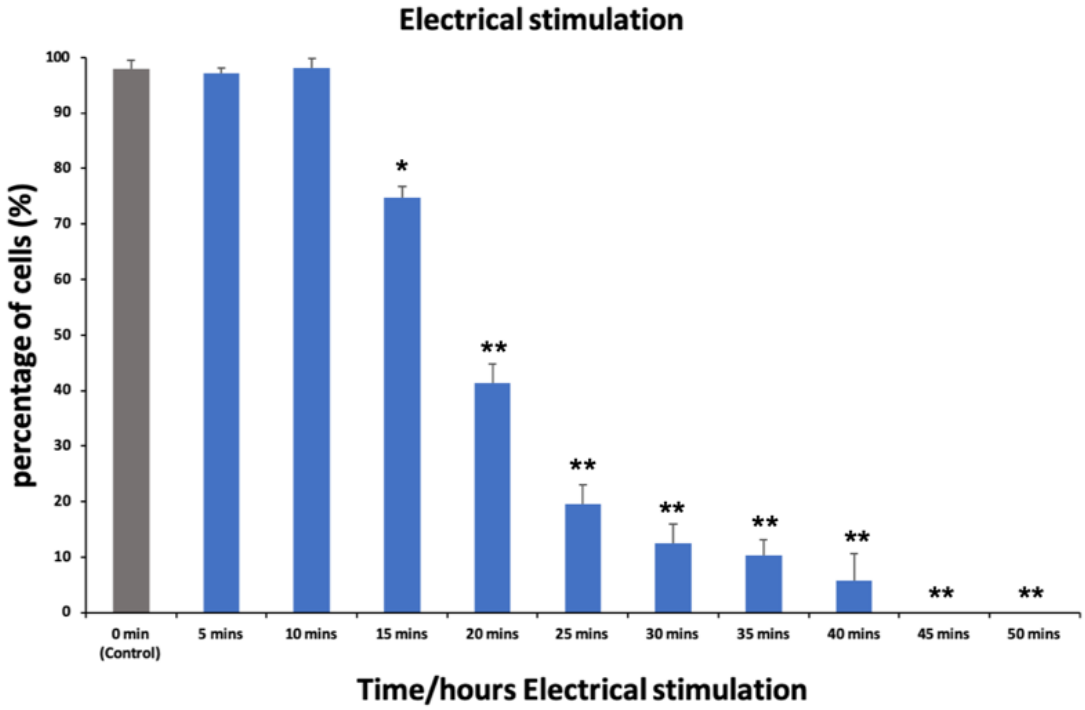


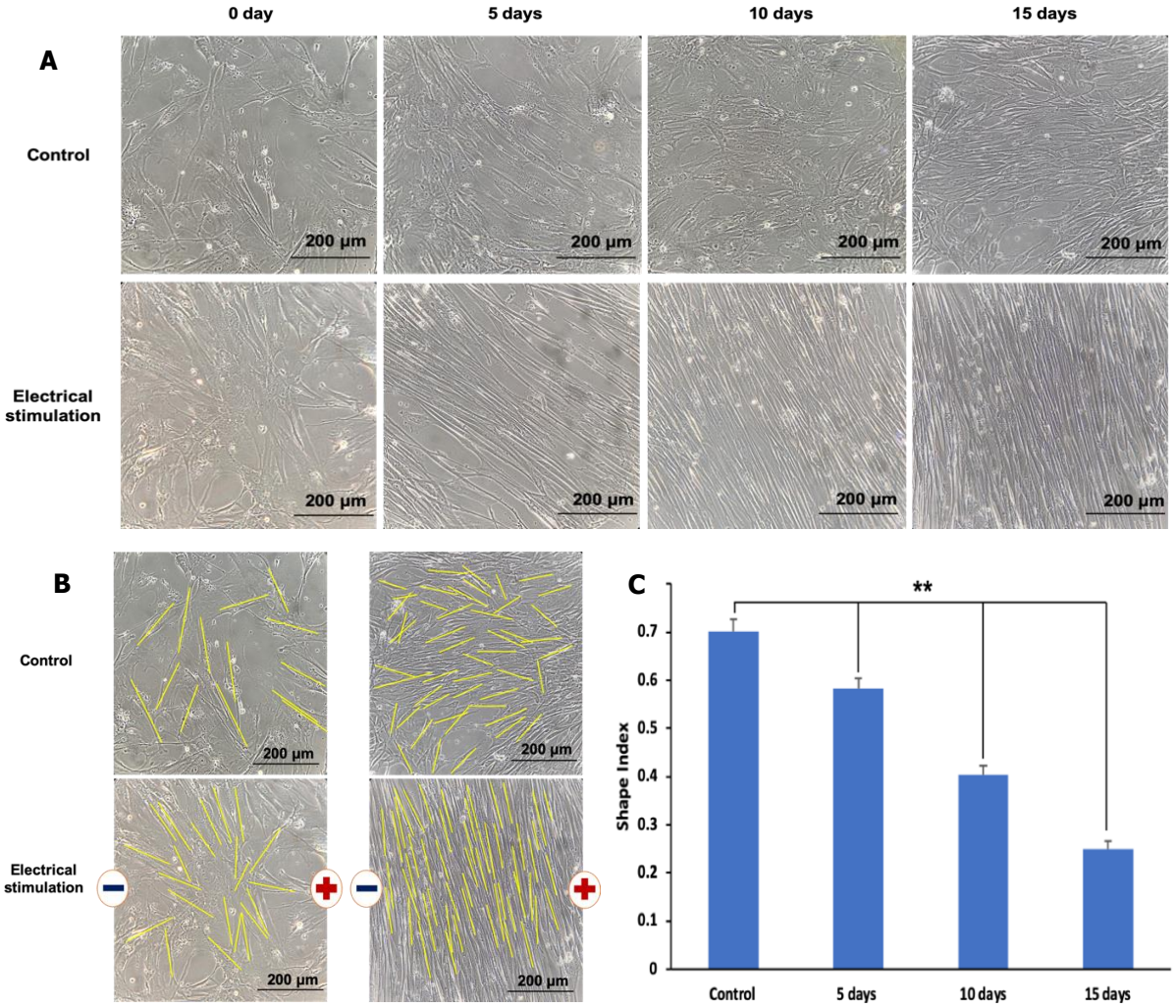
Figure 4. Impact of electrical stimulation on the viability of UC-MSCs (* P < 0.05, ** P < 0.01)

This assay was performed to evaluate the proliferation and viability of UC-MSCs after electrical stimulation in different time periods (11 groups: 0 mins (control) - 50 mins) (Figure 3). A comparison of the viability of electrically stimulated group with the control group (non-stimulated) confirmed that 5-mins and 10-mins of electrical stimulation has no considerable negative impact on UC-MSCs viability. In 15-mins electric stimulation group, the number of surviving cells began to different, the cell density decreased by 23.3% compared to the control group (* p<0.05). A significant difference was shown after 20 minutes of electrostatic stimulation, cell density decreased by nearly half compared to the control (56.68%) and in the 45-mins and 50-mins group the cells were completely dead (** p<0.01). Hojjatollah N et al(10)finding confirms that human cardiosphere-derived cells (hCDCs) differentiate to committed cardiomyocytes when hCDCs receive an electrical stimulation in 10 minutes. From

thatoutcome and the result of cell viability assay, we hypothesis that electric stimulation of UC-MSCs for 10 minutes per day to study the ability of UC-MSCs to differentiate into cardiomyocytes.

Morphological changes of the cells: response to the received stimulation

The morphological analysis was observed under a microscopeshowed that the morphology of UC-MSCs in the ES group began to change longer than in the control group and they had a similar orientation after the electrical stimulation after 5 days (Fig. 4A). By day 15, UC-MSCs were more elongated and spindle-shaped, the yellow lines in theES group show that UC-MSC were aligned mostly perpendicular to the direction of the current (Fig. 4.A, B). The shape index, which is an Image J analysis index, decreased considerably down to 0.25, by 10 min of stimulation after 15 days (Fig. 4C).



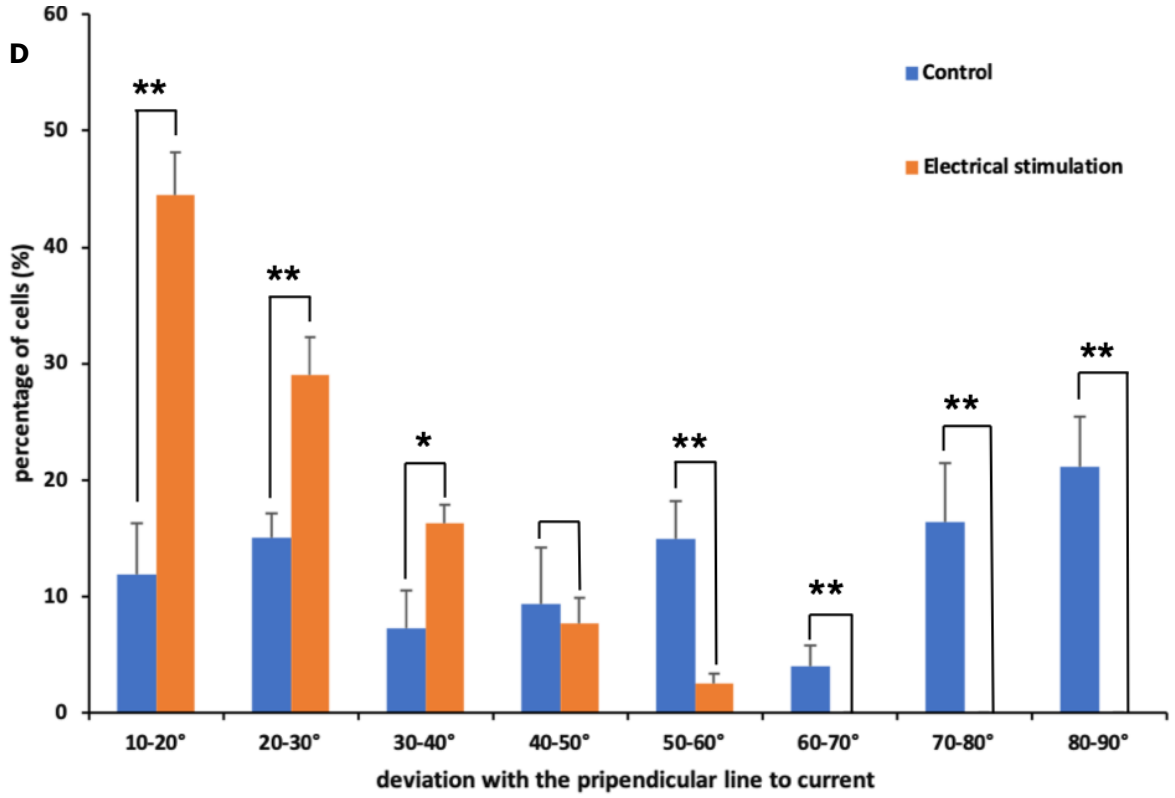


Figure. 5: Impact of electrical stimulation on the morphology and deviation of UC-MSCs and shape index. (A) the morphology of UC-MSCs is elongated under the impact of electrical stimulation after 15 days (scale bar: 200 μm). (B) The yellow lines on the cells represent the cell vectors axis. (C) Shape index of the UC-MSCs after electrical stimulation 5 min, 15 days (**P<0.01). (D) The orientation of UC-MSCs populations with concerning the passing current between electrodes shows the difference of the ES group compared to the control group

Impact of respect to the electric current to the cell orientation was investigated by measuring the θ angle between cell vectors and the electric current direction is the cell orientation with respect to the electric current (Fig.4D). The cells in the two groups had a variety of θ angle distribution, ranging from 10 to 90°. In ES group, after ten minutes of electrical stimulation, the ratio of UC-MSC with θ angles from 10° to 90° diminished considerably (P < 0.01) compared to the control. The ratio UC-MSC with angle θ 10–20 ° was the highest (44.45%) in the ES group and this ratio decreased gradually at the angles θ 20–30 °, 30–40 °, 40–50 ° (29 %; 16.27% and 7.69%). Angle θ 60–90 °, The UC-MSC ratio is almost zero. In contrast, in the control group, the ratio of cells with θ angle of 10–90 ° varied steadily between 11.9% and 21.14%.

Rerverse transcription- PCR:

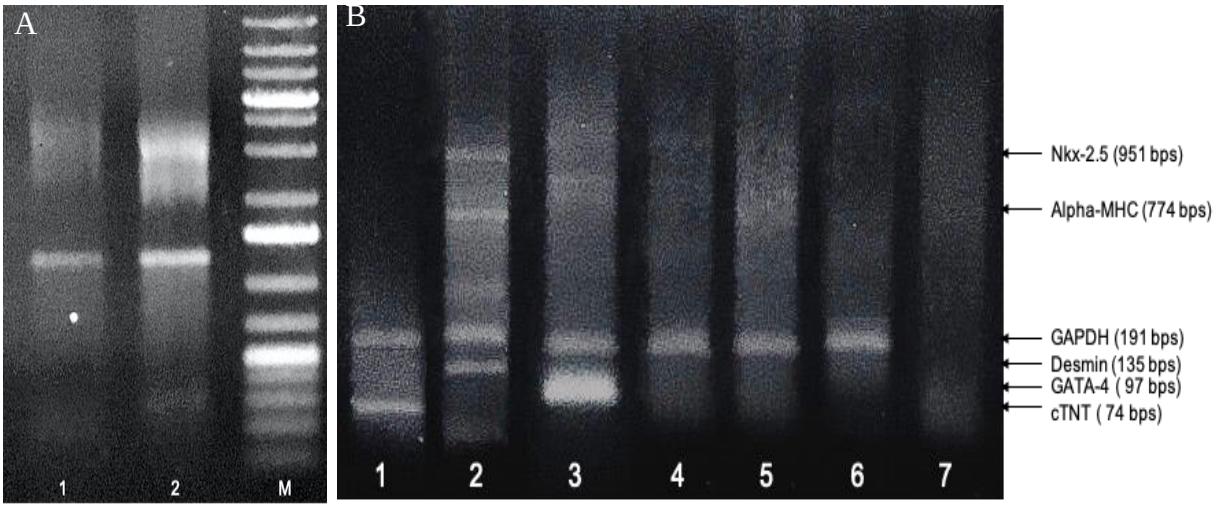


Figure 6. Semi- quantative RT-PCR
(A) Total RNA. (B) Result of RT-PCR

It is important to understand the molecular basis of the differentiate. Analyzing gene expression levels or sequencing technology would help us to understand the molecular mechanism of cellular functions. After isolated, RNA was electrophoresis and visualized (Fig 5A), Lane 1 is RNA of UC-MSCs which weren't stimulated with electrical current, lane 2 is RNA of UC-MSCs after stimulation. After first-strand synthesis and PCR reaction, products of PCR were electrophoresis and captured (Fig. 5B). This result revealed the upregulation of cardiac-specific genes like Nkx-2.5, Alpha-MHC, Desmin, GATA-4, cTNT in lane 1-3 (Fig 5B); lane 4-6 is the mRNA expression of control UC-MSCs and lane 7 is blank.

Immunocytochemistry

Immunocytochemistry examination showed cells positive for Desmin which is anearly markercardiac in electrical stimulated UC-MSCs (Fig. 6B). While that, the non-treated UC-MSCs negative for Desmin (Fig.6A).

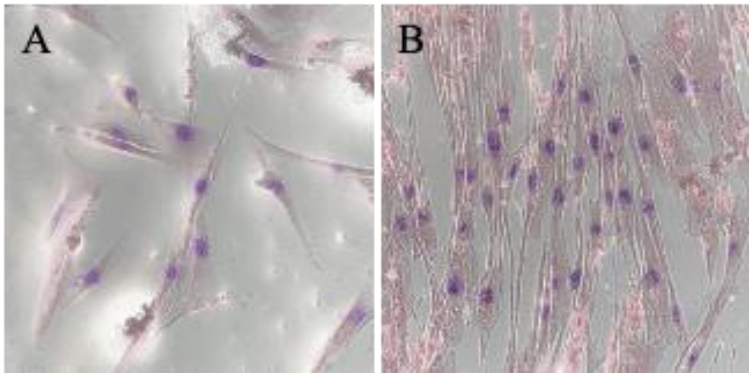


Figure 7. Immunohistochemistry stain for Desmin. (A) Control UC-MSCs (B) Treated UC-MSCs

DISCUSSION:

UC-MSCs is an appropriate source for cardiomyocyte-like cell differentiation. First of all, the MSCs are isolated with traditional strategies, were announced about the appearance of GATA4 and NKX2.5 mRNA at a low level by beforehand study(15). On different hands, our work shows the nonappearance of GATA4 and NKX2.5 mRNA in untreated UC-MSCs, which is additionally like some afore-publishes(16). This chaos can go from the origin of MSCs on the grounds previous researches often focuses on bone marrow MSCs instead of UC-MSCs like this work.Similarly, the expression of the genes in BMSCs and UC-MSCs was demonstrated to be not quite the same as one another(17), while BMSCs more display the gathering of osteogenesis related-genes, UC-MSC might be a superior hotspot for cardiomyocyte - related work(18).

In our methodologies, we differentiated cells by electric stimulation andwe attempt to perform pre-cardiovascular differentiation which can fit for cell therapy in some cardiovascular sickness since some past reports that undifferentiated MSCs can become arbitrary progenitorcells or differentiated cells in disease microenvironmentso can cause hazardous results (19)and cardiomyocyte-like cell going before transplantation would achieve improved myocardial recuperation and recovery of heart work (20). At first, the morphological measure uncovers the reaction of UC-MSCs after got electrical stimulation. The past works announced that MSCs elongated and aligned with relevant orientation, which is opposite to MSCs got 5-aza treatment(21). In line with these researches, UC-MSCs in this work after electrical stimulation exhibit elongation and alignment with a clear orientation similar to the native heart tissue. The elongation and alignment of treated UC-MSCs were represented respectively through shape index and θ angles, this data was analyzed base on ImageJ software. This result suggested electrical stimulation is an alternative method for non-specific demethylation like 5-aza on cardiac-linage differentiation.Moreover, with an effect of aforementioned monophasic electrical current, UC-MSCs were gained result like authors's desirement. This electrical current changed the epigenetic landscape exhibit through the upregulation of some cardic-specific genes. Among these, NKx2.5 is probably the soonest marker of cardiogenesis and is thought to work in blend with GATA-4, an early marker shown to regulate a portion of the genes engaged with heart muscle differentiation and function during early-stage advancement(22). Not only that, but Desmin which is a early maker also rocket upregulate verus the control group. These things demonstrate that electrical current in this research is a epigenetic modulator, this might be from DNA demethylation of the CpG islands of the promoter regions of Nkx2.5 and GATA4 clears the path to access these promoters for active transcription(23). Also, cTNT and alpha-MHC (myosin heavy chain) which is the later marker are accounted for related to morphogenesis, myogenesis, the gathering of muscle proteins and the coordination of contractile reaction in the creating myocardium after mid-fetal turn of events(24) . Consequently, the exhibit of these markers could be confirmations of differentiation into cardiomyocyte-like cells(25). Within the expression of Desmin mRNA after electrical stimulation,

immunohistochemistry assay was performed and verified the exhibit Desmin protein in the cytoplasm.

The pre - differentiation of UC-MSCs into cardiomyocytes by utilizing our gadget can recommend many exploration thoughts later on. The cardiomyocyte-like cells in the result of this paper thoroughly can use in research *in vivo* about capacity to treat for post-heart attack animal model soon on the grounds the consequence of this exploration and the support documents which were refered to.However , if that was actual, the dose of these cells in the next reseach is a big concerned issue. Reseach on transplantation of cardiomyocyte-like cells promise to be a helpful treatment for heart attack, especially after the emergency, this thing aim to reduce fibrosis state after heart attack which was announced can lead to heart failure in a long time of lifelong.Besides, our device can be considered to use directly in clinical for some cardiovascular diseases in the future. Although it is only a hypothesis now, it can be applied after by many research on it in the near future.On the total, the cardiomyocyte-like cells which was generate by our work can be a appropriate treatment source for pathophysiology of heart and our device_ Hue Biostimualtor can be have more mission in the future.

In conclusion, our research was successfully build up a low-cost Electrical stimulator in cell culture which name Hue BioStimulator. Especially, we demonstrated the efficient effect of it in pre-cardiac differentiation with a specific monophasic electric current. Parameters of used electrical field is strength: 5V/cm, cycle duration: 0.5 s, pulse width: 5 ms. Finally, after stimulation with our device UC-MSCs undergo cardiac pre-differentiation exhibition through cardiac-specific genes upregulation and desmin protein expression. These results revealed many applicated suggestion of these cells for treatment and the more missions of Hue Biostimulator in the near future.

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ISOLATED POST-TRAUMATIC RADIAL HEAD DISLOCATION, A RARE AND EASILY OVERLOOKED TRAUMA - 2 CASE REPORTS

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INTRODUTION

Isolated post-traumatic radial head dislocation is a rare and easily overlooked injury due to atypical symptoms, clinically, patients are often diagnosed late and misdiagnosed as soft tissue injuries. sprains. Patients need a meticulous physical examination and bilateral comparison, two-view x-rays to determine the abnormality in the relationship between radial head and capitellum [1,2]. The causes of the dislocation can possibly be congenital, sudden jerking of the forearm in extension or a part of a complicated elbow injury such as a Monteggia fracture; however, Isolated post-traumatic radial head dislocation with the absence of other associated injuries is rare and easily overlooked. The early isolated post-traumatic radial head dislocation can be treated conservatively with good outcomes [2.10]. However, in delayed lesion, annular ligament is fibrotic and stretched, cause difficulty in reduction, so surgery is needed to place the radial head back to the correct anatomical position, restore annular ligament and immobilization with long arm cast. [3] We report 2 cases of 7-year-old and 12-year-old female children with a diagnosis of delayed isolated post-traumatic radial head dislocation with missed initial diagnosis, surgically treated by joint resetting, annular ligament repair with suture and percutaneous K-wire fixation at Thai Binh Medical University Hospital, Vietnam.

Keywords: *Delayed isolated post-traumatic radial head dislocation, missed initial diagnosis.*

I. CASE 1

A 7-year-old female patient felt with her left hand on the floor. She was taken to the district hospital and diagnosed with soft tissue injuries, treated with arm sling, restricted arm mobility and given a prescription for outpatient treatment. However, after 20 days, the patient still felt painful in the left elbow, the left elbow joint was limited, the patient was taken to the Department of Trauma and Orthopedics, Thai Binh Medical University hospital, Vietnam. Examination showed that her left elbow was still swollen, tender at anterolateral aspect of left elbow, no ecchymoses, no abrasions, no lacerations was found. The child could pronate her left hand to 90 degrees without difficulty, but hesitant to supinate due to pain, she lacked about 20 degrees to full supination left to be fully back. Children was not able to fully stretch her injured arm, about 30 degrees left to fully extend. She had pain with flexion beyond 50 degrees. She was too painful to stop at 90 degrees. The sensory and motor examination was normal in medial, ulnar and radial distributions and innervations. Radial pulse in both wrists were palpable and the same. She had no accidents apart from the abovementioned fall.

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Date of scientific judgment: 23/10/2020
Reviewed date: 30/10/2020

Figure 1A ***Figure 1B*** ***Figure 1C***

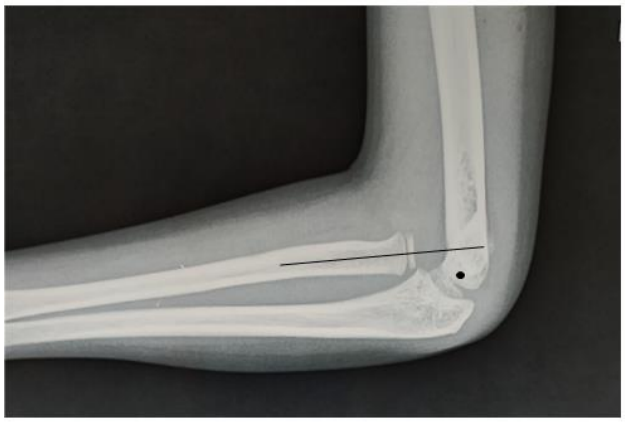
Figure 1A: A 7-year-old female patient had cubitus valgus in left hand

Figure 1 B: 7-year-old female patient with limited left arm flexion and pain

Figure 1 C: A 7-year-old female patient with limited left hand extension in lateral view, increasing pain

After the physical examination, we had the patient X-rayed the left elbow, and found the anterior dislocation of radial head in lateral view.

Figure 2 (A)



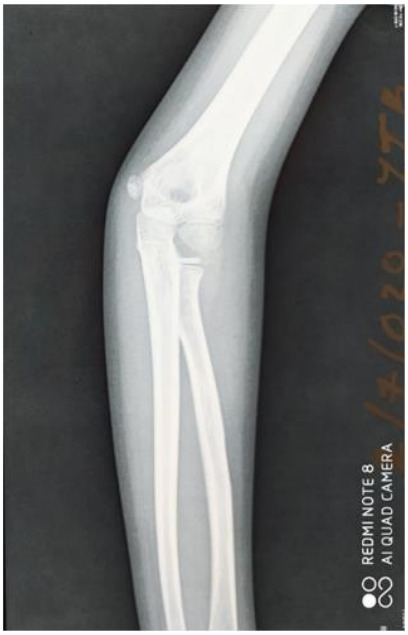


Figure 2 A ***Figure 2 B***

Figure 2 (A) Sagittal view: 7-year-old female pediatric patient: radial axis did not align with the capitellum, anterior dislocation

Figure 2 (B) Coronal view showed the occult radial head dislocation

Definitive diagnosis: Delayed isolated post-traumatic radial head dislocation in left arm. Closed reduction of the elbow was attempted with the combination of longitudinal traction on the forearm, forced supination of the forearm at 30 degrees of elbow flexion, and then flexion to 100 degrees while maintaining supination. This attempt at closed reduction failed. We decided to operate the patient: Proximal radioulnar joint resetting, annular ligament repair with suture and percutaneous K-wire fixation.

Surgical steps:

- Kocher approach was applied to reduce the joint openly.

- After exposure, the annular ligament was noted to have slipped posterior to the radiocapitellar joint. The annular ligament was compressed between the radial neck and capsule, pushing the radial head anteriorly when the capsule was tightened in flexion. The ligament could not be pulled over the radial head when intact. The annular ligament was transected and subsequently repaired distally at the level of the radial neck, creating a sling to prevent recurrent subluxation. The elbow was casted in supination and 90° of elbow flexion.

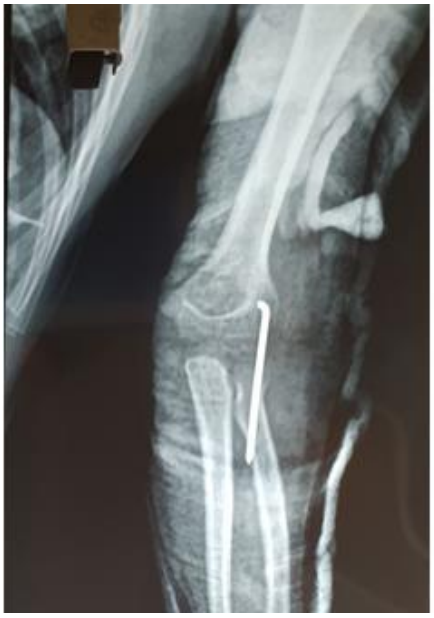


Figure 3 A

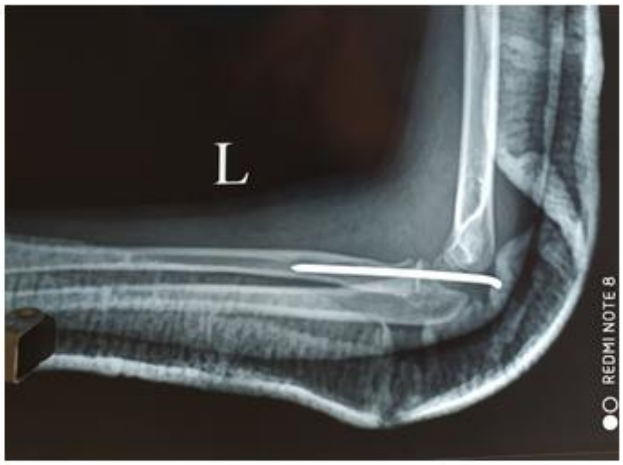


Figure 3 B

Figure 3 A: 7-year-old female patient, radiograph after straight post-operative coronal x ray.

Figure 3 B: A 7-year-old female patient, post-operative sagittal x ray

K-wire was removed after 4 weeks and after 8 weeks, the cast was removed. Then the patient was rehabilitated in the left elbow for 2 months. Re-examination after 14 weeks postoperatively, she had full range of motion in extension, flexion, pronation, and supination. She was then progressed to full activities without restriction.

Figure 4 A

Figure 4 B

Figure 4 C

Figure 4 A: 7-year-old female patient with left elbow extension posture after 14 weeks in coronal view

Figure 4 B: 7-year-old female patient with left elbow flexion posture after 14 weeks

Figure 4 C: 7-year-old female patient with left elbow extension posture after 14 weeks in sagittal view

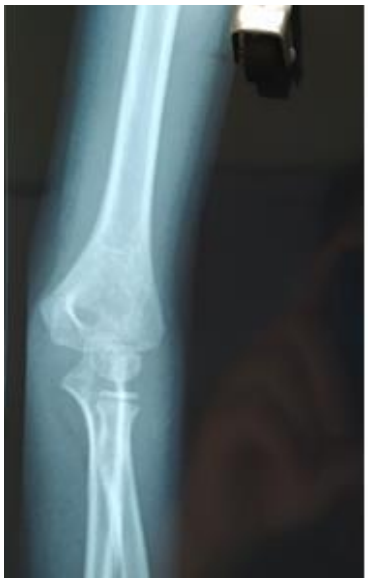


Figure 5 A

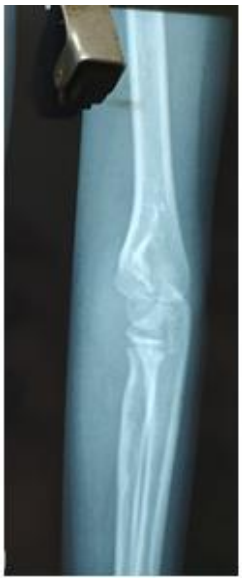


Figure 5 B

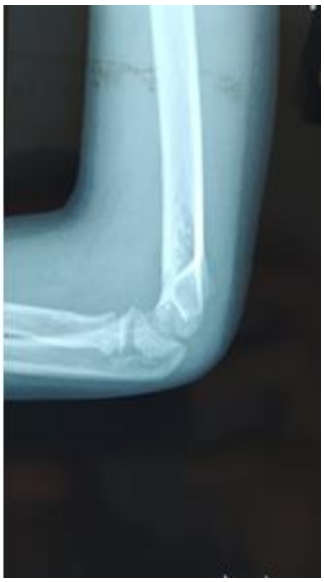


Figure 5 C

Figure 5 A: 7-year-old female patient, left elbow extension post-operative coronal x ray after 14 weeks

Figure 5 B: 7-year-old female patient, left elbow extension post-operative sagittal x ray after 14 weeks

Figure 5 C: 7-year-old female patient, left elbow flexion post-operative sagittal x ray after 14 weeks

II. CASE 2

12-year-old female child patient falls with her arms stretched on her elbows. Then, she had pain, loss of movement in the left elbow. She was not taken to a hospital, are treated at home according to traditional experience which were manipulation with medicated oil, aromatic balm. After 5 weeks of trauma, the swelling and pain, limited mobility did not decrease and was brought to our hospital by family members. Physical examination found: left elbow pain, limited movement related to radial head, no signs of nerve damage or dystrophy of the left forearm.

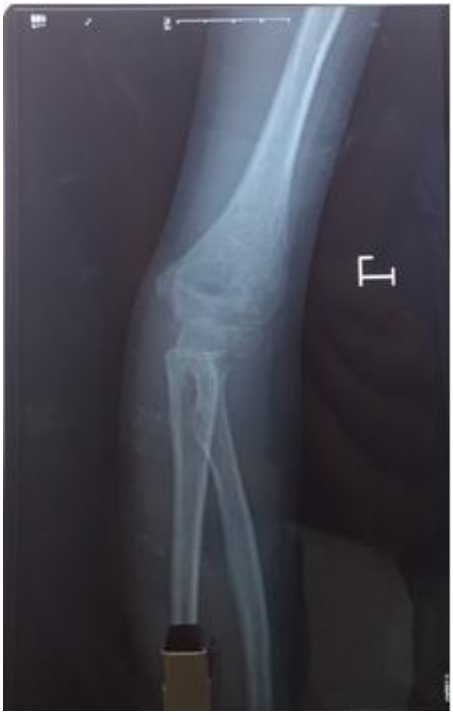


Figure 6A

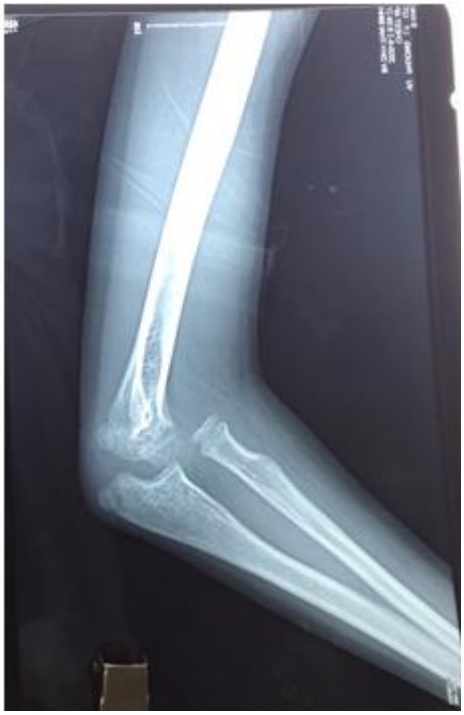
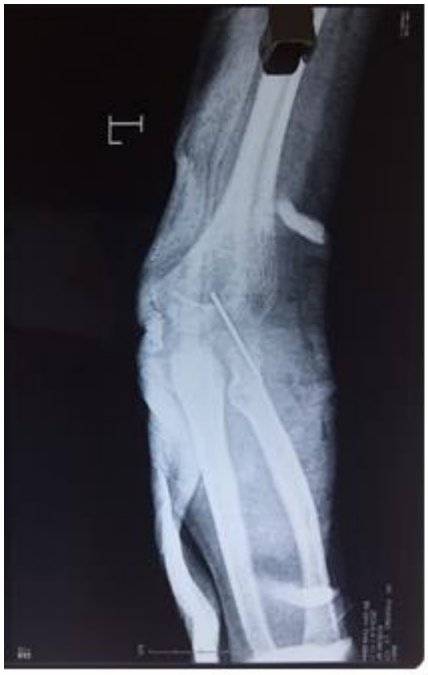


Figure 6B

Figure 6A: A 12-year-old female patient, coronal X-ray showed a dislocated radial head
Figure 6B: A 12-year-old female patient, sagittal X-ray showed a dislocated radial head

Diagnosis: Delayed isolated post-traumatic radial head dislocation in left arm, 5th week. Surgery: Proximal radioulnar joint resetting, annular ligament repair with suture and percutaneous K-wire fixation. After surgery, the patient was placed in long arm cast for 8 weeks, K-wire was removed after 4 weeks, and the child began to perform rehabilitation exercises.

After 3 months of surgery, the patient could extend fully. After 4 months, she had full range of motion in extension, flexion, pronation, and supination. She was then progressed to full activities without restriction.



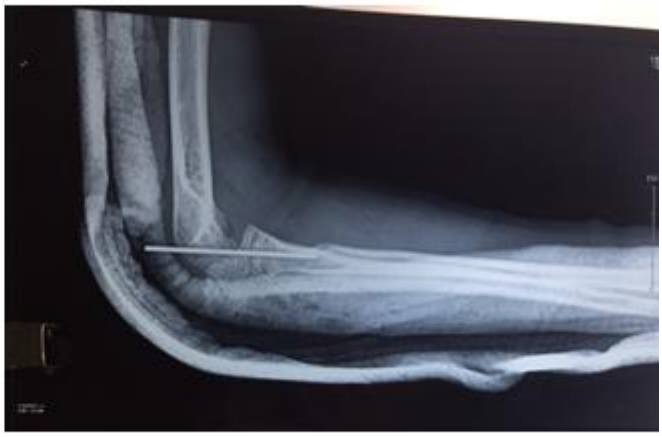


Figure 7 A ***Figure 7 B***

Figure 7 A: 12-year-old female patient, post-operative coronal x-ray: 90 degree left elbow flexion

Figure 7 B: 12-year-old female patient, post-operative sagittal x-ray: 90 degree left elbow flexion

III. DISCUSSION

Injuries about the elbow are extremely common in children. Hanlon and Estes [1] estimated that upper extremity injuries account for 65% of all fractures and dislocations in children [2,3]. In children younger than 7 years, elbow injuries account for about 30% of all limb fractures.2 Traumatic injuries to the elbow occur more often in the skeletally immature than they do in adults [4,5]. Historically, radial head subluxation has been estimated to occur in roughly 25% of all elbow injuries in children younger than 10 years. [6]

Delayed isolated post-traumatic radial head dislocation is easy to omit in children due to poor initial symptoms, easily mistaken for soft tissue injuries or sprains, plus traditional treatment methods in rural Vietnam like rubbing or manipulating with medicated oil, aromatic balm, bear gall,... make the affected area quickly swollen, the functional and physical symptoms will be difficult to examine. Furthermore, the less proficiency of local doctors in read X-ray interpretation also contributes to the omission of this injury.

Precise history-taking, thorough physical examination, and plain films are essential to making a correct diagnosis of delayed isolated post-traumatic radial head dislocation. Several entities such as radial head fracture, coronoid process fracture, congenital radial head dislocation or Monteggia fracture must be distinguished and excluded [7,8,9]

The causes, mechanism, patho-physiology and the time from the injury to the admission are the key factors in determining final treatment. If early detection, isolated post-traumatic radial head dislocation can be reduced closely and immobilized for 6 weeks in cast that is the suitable treatment and usually yields good outcomes [10]. In the case of missed and delayed, closed reduction is often ineffective, so indication of proximal radioulnar joint resetting, annular ligament repair with suture is considered, 6 week postoperative immobilization following active rehabilitation exercises Currently, there are several methods to treat delayed isolated post-traumatic radial head dislocation, such as annular ligament suture, ligament reconstruction with strip of triceps tendon, palmaris longus muscle or supinator muscle's fascia. However, in 2 abovementioned cases, the patient came to examined after about 3 and 5 weeks, we evaluated that the annular ligament and others are still in good condition, so suture to repair annular ligament is more feasible and yields desired outcomes [11,12].

Both of our patients, although detected late (3 weeks and 5 weeks), those patients were rubbed and manipulated with medicated oil, aromatic balm, so he had fibrosis, and conservative treatment failed. Finally, we applied the procedure for fibrosis debridement, suture annular ligaments together with K-wire fixation for 4 weeks and 8 week cast immobilization after surgery, when removing the powder for patients to practice active rehabilitation. Post-operative follow-up showed that 2 children could pronate and supinate maximally, painlessly and radial head was in correct position in X-rays

IV. CONCLUSION

Delayed isolated post-traumatic radial head dislocation is a rare and easy-to-miss injury. In cases of late detection, especially treated by traditionally methods with medicated oil, aromatic balm resulting in fibrotic ligaments and joints, conservative treatment is often not effective , because the fibrotic soft tissue cannot maintain radial head in correct position. Therefore, surgery is the optimal option for those patients.

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VIRUS VACCINE CAUSE SYNCYTIAL FORMATION AND MORPHOLOGY CHANGE IN HUMAN CANCER CELLS

Ho Anh Son*, Nguyen Van Chuyen*, Le Duy Cuong**

ABSTRACT

Objectives: observing the syncytial formation and morphology change in cancer cells cause by measles (MeV) and mumps (MuV) virus vaccine. **Subjects and methodology**: human colon cancer HT-29 cells were infected by MeV and MuV, then the infected cell was observed under light microscope to find the syncytial formation. HT-29 tumor slices were checked with electron microscope to find out the cancer cell morphology change. **Results**: MeV and MuV infection cause apoptosis for cancer cells. The cells were formed to syncytia and lysis. The HT-29 tumor cell morphology was observed as shrinkage, loss of microvilli, chromatin condensation and changes of nuclear contours, nuclear fragmentation, and getting lipid droplets in cell plasma. **Conclusion**: Measles and mumps virus vaccine cause syncytial formation and morphology change in human cancer HT-29 cell line.

Keywords: *HT-29, oncolytic, measles virus vaccine, mumps virus vaccine, morphology.*

I. INTRODUCTION:

Oncolytic virus (OV) is an approach that turns replication of virus into arms to kill cancer cell meanwhile they almost do not affect normal cells. OV has many advantages comparing with the traditional cancer therapeutics including: reduced adverse effects, the possibility of broad applicability to many cancer types, and a self-amplifying mode of anti-tumor activity by producing more therapeutic virus in tumors. The OV that use live attenuated measles vaccine virus (MeV) and mumps vaccine virus (MuV) to treat effectively human cancer cells has been demonstrated. Many clinical trials using these viruses via different deliveries such as: intratumoral injection, intravenous injection, intraperitoneal injection and so on to treat effectively various cancers: colorectal cancer (CRC), ovarian cancer, cutaneous lymphoma T cell, and so on have been reported with satisfactory results. Global vaccination programs with MeV, MuV established a safe data worldwide. Because of the successful measles and mumps vaccination programs which give lifelong protection, more than 80% of the people in the world are currently measles and mumps immunes 1. Even if one considers the worst-case scenario where in an attenuated measles and mumps viruses used for cancer therapy might revert back to pathogenic strains, the risk of virus transmission from patients to carriers and then into the population is limited by the high prevalence of anti-measles immunity. Besides, many preclinical and clinical trials use MeV and MuV to treat various cancer cells are shown very few side effects. Utilization of oncolytic MeV and MuV as the potential virotherapy for cancer treatment is being studied in many developed countries. But, up to now, there is no any study for assessment of combination of MeV and MuV against human CRC. Studying anti-cancer efficacy of combination of MeV and MuV against human CRC in experiment will

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initiate clinical trials using MeV and MuV combination to treat CRC patients.

As mentioned above, we carry out the study to assessment the apoptosis of combination of MeV and MuV in human colon cancer HT-29 cells line *in vitro* and *in vivo*.

II. SUBJECTS AND METHODOLOGY

2.1. Subjects

The HT-29 cell line (ATCC, Manassas, VA, USA).

MeV-Edmonton and MuV-Urabe strains derive from Priorix vaccine (GlaxoSmithKline, UK).

2.2. Method

2.2.1. Observation of cancer cell syncytial formation

HT-29 cell line derived from storage in liquid nitrogen or at -80°C, quickly thaw vial, seeding in culture plates (20x150mm). Changing the culture media every 2 days. 7-10 days after cultivation, when HT-29 cells reached a >90% confluent monolayer. HT-29 cells were collected and counted on the haemocytometer chamber. Cell concentration was adjusted to of $2x10^3$ cells/ml. 200mcl of this suspension was pippeted into each well on 96-well plates. Storing these cell-seeded 96-well plates in 37°C incubator with 5% CO2. A day after cell cultivation, check these cell-seeded 96-well plates by light microscope, the cells cling to bottom of the wells and grow. MeV and MuV solutions were added to each 96-well plates. On the 3rd, 4th, 5th and 6th day after viral infection, the infected cell was observed under light microscope.

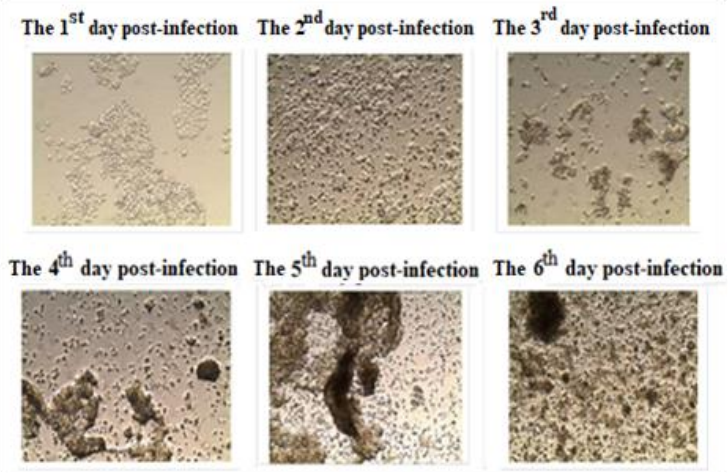
2.2.2. Observation the cancer cell morphology

Nude mice was injected HT-29 cancer cell to form the tumor. Then, animal was treated by MeV and MuV. HT-29 tumors were relocated. Tumor slices were prepared in Faculty of Cell Morphology, 69 Institute. Cell morphology was taken by transmission electron microscopy (JEM 1400, JEOL, Japan).

III. RESEARCH RESULTS

3.1. Direct oncolysis via syncytial formation in vitro

HT-29 cells were infected by MeV and MuV with viral concentration at 1.MOI. On the 2nd day post-infection, we observed beginning of morphologic changes of virus-infected HT-29 cells by light microscope, virus-infected HT-29 cells shrank and detached from the bottom of the culture plates. On the 3rd day post-infection, there were more shrank and suspended virus-infected HT-29 cells, they began to form syncytia.



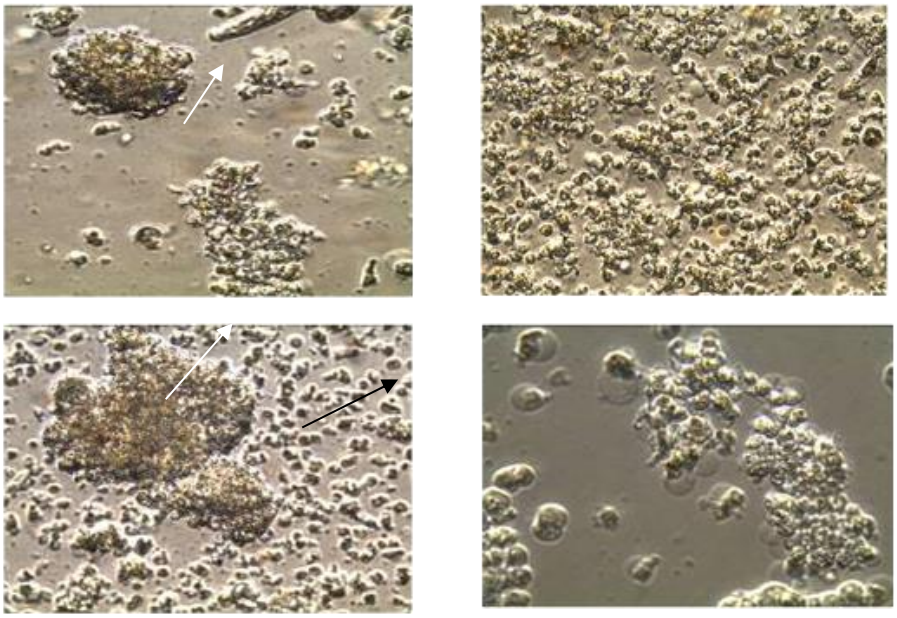


Figure 1. Syncytial formation of MeV and MuV-infected HT-29 cells

On the 4th and 5th day post-infection, syncytia enlarged and suspended around. On 6th day post-infection, syncytial formation was almost entire in culture plates (white arrows) and dead syncytium resulted in cell fragments (black arrows).

3.2.MuV and MeV induce morphologic changes in apoptotic HT-29 cells

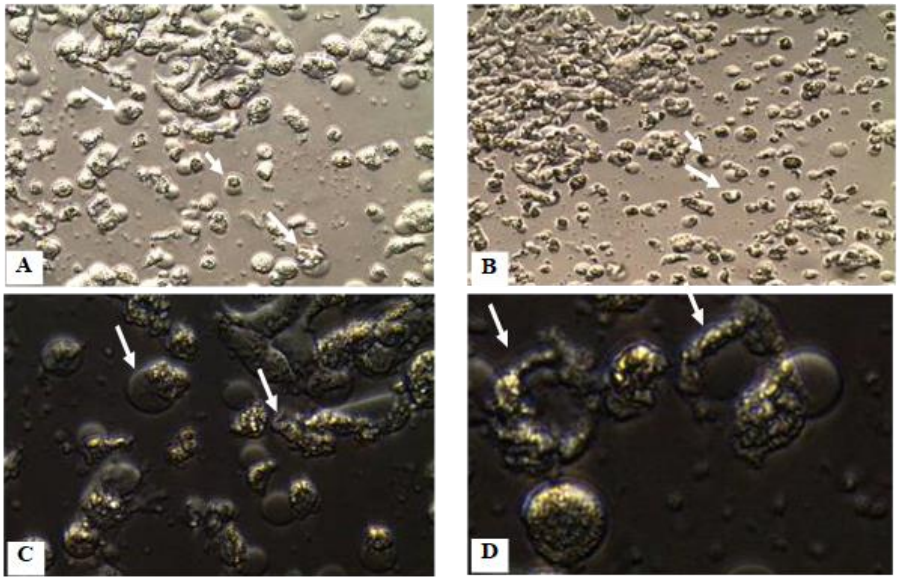


Figure 2. Observation of morphologic changes in apoptotic HT-29 cells under light microscopy

On the 3rd, 4th and 5th days post-infection, we observed morphologic HT-29 cell changes in the stages of apoptosis such as: shrank nuclei and lying on one side of cells (A, B, white arrows), shrank cells (C, white arrows), detached cells, nucleus fragmentation and braked nuclei and cell necrosis (D, white arrows) (Figure 2).

3.3. Morphologic ultrastructure of HT-29 tumor cells after treatment with MeV and MuV

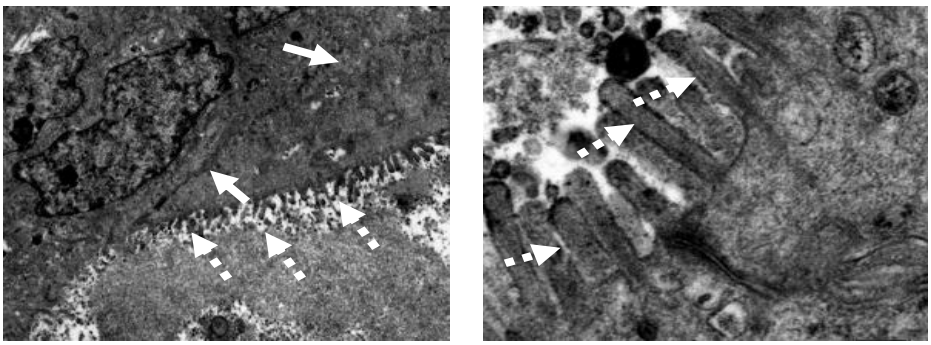


Figure 3. Morphologic ultrastructure of normal HT-29 tumor cells
White arrows: normal HT-29 tumor cells; interrupted white arrows: the microvilli of normal HT-29 tumor cells

We used transmission electron microscopy to assess the changes of virus-infected HT-29 tumor cell morphology. The results showed that the normal HT-29 tumor cells were close to each other with microvilli and intact organelles, and normal nucleus and chromatin, (Figure 3).

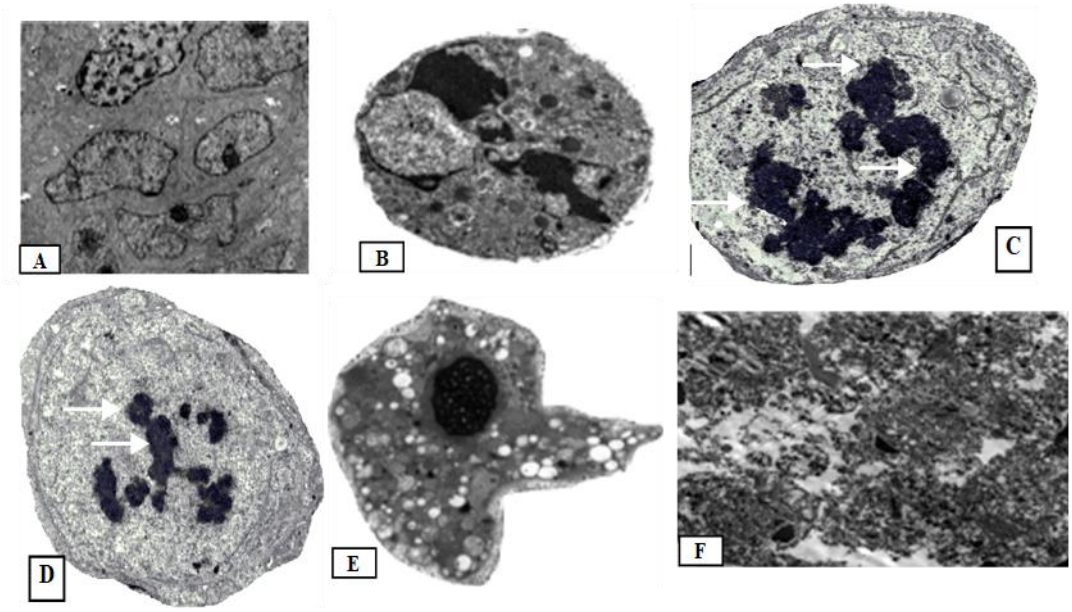


Figure 4. Morphologic ultrastructure of virus-infected HT-29 tumor cells
(A) HT-29 tumor cells; (B) Chromosome condensation in HT-29 tumor cells; (C), (D) Fragment chromosomes in HT-29 tumor cells; (E) Appearance of lipid droplets; (F) HT-29 cell necrosis.

In addition, we also observed the morphologic ultrastructure of HT-29 tumor cells that undergo stages of apoptosis pathway: cell shrinkage, loss of microvilli, chromatin condensation and changes of nuclear contours, nuclear fragmentation, and getting lipid droplets (Figure 4).

IV. DISCUSSION

4.1. MeV and MuV directly lyse HT-29 cell line through syncytial formation in vitro

MuV and MeV are able to specifically infect CRC cells, specific receptors of MeV and MuV frequently overexpress on surface of CRC cells. MuV-Urabe strain use sialoglycoproteins containing sialic acid as specific receptors on the cell surface. It was shown that overexpression of sialic acid-rich glycoproteins on the surface of CRC cells increases infection of the virus in this cell line. MeV-Edmonton strain use CD46 molecular as a specific receptor, which is a transmembrane glycoprotein that regulates activation of complement, they commonly expressed in all human nucleus cells, but is often overexpressed in CRC cells. In addition, Nectin-4 has recently been identified as a specific epithelial receptor of MeV 2. Besides the replication of enveloped viruses such as MuV or MeV requires proteases to cleave viral and membrane glycoproteins to allow the viruses to effectively enter the host cells, these proteases are overexpressed in many cancer cell lines including CRC 3.

The CPE of MeV and MuV begins immediately after H or HN protein interact with its receptor on target cells. This interaction will promote the change of structure in F protein of virus, which leads to active F protein, occurring fusion of the cell host and the viral membrane to allow the virus to enter the cell. The viral H or HN protein interacts with receptor on neighboring cell to activate membrane fusion between the virus-infected cell and neighbors. The membrane fusion between the virus-infected cell membrane and neighboring membranes form syncytium 4, 5.

4.2. Morphology of apoptotic cell death induced by MeV and MuV infection

For cancer cells, activation of apoptosis pathway has been considered a strategy for studying anticancer drugs. In this regard, studying the anticancer effects of MeV and MuV have shown that these vaccine viruses are able to inhibit proliferation of cancer cell lines by activating apoptosis without causing cytotoxicity to neighboring cells. Our findings in this study have also demonstrated that MeV and MuV produce a strong cytopathic effect on HT-29 cell line and induce apoptosis pathway. The important pathway contributes to induce oncolytic effects of MeV and MuV. In other previous studies, the characteristic of cytopathic effect induced by measles and mumps vaccine viruses *in vitro* is a syncytial formation. Nichols W.W., *et al.* (1965) studied chromosomal damage associated with the measles virus *in vitro* and the results was chromatinic condensation and then extreme chromosome breakage and fragmentation in the process of syncytial formation 7. Robbins S.J (1983) described nuclear morphology of measles virus-infected cells were swollen and the cells tended to have round rather than cuboidal morphologies. There was a correlation between cell degeneration and viral nuclear invasion. This suggested that quantification of viral nuclear invasion may be useful as a pathological marker of relative cell morbidity in persistent measles virus infection 8.

The shrunken cell fragments as apoptotic bodies, which are phagocytized by adjacent cells or degraded or extruded out of the

lumen 9. Kerr J.F.R., *et al.* (1972) observed morphological cell changes when they were undergoing process of apoptosis such as: cell shrinkage, the cells were smaller in size, dense cytoplasm, and organelles are more tightly packed. Nuclear condensation is the result of dense chromatin, which is the most characteristic feature of apoptosis 6. Ziegler U., *et al.* (2004) also used light microscope to observe cells undergoing apoptosis, including morphological changes: chromatin condensation and nuclear fragmentation. As soon as the process of apoptosis begins the cells shrink so that separate from each other, the cells start to show protrusions of the plasma membrane commonly referred to as blebs. The cells shrink, and finally the blebs separate, forming apoptotic bodies that can be observed by light microscope. Chromatin condensation starts from the inside and along the nuclear membrane, forming a crescent or ring structure. In the late stage of apoptosis, the nucleus continues to condense, eventually breaking into the cytoplasm while the cell membrane remains intact 10.

V. CONCLUSION

MeV and MuV can activate apoptotic cell death in HT-29 cell line via syncytial formation. Analysis of morphologic ultrastructure of HT-29 tumor cells after treatment with MeV and MuV showed that HT-29 tumor cells underwent apoptosis pathway.

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STUDYING OF RESULTS OF FLUORESCENCE BRONCHOSCOPY IN PATIENTS WITH SUSPECTED OF LUNG CANCER AT NATION LUNG HOSPITAL

Hoang Thi Bich Viet*, Dinh Ngoc Sy**

ABSTRACT

Bronchoscopy with 2 sources of light (white and fluorescence) is an advance in diagnosis of lung cancer. The results of many previous studies have shown that the effectiveness of fluorescent light is better than white one. Especially, the efficiency of bronchoscopy in detection of lung cancer increased clearly when combining these two sources of light.

In Vietnam, the studies, having the aim to detection of lung cancer in community actively, were less due to the lack of finance. Many studies concentrated in diagnosis and treatment of surgery, chemotherapy, radiation and target therapy but there were not many authors who studied the effectiveness of fluorescentbronchoscopy in diagnosis of suspected lung cancer. The National Lung Hospital, being the last line in the network to prevent lung cancer, receives directly patients with suspected lung cancer from other lines. Therefore, we carried out the research: *"Studying of results of fluorescencebronchoscopy in patients with suspected lung cancer"*with two aims:

1. Assessment of the results in diagnosis of lung cancer by bronchial mucosal biopsy via fluorescent bronchoscopy in patients with suspected lung cancer.

2. Comparising the effectiveness in diagnosis of lung cancer, when performing biopsy in the mucosal area of decreased fluorescent signal, with lessions which had detected by white light bronchoscopy

Method:

This research was carried out in 178 patients with risk factor and suspected of lung cancer, and 67 no suspected lung cancer patients, who had been examined and treated in the National Lung Hospital.

- All patients underwent conventional chest X-ray and were performed chest CT-scan by CT machine with 16 slices and performing bronchoscopy with 2 resources light (*fluorescent and white light).*

Results:

- The results showed that the proportion of lung cancer in the group of suspected ones was very high (76.40%).

- The diagnosis of lung cancer by bronchial mucosal biopsy via fluorescent brochoscopy in objects with suspected lung cancer:

+ The sensitivity and specificity in diagnosis of lung cancer of the decrease of signal in fluorescent bronchoscopy is 83.82% and 65.13%, higher than that of bronchoscopy with white light (72.79%; 69.72%).

+ The effectiveness in diagnosis of lung cancer, when performing biopsy in the mucosal area of decreased fluorescent signal, was significant higher in comparison with white light bronchoscopy, with the proportions following 97/152 (63.82%) and 39/132 (29.55%) ($p<0.05$).

+ When combining two sources of light, the proportion of diagnosed lung cancer were 72.06%.

Conclusion:

The rate of lung cancer, diagnosed by fluorescent bronchoscopy, were 60.20%, more

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than 20% when comparing with white light bronchoscopy

The decrease of signal in fluorescent bronchoscopy had the sensitivity and specificity in diagnosis of lung cancer higher than bronchoscopy with white.

Keywords: *Bronchoscopy, fluorescent bronchoscopy, National Lung Hospital.*

I. BACKGROUND

Bronchoscopy with 2 sources of light (white and fluorescence) is an advance in diagnosis of lung cancer. The results of many previous studies have shown that the effectiveness of fluorescent light is better than white one. Especially, the efficiency of bronchoscopy in detection of lung cancer increased clearly when combining these two sources of light.

In Vietnam, the studies, having the aim to detection of lung cancer in community actively, were less due to the lack of finance. Many studies concentrated in diagnosis and treatment of surgery, chemotherapy, radiation and target therapy but there were not many authors who studied the effectiveness of fluorescentbronchoscopy in diagnosis of suspected lung cancer. Therefore, we carried out the research: *"Studying of results of fluorescencebronchoscopy in patients with suspected lung cancer"*with two aims:

1.Assessment of the results in diagnosis of lung cancer by bronchial mucosal biopsy via fluorescent bronchoscopy in patients with suspected lung cancer.

2. Comparising the effectiveness in diagnosis of lung cancer, when performing biopsy in the mucosal area of decreased fluorescent signal, with lessions which had detected by white light bronchoscopy

II. RESEARCH SUBJECTS AND METHODS

2.1. Research subjects

This research was carried out in 178 patients with risk factor of lung cancer (n1), having lesions of suspected lung cancer on conventional chest X-ray image and 67 no suspected lung cancer patients (n2). The duration of study was from Oct 2016 to Dec 2018.

2.1.1. Selection criteria

- Patients with risk of lung cancer (male, ≥ 40 years old, smoking ≥ 10 pack-year).
- Having clinical symptoms (cough, sputum, hemoptysis, dyspnea, chest pain, weight loss...).
- Having results of tests: blood count, function of liver and kidney, coagulation, ECG, respiratory function which guaranteed for bronchoscopy.
- Having lesions of suspected lung cancer on conventional chest Xray image: nodule, mass, obstructive pneumonia, atelectasis, mediastinal lymph nodes and pleural effusion.

2.2. Research methods

Study design: Case study, cross sectional description.

Sample size and sampling methods:

Sample size: Apply the formula for calculating sample sizes for descriptive research

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

n: Minimal sample size for the group of patients with suspected lung cancer.

$Z_{1-\alpha/2}$:the corresponding value of the confidence limit coefficients, with the confidence being 95% then$Z_{1-\alpha/2}$= 1.96 .

p: the estimated population rate. Based on the results of the KC.10-29/11-15 (State Level

Project) the incidence of lung cancer in subjects with high risk of lung cancer, screening in community, was 10.6%. [1]

d:Expected error, in this study, it should be d = 0.05.

After calculation of sample size, the result was n = 146.

2.2.3. Data processing and analysis

Management and analysis of the data cording to the method of Medical statistics by the STATA 14.0 program.

+ Calculate mean($\overline{X}$) and standard rate (SD), min, max, %.

+ Compare the difference between groups, proportions and average numbers in pairs by Paired-SamplesT-test, test χ^2. The difference was statistical when p < 0.05.

+ Evaluate the value of bronchoscopy with two light sources: sensitivity, specificity, negative and positive predictive value.

III. RESULTS AND DISCUSIONS

3.1. The results of lung cancer diagnosis by bronchial mucosal biopsy via fluorescence bronchoscopy in 178 subjects with suspected lung cancer and in 67 no suspected lung cancer

Table 3.1. The proportions of bronchoscopic detected lesions by two light sources of studied patients

The bronchoscopic images	Suspected lung cancer (n_1= 178)		No suspected lung cancer (n_2= 67)		Total	P
	n_1	%	n_2	%		
Lesions detected via white light bronchoscopy	101	**56,74**	31	46,27	132	0,00
Decreased fluorescent signal	135	**75,84**	17	25,37	152	0,00

The proportion of lesions detected via white light and decreased fluorescent signals in patients with suspected lung cancer, was higher than that in the no suspected lung cancer group, statistically (p<0.01).

In general, in total of 245 subjects with risk of lung cancer, the proportion of decreased fluorescent signal was higher in comparison with the rate of lesions in white light bronchoscopic images. Thorough analysis in the patients with suspected lung cancer and patients with diagnosis of lung cancer also had similar results. This result proved that decreased fluorescentsignal was more valuable in detecting lesions than white light in diagnosis of lung cancer, similar to other previous studies by Li.Y et al. (2010), Chen.W et al. (2011).[2][3]

Table 3.2. Characteristics of lesions via fluorescent bronchoscopy of studied patients

The bronchoscopic images	Suspected lung cancer (n_1= 178)		No suspected lung cancer (n_2= 67)		P
	n_1	%	n_2	%	
Decreased fluorescent signal	135	**75,84**	17	25,37	0,00
Normal	43	24,16	50	74,63	0,00
Total	178	100	67	100	

The proportion of decreased fluorescent signals in patients with suspected lung cancer was higher than that in the non-suspected lung cancer group (p<0,01).

Fluorescence bronchoscopy based on the principle that tissue areas with thick mucous membranes or vascular hyperplasia produce images of reduced fluorescence signals. The assessment of whether or not having lesions when fluorescence bronchoscopy is based on reduction or no reduction of fluorescence signal. Therefore, it is possible to detect lesion when fluorescence bronchoscopy will be higher.[4]

Of 178 patients in the group 1, 136 ones were diagnosed lung cancer and 42 ones with no lung cancer.About the results of white light bronchoscopy, the proportions of patients with lung cancer having tumour, infiltration and compression were significant higher than that in the group of patients without lung cancer (p<0.05). [5[. The patients in the group with lung cancer had a higher rate of mucosal congestion in comparison with the group without lung cancer, but insignificantly (p>0.05).

3.2. Comparison the results of diagnosis by bronchial mucosal biopsy via fluorescence bronchoscopy in subjects with suspected lung cancer

Table 3.3. Comparison of the diagnostic results with lung cancer of each lesion between white light and fluorescence bronchoscopy

Location of biopsy (n=245)		**Lung cancer**		
		n	**%**	**p**
White light	(1) Tumour (n=23)	17	73.91	$p_{1,6}$ =0.263
	(2) Infiltration(n=44)	20	45.45	$p_{2,6}$= 0.055
	(3) Compression (n = 45)	02	4.44	$p_{3,6}$= 0.000
	(4) Congestion (n = 20)	0	0.00	$p_{4,6}$= 0.000
	(5) No lesion (n = 113)	0	0.00	$p_{5,6}$= 0.000
Fluorescence	(6) Decreased signal (n = 152)	97	63.82	

The diagnostic effectiveness of fluorescence bronchoscopy in detection of lung cancer was much better when performing biopsy in the location of decreased signal, with 97/152 of patients (63.82%), in combination with white light bronchoscopy (29.55%). Bronchoscopy with two sources of light had the value in detection of cancer. However, decreased fluorescent signal had the higher sensitivity when comparing with lesions in white light bronchoscopy, similar to other previous studies by Chen W., et al. (2011) [3].

Table 3.4. The comparison of lesions in fluorescent bronchoscopic images between lung cancer and non-lung cancer groups

The bronchoscopic images	**Lung cancer (n_1= 136)**		**Non lung cancer (n_2= 42)**		**Total**	**p**
	n_1	**%**	**n_2**	**%**		
Decreased fluorescent signal	114	**83,82**	21	50,00	135	0,00
Normal	22	16,17	21	50,00	43	0,01
Total	136	100	42	100	178	

The proportion of decreased fluorescent signals in patients with lung cancer was higher than that in the no lung cancer group (p<0,01).With the detection of lesions in decreased fluorescent signal, the results of our study has shown that if only lesions were detected, the diagnostic sensitivity of lung cancer was 72.79% and specificity was 69.72%.With fluorescent light, if decreased signal was lesion, the diagnostic sensitivity of lung cancer was 83.82% and specificity was 65.13%.

Table 3.5.The results of lesion detection of white light and fluorescence bronchoscopy in detection of lung cancer

Lesions in bronchoscopic image		Diagnosis of lung cancer (n_1=136)		Diagnosis of nonlung cancer (n_2=109)		Total (n=245)	
		n_1	%	n_2	%	N	%
White light	Lesion	99	72.79	33	30.27	132	53.87
	No lesion	37	27.20	76	69.72	113	46.12
	Value	p=0.000; Se=72.79%; Sp=69.72% NNV= 67.25; PPV= 75%					
Fluores -cence	Decreased signal	114	83.82	38	34.86	152	62.04
	No decreased signal	22	16.17	71	65.13	93	37.96
	Value	p=0.000; Se=83.82%; Sp=65.13% NNV= 76.34; PPV= 75%					

In general, in total of 245 subjects with risk of lung cancer, the proportion of decreased fluorescent signal was higher in comparison with the rate of lesions in white light bronchoscopic images. Thorough analysis in the patients with suspected lung cancer and patients with diagnosis of lung cancer also had similar results. This result proved that decreased fluorescentsignal was more valuable in detecting lesions than white light in diagnosis of lung cancer, similar to other previous studies by Li.Y et al. (2010)[2], Chen.W et al. (2011)[3].

IV. CONCLUSIONS

Studied in 178 patients with suspected lung cancer and 67 patients with no suspected lung cancer, we had some conclusions:

1. The results of lung cancer diagnosis by bronchial mucosal biopsy via fluorescence bronchoscopy in subjects with suspected lung cancer:

-Bronchoscopy with two sources of light diagnosed lung cancer in 98 of136 patients (72.06%).

- White light bronchoscopy diagnosed lung cancer in 39 of98 patients (39.80%).

-Fluorescence bronchoscopy diagnosed more lung cancer in 59 of 98 patients (60.20%), increasing 20% more than white light bronchoscopy.

- 38/136patients (27.94%) were diagnosed lung cancer via both white lightandfluorescence bronchoscopy.

- 27,94% of patients were applied transthoracic biopsy.

2. The higher value of fluorescence bronchoscopy in comparison with white light bronchoscopy:

-In the group of patients with lung cancer, the proportion of decreased fluorescent signal were 83.82%, being higher than the rate of lesions detected via white light bronchoscopy, statistically (p=0.01)

- The efficacy in diagnosis of lung cancer of fluorescence bronchoscopy was much higher when performing biopsy in the location of decreased signal (63.82%) in comparison with performing biopsy in the lesions detected via white light bronchoscopy (29.55%) (p<0.05)

- Decreased fluorescent signal had the sensitivity and specificity in diagnosis of lung cancer of 83.82% and 65.13%. Lesions in white light bronchoscopy had the sensitivity and specificity in diagnosis of lung cancer of 72.79% and 69.72%.

- Fluorescence bronchoscopy detected about 20% of patients with lung cancer more than white light bronchoscopy.

V. RECOMENTDATIONS

Based on the results of our study, we had some recommendations:

- The proportion of lung cancer in the group of suspected lung cancer (clinical symptoms and conventional chest Xray) was very high (76.40%). It was needed to screen early for these subjects whenever they had and respiratory symptoms.

- It should be applied fluorescence bronchoscopy to detect the mucosal areas with decreased signal and could be combined two sources of light to improve the effectiveness in diagnosis of lung cancer.

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THE BASIC ANATOMY OF THE MEDIAL SURAL ARTERY PERFORATOR FLAPS

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ABSTRACT

Objectives: Describe anatomy vascular pedicles of ***medial sural artery perforator flap in Vietnamese adult.*****Subjects and study methods**: Descriptive cross-sectional study, dissect 62 lower limbs of 41 Vietnamese adult cadavers preserved by formalin in Department of Anatomy, Hanoi Medical University and Ho Chi Minh Medicine and Pharmacy University. **Results**: Origin of medial sural artery is branched constantly from popliteal artery. Common stem of artery was 8,39 ± 3,5 cmin mean length. The diameter of common stem, which was measured from origin, was 2,88 ± 0,98 mm averagely. The common stem of artery, which did not have any branch (15%), divided in to 2 branches (15%), 3 branches (30 %), 4 branches (40%) before entering muscle. Medial sural artery had 1 to 5 branches perforating up to skin. The distance from perforating branch to the knee joint (popliteal crease) was 10,12 ± 3,7 cm, the distance from perforator branch to middle posterior leg was 1,6 ± 0,96 cm**. Conclusion**: By dissection, we determine the number of perforating branches, location when perforating up to skin, length and diameters of these branches and anatomical milestones.

Keywords*: Perforating Branche, Sural, Medial sural artery perforator flap.*

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I. INTRODUCTION

In recent years, an increasing number of scholars are concerned about perforator flaps which are myocutaneous flaps used known as pedicle flaps, pedicle free flaps or free flaps nourished by musculocutaneousperforators or artery perforators. In case of establishing a perforator flap, the name and position of the perforatorwhich supplies blood to the flap has to be defined.

In 1989, the term "perforator flap" was first mentioned by Koshima và Soeda [3]. Untill now, this term has been commonly used in academic documents world-wide.

Normally, it is favorable to create perforator flaps from lower extrimities. There are several types of perforator flaps, which have been widely researched and applied by plastic surgeons and anatomists, including Descending genicular artery perforator flap,saphenousflap, medial sural artery perforator flap (MSAP flap) and lateral sural artery perforator flap (LSAP flap).

LSAP flap and MSAP flap are directly estabished from skin-gastrocnemius flap. Perforator flaps are fully nourished by musculocutaneous artery perforating branches among which medial sural artery perforators constantly exist. Therefore, this type of flap has been being continuously researched and being widely applied into practice.

Each head of gastrocnemius muscle is nourished by an artery which directly arises either from one main vascular pedicle or popliteal artery. Sural artery pierces deeply

into the muscle through the muscular centre and ends at the Acchile tendon. It gives off several sural artery perforating branches to supply blood for the covering skin.

In 2001, it was the first time that anatomical characteristics of medial sural artery perforator flap, which was investigated in 10 cadavers was on public by Cadavas and et al [6]. In this year, Hallock and et al dissected 10 cadavers to explore medial skin-gastrocnemius perforators in the USA [7]. In 2004, a research which was conducted on 20 legs of 10 caucasian cadavers to investigate musculocutaneous perforators by Thione and et al was go on the public [8].

Following that, in 2007there were several researches on perforating branches implemented including a research on 44 Asian cadavers of Okamoto (Japan)n [10]and a research of Torres and Brazillian colleagues[11].

The clinical application of free medial sural artery perforator flap on the treatment of defects of head and neck was evaluated by Kao and his colleagues in Taiwan in 2010. This research express a possitive result of this intervention.

In 2011, a research on 10 formalin cadavers using red latex and lead tetroxide pumped into the medial sural artery was implemented by Altaf in Saudi Arabia [13].

In 2012, Wong and his colleagues [14] researched on the position of main artery perforator which was reliable and stable enough forflap transpositionin 10 fresh cadavers with red latex pumping. In this year, a study on 47 lower extrimities of Asian formalin cadavers to investigate musculocutaneous perforating branches of medial sural artery was conducted by Otani and his colleagues in Japan [15].

These mentioned researches generally describes that sural artery constantly branches. The quantity of branches varies from 1 to 8 branches among which the medial sural artery gives off at least 2 branches on average. The length of the artery from popliteal crease to where it branches is between 5 and 19 cm and its length from gastrocnemius midline to where it branches is from 0.3 to 7 cm. Additionally, most of the length of the pedicle of medial sural artery perforator flap is at least 7.7cm

Both free medial sural artery perforator flaps and pedical medial sural artery perforator flaps have been being effectively used in treatment of body defects especially in head and neck region by plastic surgeons world –wide. However, there is a lack of comprehensive studies on the anatomy of artery perforating branches in Vietnam. Therefores, this research on anatomy of vascular pedicle of the medial sural artery perforator flap in Vietnamese adults was conducted.

II. RESEARCH METHODOLOGY

2.1. Subjects

The study was implemented in 62 lower limbs of 41 Vietnamese adult cadavers preserved by formalin in the Department of Anatomy, Hanoi Medical University and Ho Chi Minh Medicine and Pharmacy University.

2.2. Research method

This is a cross-sectional study using cadaveric dissection

Dissection technique: medial and lateral sural artery skin perforators were dissected. In this article, the dissection technique of medial sural artery skin preforators is described.

Anatomical milestones:

- Knee joint (popliteal crease)
- Middle posterior leg line
- Apex of Medial and lateral malleolus
- Apex of head of fibula, medial condyle of tibia

Incisions:

- Middle posterior leg incision (vertical line): starts from the peak of popliteal region, through the middle line of popliteal region, along the middle posterior caft line to the middle spot on the line linking between the medial and lateral malleolus.
- Horizontal incisions: popliteal crease and the line linking between medial and lateral malleolus

The popliteal fossa was dissected to expose popliteal vessels in order that medial sural artery was accessed. Several indexes of medial sural artery thenwere measured, including lengths and diameters of common trunk and branches. After that, a catheter was set into the artery and latex was pumped.

At the next step, the myocutanous was dissected from the midline of the gastrocnemius muscle to its two sides, limited by the line linking the medial end of popliteal crease with medial malleolus.

All of the sural artery perforators were preserved and were dissected to the main trunk to define the number, location and distribution of the perforating branches related to several anatomical milestones namely posterior midline and popliteal fossa.

Perforating veins, small saphenous vein, superfacial nerves, and superfical medial suralnerve were also preserved.

The length and diameter indexes of the perforators of which diameter were larger than 5 mm were calculated.

All the results were documented, taken photography and expressed through illustrative diagrams.

Research tools: dissection tool set, electricity meter, syringes, needled, catheter and latex

The data was analysed with statistical algorithm.

III. RESULTS AND DISCUSSION

The following observations arederived from investigation of 62 lower extrimities in 41 Vietnamese adult cadavers:

- 55/62 specimens had 1 medial sural artery, accounting for 88,71%, 7/62 specimens had 2 supplying arteries, accounting for 11,29%.
- Origins: Medial sural artery divided from the posterior side of popliteal artery, in 47/62 specimens this artery is directly divided from popliteal artery, accounting for 75,8%. The number of cases in which medial sural artery divided from the same source vessel with lateral sural artery is 15/62 specimens, accounting for 24,2%.
- Dimension: the artery runs downward, descends beneath the medial gastrocnemius shealth.
- Mearsurements:

The length of main truck from the origin pedicle to the last muscle navel is around from 12.57 mm to 17.74 mm, mean ± SD is 14,75 mm ± 3,5 mm

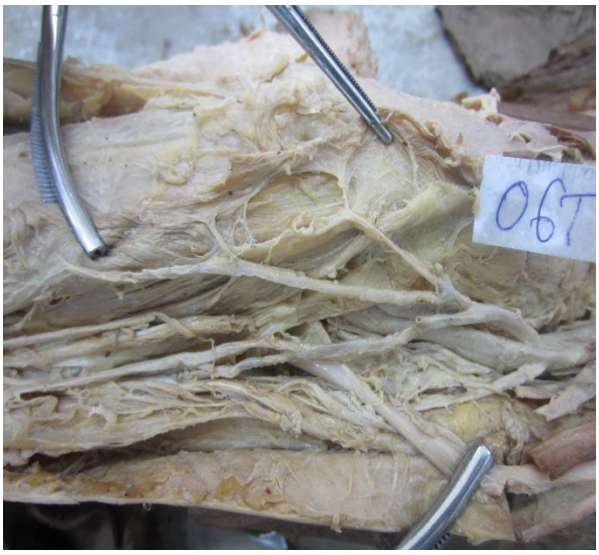
The diameter of main truck at the origin, where popletial artery gives off the sural artery, is 1.74mm for minima and 4.88 mm for maxima, so mean ± SD is 3,4 ± 0,92 mm

- Branches of main truck of medial sural artery: 15% no branches, 15% gives off 2 branches, 30% gives off 3 branches and 40%

gives off 4 branches before piercing into the muscle.

- Perforating branches of medial sural artery: 100% gives off at least 1 branches and the number of the branches peaks at 5 perforators. Mean ± SD is 3,35 ± 0,91 perforators.

The distance from the perforators to posterior caft midline is fluctuated from 0.39 cm to 6.7cm and mean ± SD is 1,6 ± 0,96 cm



Firgure 1: Medial sural artery

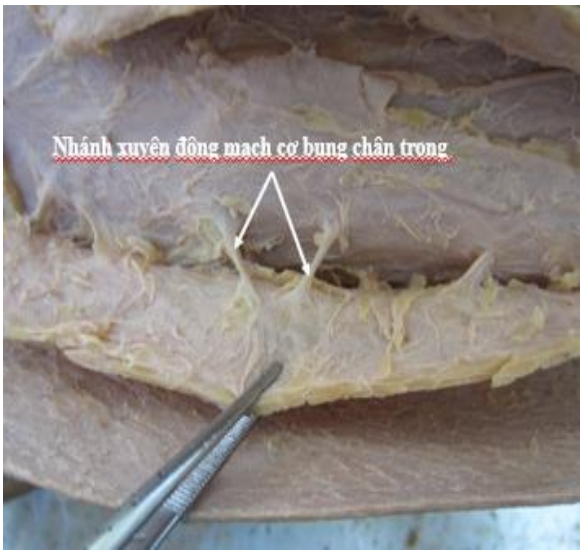
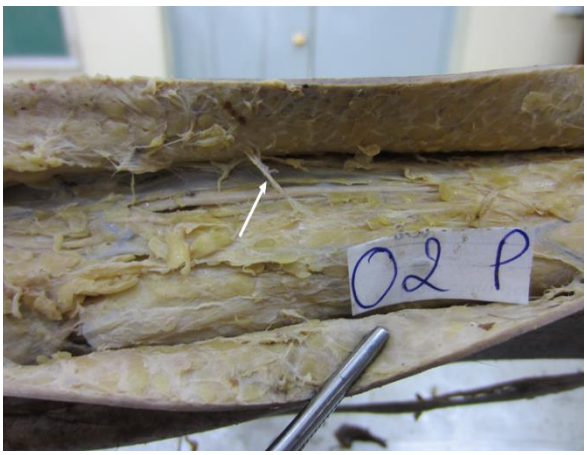


Figure 2: Skin perforating branches of medial sural artery



Firgure3: Skin perforating branch

Firgure 4: Three skin perforating branches

IV. DISCUSSION

Similar to other international and national researches, this study shows that medial sural artery perforator constantly exists in all of the dissecting specimens. In our study, 100% of medial sural artery is a branch of popliteal artery and the mean external diameter of medial sural artery at the point that popliteal branches is 2,88 ± 0,98 mm. In comparison with the research of Altaf [13], only 70% of medial sural artery is a branch of popliteal artery and 30% has a main truck with lateral sural artery; besides, the external diameter of medial sural artery in Altaf's research is

smaller than its in our study, only 3,01 ± 0,02 mm.

The main pedicle of medial sural artery, which is from its origin to the gastrocnemius muscle, is on average 8,39 mm ± 3,9 mm long. In 15% of cases, this pedicle gives off no branches, whereas, 15% of other pedicles gives off 2 branches, 30% of the pedicles gives off 3 branches and 40$ of the rest one has 4 branches.

According to the study which was investigated on 20 lower limbs of 10 Caucasian cadaversby Thione and his colleagues [8], the main pedicle of medial sural artery gives off 2 branches namely lateral and medial branches which both run into the muscle and branches several perforators. The mean external diameter of medial sural artery is 2.2mm (fluctuates between 2.3mm and 3.0 mm), smaller than our study's result (12mm).

These following indexes are the results of several previous researches which are conducted to investigate the anatomy of medial sural artery and its perforating branches:

Author	Number of Specimen	Preforators		Origin from joint line (cm)	Distance from perforator branch to posterior leg midline (cm)
		n	Mean		
Cavadas [6]	10	1 – 4	2,2	8,5-19	
Hallock [7]	10	2 – 7	4,6±1,8		
Thione [8]	20	38/20	1,9	7 – 18	
Shim [9]	40			The 1st perforators beneath popliteal crease: 9,68±1,08	Perforators lies along the line linking midspot of popliteal crease to medial malleolus
Okamoto [10]	44	1 – 5		5 – 17,5	1,5 – 4,5 cm
Torres [11]	12	2 – 4	2,9	10,7 – 14	
Kao [12]	26	1 – 5	2,7	6 – 18	0,5 – 7 cm
Altaf [13]	20	1 – 5	2	10,2 – 15,9	
Wong [14]	10	3 – 8	4,4	6 – 22,5	2±0,5 cm
Otani [15]	47	1 – 5	2,4	5 – 17,5	0,5 – 4,5 cm
Ours	62	1 – 5	3,35	5,1 - 18,73	0,39 – 6,7 cm

As can be figured out in the table above, in all of the cases, the medial sural artery gives from 1 to 5 perforating branches and median value is 2 branches per specimen. This is as the same as the results in Wong's research [14] and Hallock's research [7] which are 4.4 branches and 4 branches respectively.

The distance from perforator to popliteal crease also varies. The longest distance is 22.5cm in Wong's research [14] and the nearest is 5 cm in Otani's research [15]. In out study, the perforators sit at the inner side of the gastrocmenius midline and reach 0,5 – 7 cm proximal to this midline, similar to other studies.

To conclude, the importance of artery perforators in general and medial sural artery perforators in particular has been proven and admitted through a large number of

researches worldwide. It is acknowledged that these perforators play an crucial role in supplying blood to muscle and musculocutaneous tissue. Therefores, depending on this perforators, pedical or island musculocutaneous flap and myocutaneous flap is going to be used in the treatment of body defects especially in the region of head and neck.

V. CONCLUSION

The medial sural artery constantly originates from popliteal artery, supplies blood for medial gastrocnemius muscle. The skin area covering this muscle is nourished by one of five perforators of the medial sural artery. The perforating flaps can be created using medial sural artery perforating branches.

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SOME CHARACTERISTIC OF DISEASE PATTERN IN COMMUNITY OF MO RAI COMMUNE, SA THAY DISTRICT, KONTUM PROVINCE

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ABSTRACT

Objectives: Describe some characteristics of a disease pattern in community of Mo Rai border commune, Sa Thay district, Kon Tum province. ***Methodology:*** a cross-sectional descriptive study combining cross sectional investigations and retrospective secondary analysis. ***Results:*** The percentage of households in Mo Rai commune with at least 1 sick person per month was 26,0%. The highest incidence is found in children under 10 years old (37.65%), in the group of 10 - 19 years old (16.86%) and over 60 year-old group(17.26%). The main acute illness in Mo Rai commune requiring treatment is flu syndrome type 3 (27,59%), pneumonia - bronchitis (17,24%) and diarrhea (10,34%). The highest percentage of chronic diseases requiring treatment in the community is gastric disease (8,228%), skin disorders (8,9%) and osteoarthritis (5,48%). Infectious diseases are common and account for a high percentage of those treated in health care system. Infectious diseases in the expanded program on immunization markedly decreased. Non-infectious diseases such as tumors, those related to hematopoietic organs and metabolic endocrine disorders are increasing. ***Conclusion:*** Disease pattern of patients treated in Mo Rai commune is that of developing countries.

Keyword: *Mo Rai commune, disease pattern*

I. INTRODUCTION

In term of Central Highlands geography, there are 28 communes and 12 districts of 4 provinces: Kon Tum, Gia Lai, Dak Lak, and Dak Nong bordering Laos and Cambodia. Of which, there are 530 km of borderline. This is the area with the most difficult socio-economic conditions and transportation in the Central Highlands. Ensuring the health of people in the border areas of the Central Highlands still mainly depends on grassroots healthcare network.

Mo Rai commune is a border commune in Kon Tum province with a large area (585.5 km2), low population density (4,600 people), mainly ethnic minorities, far from the district center (65km) and it shares a 33kilometer international borderline with Cambodia. Economic conditions and access to health services face many difficulties. Therefore, in order for the grassroots health system to take the initiative and improve its effect, the health sector needs to have a deep understanding about the disease pattern of the community. Stemming from the above problems, Stemming from the above problems, the study is conducted with the following objective:

Describe some characteristics of a disease pattern in community of Mo Rai border commune, Sa Thay district, Kon Tum province.

II. SUBJECTS AND METHODOLOGY

2.1. Location, time and subjects

2.1.1. Subjects

- Household:

+ Householder or the person with the best information about using family health services.

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+ The person has been sick within 4 weeks or who is well-informed about the health status of the sick person.

- Medical records, disease statistics book

2.1.2. Research location and period:

- Research location: Mo Rai border commune, Sa Thay district, Kon Tum province

- Research period: 6/2019-6/2020

2.2 Methodology:

2.2.1. Study design:

Cross-sectional descriptive research: investigating and describing some characteristics and structure of disease pattern of the community in Mo Rai commune, Sa Thay district, Kon Tum (2019).

2.2.2. Sample size

Sample size to assess the people's disease situation:

$$n = Z_{1-\alpha/2}{}^2 x \frac{p(1-p)}{d^2} x\ DE$$

In which: n is the sample size

$Z_{(1-\alpha/2)}$: Confidence interval 95% ($Z_{(1-\alpha/2)}$ = 1,96).

p: the rate of sick person within 4 weeks of using health services. According to research results of Nguyen Thi Thang (2017),[1] the rate of sick people within 4 weeks of using health services was 26.5% (p = 0.265). Choose p = 0.265

q = 1- p.

d: Absolute error selected by the study, choose d = 0.05.

DE: design effect.

Thus, n = 299 people.

Take design effect DE = 2; We have n is 299 x 2 = 598 households. In fact, the study was conducted in 600 households.

2.2.3. Content and research index:

- **Sick person**:

Satisfy two conditions:

+ Suffering from any disease / symptom in 4 weeks from the time of interview and before.

+ Disease / symptoms persist for at least a day and affect life activities

- **Chronic illness or disease**

All illnesses or symptoms that last for more than 3 months, with or without a diagnosis by a healthcare professional, are considered chronic illnesses or diseases. The table (ICD -10) classifies the following diseases or chronic diseases:

+ Circulatory system diseases.
+ Nervous system diseases.
+ Musculoskeletal diseases
+ Respiratory diseases.
+ Eye diseases.
+ Metabolic endocrine diseases

- **Acute disease**: is a disease that have very quick onsets and typically last for only a brief period. The disease can then go away on its own or develop and progress to chronic or dangerous complications.

- **ICD X Table**: determine the disease structure according to 21 chapters of ICD-X

III. RESULTS:

3.1. The disease structure in the residential community of Mo Rai commune

Table 3.1. Rate of households with sick member during the month

Index	Indigenous People	Workers of Company 78	Total
Number of surveyed households	400	200	600
Number of households with sick member(s)	114	42	156
Rate of households with sick member (%)	28,5	21,0	26,0
p	>0,05		

The proportion of households with at least 1 sick member in the last month in Mo Rai commune was 26.0%. The proportion of indigenous households with with infected member was greater than that of workers of Company 78, the difference was not statistically significant (p> 0.05)

Table 3.2. Number of patients in each household per month

Number of patients	Indigenous people (n=114)		Workers of Company 78 (n=42)		Total (n=156)	
	n	Percentage (%)	n	Percentage (%)	n	Percentage (%)
1 person	48	42,10	21	50,00	69	44,23
2 people	57	50,00	18	42,85	75	48,08
≥ 3 people	9	7,89	3	7,14	12	7,69
Sum	114	100	42	100	156	100

Analysis of the number of patients in each household last month showed that most of them are households with 1-2 sick people , accounting for 92.31%; Only 7.69% of households had 3 or more people catching a disease.

Table 3.3. Prevalance in the month by age group (n=255)

Age group	Indigenous people (n=189)		Workers of Company 78 (n=66)		Total (n=255)	
	n	Percentage (%)	n	Percentage (%)	n	Percentage (%)
< 10	75	39,68	21	31,81	96	37,65
10 - 19	30	15,87	13	19,69	43	16,86
20 - 29	6	3,17	4	6,06	10	3,92
30 -39	9	4,76	7	10,60	16	6,27
40 - 49	8	4,23	5	7,57	13	5,10
50 - 59	24	12,69	9	13,63	33	12,94
≥60	37	19,57	7	10,60	44	17,26
Sum	189	100	66	100	255	100

Analysis of the prevalence of any disease in the past month by age groups in the community shows that the highest rate is in the group of children under 10 years old, accounting for 37.65% and 16.86% in the 10-19 year-old group. In the age group of the elderly over 60 years old, the rate is 17.26%.

Table 3.4. Distribution of cases within 4 weeks by some individual characteristics (n=255)

Index		Indigenous people (n=189)		Workers of Company 78 (n=66)		Total (n=255)	
		n	Percentage (%)	n	Percentage (%)	n	Percentage (%)
Sex	Female	91	48,14	41	62,12	132	51,76
	Male	98	51,85	25	37,87	123	48,24
Ethnics	Jrai	142	75,13	52	87,78	194	76,2
	Rơ Mâm	47	24,86	14	21,22	61	23,8
Educational level	Literacy	36	19,04	12	18,18	48	18,82
	Primary	49	25,92	9	13,63	58	22,74
	Secondary	46	24,33	14	21,21	60	12,52
	High school	34	17,98	20	30,30	54	21,17
	University/College	0	0	0		0	0,00
	Illiteracy	24	12,69	11	16,66	35	13,72

Index		Indigenous people (n=189)		Workers of Company 78 (n=66)		Total (n=255)	
		n	Percentage (%)	n	Percentage (%)	n	Percentage (%)
Professionals	Officer	15	7,93	7	10,60	22	8,62
	Seller	21	11,11	11	16,67	32	12,54
	Worker	15	7,93	7	10,60	22	8,62
	Farmer	112	59,25	30	45,45	142	55,68
	Housewife	1	0,52	1	1,51	2	0,78
	Others	25	13,22	10	15,15	35	13,72
Sum		189	100	66	100	255	100

The prevalence of women is higher than that of men. Specifically, the rate of sick women accounts for 51.76%, and that of men is 48.24%. The number of patients is concentrated in the Jrai group (76.2%) and the Ro Mam group (23.8%).

There is not much difference in morbidity rate among groups with different education levels. The number of sick people is mainly concentrated in farming occupations.

Table 3.5. Distribution of the proportion of patients with treated chronic illnesses (n=146)

Type of disease	Indigenous people (n=100)		Workers of Company 78 (n=46)		Total (n=146)	
	n	Percentage (%)	n	Percentage (%)	n	Percentage (%)
Cardiovascular	0	0	1	2,17	1	0,68
Osteoarthropathy	6	6,00	2	4,34	8	5,48
Stomach	6	6,00	6	13,04	12	8,22
Hepatobiliary	1	1,00	1	2,17	2	1,37
Colon	4	4,00	2	4,34	6	4,11
Chronic bronchitis	3	3,00	2	4,34	5	3,42
Asthma	2	2,00	2	4,34	4	2,74
Neurological	4	4,00	4	8,69	8	5,48
Mental	2	2,00	0	0	2	1,37
Dermatology	9	9,00	4	8,69	13	8,90
Kidney - Urology	8	8,00	4	8,69	12	8,22
Endocrine	5	5,00	2	4,34	7	4,79
Others	5	5,00	3	6,52	8	5,48

Among treated chronic diseases, diseases of stomach, kidney-urology and dermatology account for a high proportion (8.22%, 8.22% and 8.9%, respectively). The lower rate of treatment is osteoarthropathy (5.48%), cardiovascular and hepatobiliary diseases (from 0.68 to 1.37%).

Table 3.6. Distribution of the proportion of patients with an acute illness and being treated (n=203)

Type of disease	Indigenous people (n=131)		Workers of Company 78 (n=72)		Total (n=203)	
	n	Percentage (%)	n	Percentage (%)	n	Percentage (%)
Flu	39	29,77	17	23,61	56	27,59
Pneumonia, bronchitis	24	18,32	11	15,27	35	17,24

Type of disease	Indigenous people (n=131)		Workers of Company 78 (n=72)		Total (n=203)	
	n	Percentage (%)	n	Percentage (%)	n	Percentage (%)
Diarrhea	16	12,21	5	6,94	21	10,34
Hepatobiliary	7	5,34	6	8,33	13	6,40
Kidney - Urology	4	3,05	1	1,38	5	2,46
Otorhinolaryngology	3	2,29	3	4,16	6	2,96
Odonto-stomatology	4	3,05	3	4,16	7	3,45
Eye	1	0,76	1	1,38	2	0,99
Neuropsychiatry	7	5,34	6	8,33	13	6,40
Skin-mucosa	8	6,10	7	9,72	15	7,39
Injury	2	1,52	1	1,38	3	1,48
Undefined reason	9	6,87	5	6,94	14	6,90

The high prevalence of treated acute diseases is respiratory and digestive diseases such as flu syndrome 27.59%; bronchitis and pneumonia 17.24% and diarrhea 10.34%; skin and mucous diseases 7.39%. Others account for a lower percentage.

3.2. The disease structure in health facility:

Table 3.7. Classification of diseases based on the ICD-10 document

Chapter	Year 2015	Year 2016	Year 2017	Year 2018	Year 2019	Total
I	17	497	796	454	558	2322
II	1	15	11	9	5	41
III	0	8	6	6	0	20
IV	0	3	11	5	4	23
IX	4	39	62	56	48	209
V	0	1	1	1	4	7
VI	17	139	150	112	100	518
VII	3	80	137	124	118	462
VIII	10	114	149	133	181	587
X	66	876	1397	1552	1268	5159
XI	63	518	604	545	485	2215
XII	11	171	239	259	84	764
XIII	9	69	130	195	228	631
XIV	16	118	138	139	93	504
XIX	46	533	572	497	209	1857
XV	5	83	64	16	26	194
XVI	1	4	4	1	0	10
XVII	0	1	0	0	0	1
XVIII	4	153	158	219	62	596
XXI	40	379	409	295	68	1191
Total	313	3801	5038	4618	3541	17311

The year with most number of patients hospitalized was 2017 (5038 people), 2018 with 4618 people. The smallest number of patients admitted to hospital was in 2015 (313 people).

Chapter X: Diseases of the respiratory system (5159 admissions) account for the largest proportion and it is followed by chapters I, XI, XIX. The chapters that have a very small number of patients are Chapters II, III, IV, V, XVI and XVI.

IV. DISCUSSION

4.1. The disease structure in the residential community of Mo Rai commune

The rate of households in Mo Rai commune having at least 1 sick member in the month is 26.0%. The most common infectious diseases are diarrhea and respiratory illnesses. Diseases with a high incidence are influenza-like illness, respiratory diseases such as pneumonia, bronchitis and gastrointestinal diseases. The high prevalence of treated acute diseases is respiratory and digestive diseases such as flu syndrome 27.59%; bronchitis and pneumonia 17.24% and diarrhea 10.34%; skin and mucous diseases 7.39%. Among treated chronic diseases, diseases of stomach, kidney-urology and dermatology account for a high proportion (8.22%, 8.22% and 8.9%, respectively)

Research by Vo Van Ty et al (2012) at Thong Nhat Hospital shows that the disease model is characterized by: circulatory system illness (23.9%); respiratory system diseases accounting for 14.6%; digestive system diseases accounting for 14.4%. 9.4% number of patients are diagnosed with bacterial and parasitic infection [2]. According to a study by Dye C. (2014),the global burden of disease is shifting from infectious diseases to non-communicable diseases [3]. A statement by Tang S. et al also affirms that China's non-communicable disease control is the biggest challenge.t [4], Non-communicable disease is also a burden in Japan [5] and India [6] and Malaysia [7]. There are programs and measures to prevent NCDs such as: control tobacco, nutritional policies, improve physical performance, reduce harmful alcohol use, and strengthen the capacity of healthcare system. [8]...

Thus, the burden of disease has shifted sharply to non-communicable diseases, but communicable disease continues to be an issue of concern and a challenge for the health system in the coming years.

4.2. The disease structure in health facility

Does the medical evaluation of disease patterns in muscle reflect the disease pattern in the community? The research results show that the common patterns of disease in clinics and commune health stations have many similar characteristics, reflecting the disease pattern in the community. Graph 3.1 shows results: Chapter X: Diseases of the respiratory system (5159 admissions) account for the largest proportion and it is followed by chapters I, XI, XIX. The chapters that have a very small number of patients are Chapters II, III, IV, V, XVI and XVI. Specifically:

- Group of respiratory diseases (Group X) ranked first.
- Group of infectious diseases (Group I) ranked second.
- Group of digestive diseases (Group XI) ranked third.
- Group of trauma poisoning (Group XIX) ranked fourth.
- Factors affecting health and health service access (Group XXI) ranked fifth.

Research results on disease models in health facilities correspond to research results in the community. The structure of the disease model is still the model of developing countries. Infection diseases started to decline but remained high. There is a new shift in the disease structure, of which the group of trauma, poisoning, diseases of the hematopoietic system, tumor disease, and mental illness have emerged and replaced the group of nutritional and infectious diseases.

V. CONCLUSION

The disease structure in the residential community of Mo Rai commune: The rate of households in Mo Rai commune having at least 1 sick member in the month is 26.0%. The highest incidence is found in children under 10 years old (37.65%), in the group of 10 - 19 years old (16.86%) and over 60 year-old group(17.26%). The main acute illness in Mo Rai commune requiring treatment is flu syndrome type 3 (27,59%), pneumonia - bronchitis (17,24%) and diarrhea (10,34%). The highest percentage of chronic diseases requiring treatment in the community is gastric disease (8,228%), skin disorders (8,9%) and osteoarthritis (5,48%).

The disease structure in health facility: Infectious diseases are common and account for a high percentage of those treated in health care system. Infectious diseases in the expanded program on immunization markedly decreased but they are popular and remains high proportion. Non-infectious diseases such as tumors, those related to hematopoietic organs and metabolic endocrine disorders are increasing

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AN EVALUATION OF THE ANTERIOR OPEN BITE TREATMENT WITH STRAIGHT WIRE AND INTERARCH RUBBER BAND

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ABSTRACT

The goal of this study is to evaluate the stability of the results of using straight wire and interarch rubber band in nonsurgical anterior open bite treatment in 69 patients. The results of the treatment were evaluated clinically at three points: pre-treatment (T1), post-treatment (T2) and follow-up (T3). The patients were divived into two groups: short-term (1-2 years post treatment follow up) and long-term (2-5 years post-treatment follow up). All patients achieved good results at the end of the treatment (T2). The stability of anterior open bite treatment was 89.85% at the point of T3. Open bite relapse was found in 7 out of 69 patients, accounting for 10.14%. The treatment outcomes showed no variations between short term vs. long term groups. The variation between overjet post treatment and open bite anterior post treatment had significantly correlation p <0.001. The use of straight wire and interarch rubber band to intrude posterior teeth apically and extrude anterior teeth incisially have achieved remarkably result and asethetic improvement on the face.

Keywords: *Anterior open bite, Treatment of anterior open bite.*

I. INTRODUCTION

Open bite is malocclusion when one or more teeth do not come in contact with the occlusal table and do not occlude with any of the opposing teeth. Open bite usually occurs at anterior teeth, but can also be seen in posterior teeth or a combination of both. This malocclusion is common at the age of permanent teeth (mixed dentition stage). The causes of open bites are often hereditary, unfavorable teeththh and jaw growth, tongue posture, sucking habits and obstruction of nasal breathing [Solow, Kreiborg 1977; Proffit et al. 1983; Nanda 1988; Brenchley 1991; Vig 1998]. Treatments of open bites are challenging due to its recurrence, especially in elderly patients due to many factors involved in open bites [1,2,3]; Therefore; most orthodontists recommend open bite treatment should be at the stage of mixed dentition to increase the likelihood of success, limit the vertical dimension development of occlusal changes, and reduce open bite relapse. The goal of open bite treatment is to restore aesthetic function because patients lack confidence in communicating with their appearance, to reduce the vertical dimension. In addition, open bite treatment is to restore the function of pronunciation, mastication and to prevent the posterior teeth from being worn by constant grinding that results in frequent soreness; which affects the quality of life.

Clinically from 2008 to 2020, a prospective cohort study was conducted to treat 69 patients with anterior open bites in order to: assess treatment results and the stability of the use of straight wire and interarch rubber bands in anterior open bite treatment, 1 to 5 years post treatment follow up.

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II. PARTICIPANTS AND METHODS

2.1. Participants:

- *Group 1*: Patients (from 9 years and older) with anterior open bite malocclusion, orthodontic intervention using a straight wire and interarch rubber band; monitoring time from 1-2 years.

- *Group 2*: Patients (from 9 years and older) with combination anterior and posterior open bite malocclusion, orthodontic interventions using straight wire with interarch rubber band; monitoring time from 2-5 years.

Criteria for choosing patients: Patients 9 years of age or older, with anterior open bite and acceptable facial aesthetics with unnecessary orthodontic surgery. Agreement to participate in orthodontic treatment.

Exclusion criteria: Patients are less than 9 years of age or greater 25 years of age with more than 10 mm open bites.

2.2. Methods: Study design: Future Cohort study, patients were chosen for treatment from 5/2008 - 2/2020.

***2.3.* Collected information:** All study patients were recorded information in the research sample:

- Clinical examination was performed to identify malocclusion. In addition; cephalometric, panorex X-rays, radiology analysis, straight frontal and lateral photographs, diagnostic casts were taken at different points of treatment T1, T2, T3.

- Treatment plan was made and discussed with the patient.

- Treatment plan was implemented: All patients participating in the study were treated using straight wire and interarch rubber band. Duration of the treatment was from 2 - 3.5 years depending on each particular case. Post orthodontic treatment, all patients must commit to wear a Hawley retainer in their upper and lower jaw fixed by connecting teeth between 33 and 43. For the first 2 years, wear night-time removable retainer and after that 2 years, patients only wear retainer one night per week.

- Group 1: Short term (ST) post treatment monitoring: 1-2 years.

- Group 2: Long term (LT) post treatment monitoring: 2-5 years.

- Treatment monitoring: During treatment, patients come monthly to check and adjust until the bite is stable, functional and aesthetic. Study cast models, cephalometric and panorex X-rays were taken at different times (T1, T2) for comparison. Post treatment, the patients were re-examined every 6 months, and study cast models, cephalometric and panorex X-rays were taken at every year until 5 years post treatment.

2.4. Processing statistics: Using SPSS for Windows 22.0 software.

2.5. Evaluation of results: Differences in pre- and post -treatment results at T1, T2, and T3 were analyzed with independent t-test and chi-square test, p was less than 0.05. considered statistically significant. Cephalometric X-rays were analyzed using FACAD® software program (Ilexis AB, Sweden) to check the difference before and after treatment at times T1, T2, T3.

III. RESULTS

3.1. Patient information:

- Total number of patients: 69 including 25 men (36.2%), 44 women (63.8%). Youngest age: 9, oldest: 25, average age14.2 years old ± 4.1.

- Patients with different class of occlusion according to Angle: Class I (30 patients,

accounting for 43.5%), Class II (17 patients, accounting for 24.6%), and Class III (22 patients, accounting for 31.9%).

- The number of patients belonging in short-term post treatment monitoring group (ST): 38 patients, accounting for 55.07% and the number of patients belonging in long-term post treatment monitoring group (LT): 31 patients, accounting for 44.93%.

3.2. The average value of open bites patients at times T1, T2 and T3.

Table 1

	Number of patients	Minimum value	Maximum value	Average value	Standard deviation
Open bite (T1)					
ST	38	- 9 mm	- 2 mm	- 3.958 mm	±1.894
LT	31	- 9 mm	- 2 mm	- 4.500 mm	±2.049
Open bite (T2)					
ST	38	1 mm	2,5 mm	1.723 mm	±0.341
LT	31	1 mm	2,2 mm	1.674 mm	±0.359
Open bite (T3)					
ST	38	0.8 mm	2.5 mm	1.686 mm	±0.377
LT	31	- 1 mm	2.2 mm	1.465 mm	±0.647

- The variation in the average numbers of open bites between two times (T1) and (T2) is statistically significant with p <0.001.

- The variation in the average numbers of open bites between two times (T2) and (T3) is not statistically significant with p> 0.05.

3.3. Overjet average value at points T2 and T3.

Table 2

Overjet value	Number of patients	Minimum value	Maximum value	Average value	Standard deviation
Overjet post treatment	69	1.1 mm	2.6 mm	1.961 mm	±0.330
Overjet value stability post treatment	51 (73.91%)	1.0 mm	2.6 mm	1.945 mm	±0.348
Overjet value relapse post treatment	7 (10.14%)	- 9 mm	- 3 mm	- 0.529 mm	±0.236
Overjet value post treatment-T3 in comparison toT2	11 (15.94%)	1.9 mm	2.8 mm	2.427 mm	±0.214

Post treatment (T3), 7 patients (10.14%) relapsed with average value of overjet: - 0.529mm ± 0.236 and 11 patients (15.94%) had average value of overjet 2.427mm ± 0.214.

3.4 . The number of open bite patients post treatment were determined after the overjet value change at T3 in comparison to T2.

Table 3

Overjet post treatment (T3 vs.T2)	Number of open bite patients post treatment			Number of patients
	Stability	Relapse (+)	Relapse (-)	
Stability	62 (89.85%)			62
Relapse		6 (8.69%)	1 (1.44%)	7
More in T3 vsT2				
Total	62	6	1	69

Within 07 patients (10.14%) who had open bite relapse, 01 had negative open bites, accounting for 1.44%. A small but statistically significant increase was found in patients with anterior open bite from stage T2 to T3 (p <0.001).

3.5. The number of patients with overjet value change post treatment at T3 in comparison to T2.

Table 4

Post treatment results	Anterior open bite (T3 vs T2)	Number of patients with overjet value change post treatment		Number of patients
		No change	Change	
Stability	62 (89.85%)	51 (73.91%)		51
Relapse	7 (10.14%)		7 (10.14%)	7
More in T3 vs. T2		11 (15.94%)		11
Total		62	7	69

The variation between overjet post treatment and open bite anterior post treatment had significantly correlation p <0.001.

3.6 The average value of anterior open bite patients classified based on Angle class of occlusion at T1, T2 and T3.

Table 5

		Classification of Angle class of occlusion		
		Class I	Class II	Class III
T1	ST	- 3.84 mm	- 3.88 mm	- 4.18 mm
	LT	- 4.5 mm	- 4.39 mm	- 4.58 mm
T2	ST	1.69 mm	1.86 mm	1.53 mm
	LT	1.69 mm	1.86 mm	1.53 mm
T3	ST	1.65 mm	1.7 mm	1.47 mm
	LT	1.63 mm	1.53 mm	1.28 mm
p-value	p (T2-T3) = 0.25			

The difference in the average value of open bites patients at time T3 compared with T2 according to occlusal classification Angle was not statistically significant with p = 0.25.

IV. DISCUSSION

Results: The main goal of treatment is to eliminate open bites in order to achieve aesthetics, satisfactory smile and mastication function. In order to achieve a stable outcome, the elimination of abnormal tongue posture has been instructed to the patients during the treatment period. Maintaining the facial aesthetics was originally planned because it was considered an important goal that both patients and physicians should work towards. The recommended treatment method is to rapidly expand the upper jaw together with intruding posterior teeth apically and extruding anterior teeth incisally using straight wire whose both ends were bended an angle of 45 degrees at to stimulate the lower jaw bone rotate itself to close the occlusion [3]. Particularly, the braces on anterior teeth were attached closely to the gums 0.5 - 1mm and the braces on posterior teeth were attached near the occlusal surface. This would help to close the anterior open by extruding anterior teeth and intruding of posterior teeth.

The results in Table 1 indicated that the group of patients belonging in short-term post treatment monitoring had the average value at the time (T1): - 4.500 mm ± 2.049, post treatment the change of open bites average value increased: 5.965mm ± 2.235 (T2) and 5.644mm ± 2.271 (T3). The difference in average values between the two times (T1) and (T2) was statistically significant with p <0.001. The long-term post treatment monitoring had the average value at the time (T1): - 4.500 mm ± 2.049, post treatment the change of open bites increased: 6.174 mm ± 2.408 (T2) and 5.965mm ± 2.696 (T3). The difference in average values between the two times (T2) and (T3) was not statistically significant with p> 0.05. Data analysis showed that the model responded over time to all subjects, and the change in open bites was not related to age or gender. Out of 69 patients, 62 patients (89.85%) were stable and 07 patients (10.14%) had open bites relapse at T3 time. However, of these, only 01 patient had negative open bite (1.44%). A small but statistically significant increase was found in patients with anterior open bite from T2 to T3 period (p <0.001). In both ST and LT groups, there was no statistically significant difference between the groups.

Overall, all patients received good results at the end of intensive treatment (T2). The long-term stability of the results of open bite treatment was found to be 89.85% (T3). Compared with Silvia Geron Atalia Wasserstein Zachi Geron [6] in 2013, the study of 39 cases of anterior open bite treatment in adults with orthodontics at lingual surfaces showed that stable results post treatment accounted for 87.5% (T3), 12 patient relapses (5%). The treatment of open bite first depended on the severity of the open bite and the age of the patient. For elderly patients, surgery is often necessary and the stability of results is in the range of 75 - 85% (Bailey et al., 2004; Ding et al., 2007; Espeland et al., 2008; Stansbury et al., 2010; Teittinen et al., 2012). Kuroda S, Sakai Y, Tamamura N, Deguchi T, Takano-Yamamoto T. [3] in 2007 studied the treatment of anterior open bite by intruding the posterior teeth apically with osteoporosis anchorage devices and maxillofacial surgery. The results showed that incisors were significantly extruded in surgical treatment subjects (4.6 mm, P <0.01). There was no significant difference in treatment results between osteoporosis anchorage and surgery. These results showed that intruding posterior teeth with anchorage devices to treat open bites is simpler and more useful than maxillofacial surgery.

Maintaining treatment results: Patients were divided into two monitoring groups: the ST group had 38 patients (55.07%), the LT group had 31 patients (44.92%). In the LT group, 5 patients were followed up to 3 years after treatment, 12 patients were followed up to 4 years and 14 patients were followed up to 5 years after treatment. The average of follow-up time for this group was 4.290 years ± 0.322 and average time of follow-up time for both ST and LT groups was 3.028 ± 0.9855 years. In comparison to authors like Aldo Otazú Cambiano, Guilherme Janson, Diego Coelho Lorenzoni, Daniela Gamba Garib, Dino Torres Dávalos [1] The average duration of follow-up for was 3 years because the clinical stability at 3 years post treatment can be compared by cephalometric film superposition method; which shows a reduction in the longitudinal bone size due to counterclockwise rotation, increases the SNB angle and reduces the angle of the ANB.

Classification of open bite results based Angle class of occlusion: The average number of open bite patients classified based on Angle class of occlusion at the times of T1, T2 and T3 according to Table 5 showed that 30 patients (43.5%) had class I occlusion, 17 patients (24.6%) had class II occlusion and 22 patients (31.9%) had class III occlusion. Among patients with class III occlusion, the average number of open bites of both groups (ST) and (LT) was greater than the average number of open bites patients with class I and II occlusion at time (T1), but at (T2) and (T3) the open bite values were less than 2 groups of class I and II occlusion. However, this difference was not statistically significant p = 0.25.

Post treatment relapse problem: The method of extruding the anterior teeth incisially and intruding the posterior teeth apically using straight wire and interarch rubber band is considered a simple, easy to perform one, but requires patient's compliance, bending technique and physician's troubleshooting experience during the open bite treatment duration. After nearly two years of treatment, anterior open bite in 69 patients had significant reduction at anterior teeth as interarch rubber bands were used for 20 hours a day for 12 months. After this period, the interarch rubber band was only used to maintain results at night for about 10 months. The problem of relapse post treatment was mostly due to the emergence of maxillary posterior teeth and unresolved related factors. According to Park, HS, Kwon, OW, and Sung, JH. [5] The long-term stability of bones and teeth in anterior open bite treatment was 3.2 years regardless of methods of treatments: surgery or no surgery. In our study, the long-term post treatment monitoring group (LT) had an average value at the time of (T1): - 4.500 mm ± 2.049 after treatment, the average change of open bite increased: 6.174 mm ± 2.408 at the time of (T2) and 5.965mm ± 2.696 (T3). The difference in average values between the two times (T2) and (T3) was not statistically significant with p> 0.05. Most authors believed that instability could be complicated by the neuromuscular system, weak mastication force, breathing by mouth, lips not closed in a resting position, etc. Therefore, practicing and establishing the new correct tongue posture, increasing mastication force by eating chewing gums are the main factors to achieve stability after the open bite treatment.

The results in Table 4 showed that at the time (T3) 62 patients (89.85%) did not change the overjet after treatment; of which 11 patients (15.94%) had average overjet greater at moment of T2: 2.427 mm ± 0.214 and 7 patients had recurrence accounted for 10.14% with average overjet value - 0.529 mm ± 0.236. When the overjet post treatment relapsed, anterior open bite also recurred in 7 patients, including 01 patient had negative open bite recurrent. The stability of overjet post treatment was significantly related to the stability of the anterior open bite with p <0.001. Nicole R. Scheffler, William R. Proffit, and Ceib Phillips [4], have found a similar association between overjet at posterior teeth and anterior open bite treated with surgery. The study also suggested that the recurrence of overjet had been associated with recurrence of open bite post-surgery regardless of surgical procedures.

V. CONCLUSION:

Using a straight wire arch with interarch rubber band in treatment of anterior open bite

in patients who are not suitable for surgical procedures has obtained good results, long-term stability 89.85%. There was 10.14% of open bite recurrence at the time of T3. The stability of overjet post treatment was significantly related to the stability of anterior open bite because when overjet post treatment was recurrent, the anterior open bite was also relapsed.

The method of extruding of anterior teeth incisially and intruding posterior teeth apically using straight wire arch and interach rubber band is considered a simple and easy method to implement. However, it requires patient compliance, bending technique and troubleshooting experience of treating physician during anterior open bite treatment duration.

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RESULTS OF SOME ANTERIOR OPEN BITE TREATMENT CASES

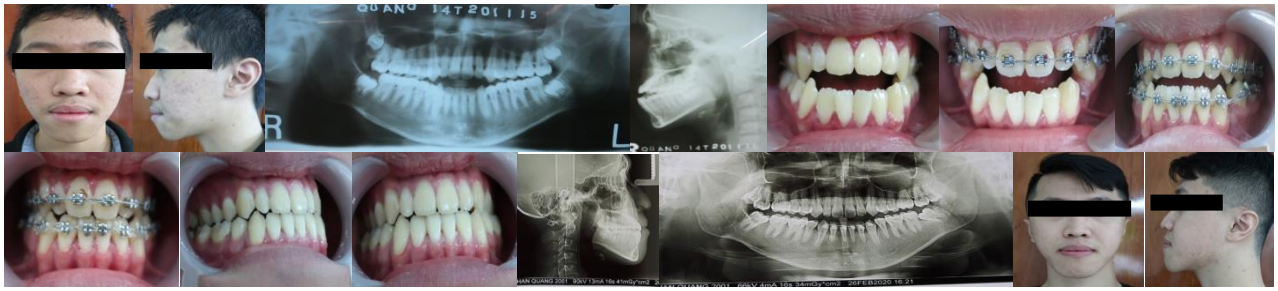

Fig.1 Photographs of pre and post treatment for patient: Phan Q. 14 years of age, male. Malocclusion class III Angle with 8 mm anterior open bite.

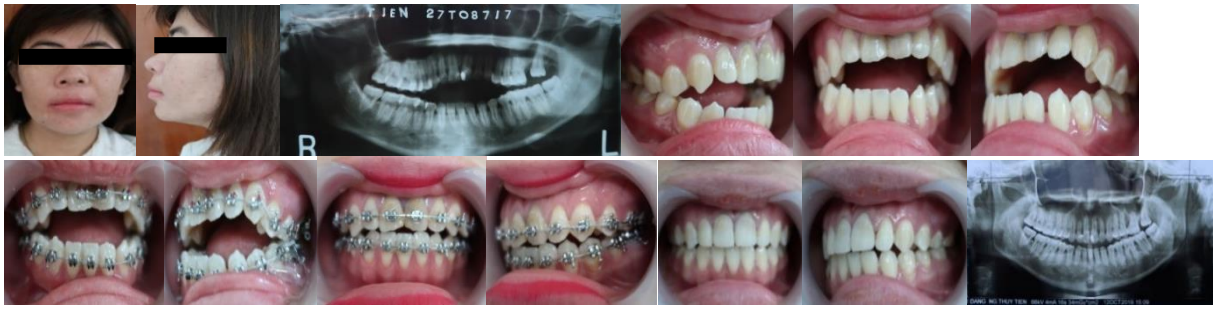

Fig.2 Photographs of pre and post treatment for patient: Dang Nguyen Thi T. 22 years of age. Malocclusion class III Angle with 9 mm anterior open bite.

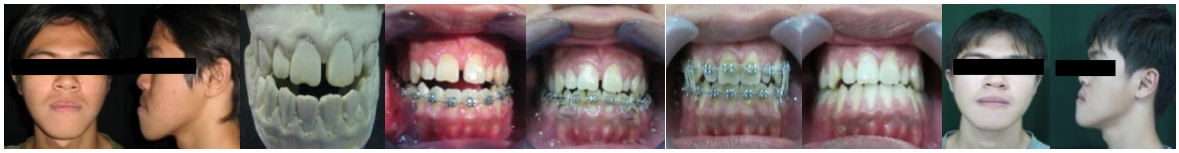

Fig.3 Photographs of pre and post treatment for patient:Truong Viet H. 17years of age. Malocclussion class III Angle with 5mm anterior open bite.

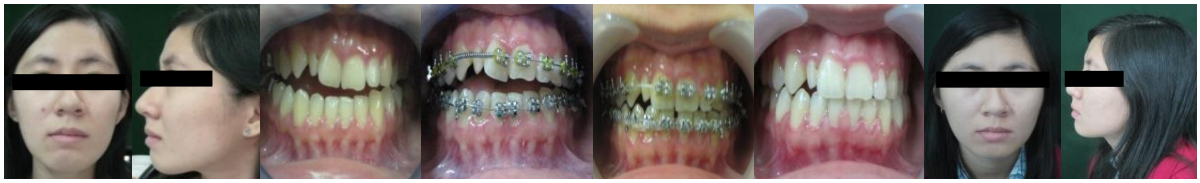

Fig.4 Photographs of pre and post treatment for patient: Le Thu H. 25 years of age. Malocclusion class I Angle with 4 mm anterior open bite.

THE RELATIONSHIP BETWEEN SERUM HOMOCYSTEINE LEVEL AND MICROALBUMINURIA IN PATIENTS WITH TYPE 2 DIABETES

Nguyen Van Chuyen*, Ngo Duc Ky**

ABSTRACT

Objectives: to find out the the relationship between serum homocysteine level and some characteristics of patients with type 2 diabetes. **Subjects and methodology**: cross-sectional study describes 130 qualified subjects, including type 2 diabetes and no diabetes with similar age, sex, and BMI. **Results**: Among 130 study participants, there were 67 patients with type 2 diabetes and 63 people without diabetes. There was no difference in age, sex, BMI in type 2 diabetes group and control group without diabetes. Average level of homocysteine in type 2 diabetic patients was 13.09 ± 6.07 µmol / l, higher than that in the group without diabetes was 8.57 ± 3.4 µmol / l, the difference was statistically significant with p <0.01. In the group of diabetic patients with MAU (+), homocysteine level was 16.4 ± 6.45 µmol / l, higher than the group of type 2 diabetes with MAU (-) of 9.89 ± 3.46 µmol / l, the difference was statistically significant with p <0.01. There is no relationship between homocysteine with HbA1c, Cholesterol, LDL - cholesterol, HDL - Cholessterol. There was a statistically significant positive relationship between homocysteine and MAU (r = 0.639; p <0.01) and triglyceride (r = 0.3; p <0.05). **Conclusion**: Homocysteine concentrations in patients with type 2 diabetes are higher than in non-diabetic patients and homocysteine concentrations increase with renal complications.

Keywords: *Type 2 diabetes, Homocysteine, MAU*

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I. INTRODUCTION:

In recent years, many authors have noticed an independent risk factor for early emergence of complications in patients with type 2 diabetes, which is homocysteine (Hcy). Homocysteine is an amino acid, formed from the reduction of methyl group of methionine. Homocysteine is a risk factor for coronary artery disease, closely related to the prognosis of cardiovascular disease in patients with type 2 diabetes, insulin resistance, and increasing risk of occurrence and severity of kidney disease and disease, retinopathy, diabetic neuropathy. [1] Several studies have shown that higher level of homocysteine increases the risk of occurrence and is associated with vascular complications in patients with type 2 diabetes. [2]. In addition, homocysteine is known to be an independent risk factor associated with the early development of kidney complications in type 2 diabetes patients. [3,4]. Therefore, in order to contribute to understanding new risk factors for type 2 diabetes, we conduct a research project with the goal: to find out the relationship between serum homocysteine levels with some characteristics. in patients with type 2 diabetes

II. SUBJECTS AND METHODOLOGY

Subjects: 130 qualified subjects, including type 2 diabetes and no diabetes with similar age,sex, BMI. Research period is from 07/2018 to 31/12/2019 at Nghe An General Friendship Hospital.

Criteria for control group selection: patients without diabetes were tested for fasting venous blood glucose test, HbA1c test and 75g glucose tolerance test.

Exclusion criteria: Kidney failure, glomerulonephritis, nephrotic syndrome, systemic lupus erythematosus, psoriasis, hypothyroidism, currently or within the last 3 month using of vitamin B drugs...

Research method: cross-sectional description with comparison with control group. Convenient sample size without probability.

Research steps: Clinical examination and clinical tests such as blood glucose, HbA1c, creatin, blood electrolytes, microalbuminuria, and blood lipids components. Using a system of COBAS 6000 test machines from Roche. Data are processed based on SPSS 16.0.

III. RESEARCH RESULTS

3.1. General characteristics of the research team

Table 1: General characteristics of type 2 diabetes group and control group

Characteristics	Type 2 diabetes group (n = 67)	No diabetes group (n = 63)	p
Age (năm)	65,58 ± 10	62,57 ± 9,2	>0,05
BMI (Kg/m²)	21,37 ± 2,6	21,45 ± 1,8	>0,05
THA (%)	56,2	43,8	>0,05
Glucose (mmol/l)	9,2 ± 4,5	5,3 ± 0,6	<0,01
HbA1c (%)	7,5 ± 1,6	5,1 ± 0,2	<0,01
Creatinin (µmol/l)	79,48 ± 29,51	76,08 ± 16,43	>0,05
Cholesterol (mmol/l)	4,3 ± 2,2	4,11 ± 2,15	>0,05
Triglycerid (mmol/l)	1,77 ± 2,51	1,15 ± 0,92	>0,05
LDL – Cholesterol (mmol/l)	2,56 ± 1,43	2,49 ± 1,72	>0,05

Comment: there was no difference in age, BMI, THA, lipid components in type 2 diabetes group and control group (p> 0.05).

Table 2. Gender characteristics of the study

Characteristics	Type 2 diabetes group (n = 67)	No diabetes group (n = 63)	p
Male (%,n)	53,1 (43)	38,9 (38)	>0,05
Female (%,n)	49,0 (24)	51,0 (25)	

3.2. Homocysteine characteristics of the study

Table 3.Homocysteine characteristics of type 2 diabetes group and group without diabetes

Characteristics	Homocystein (µmol/l)	P
Type 2 diabetes group (n = 67)	13,09 ± 6,07	<0,01
No diabetes group (n = 63)	8,57 ± 3,4	

Comment: The average homocysteine in type 2 diabetes group was 13.09 ± 6.07 µmol / l, which was statistically significant higher than the control group with 8.57 ± 3.4 µmol / l, the difference was statistically significant with p <0.01.

Table 4. Homocysteine characteristics by sex in patients with type 2 diabetes

Type 2 diabetes group	Homocystein (µmol/l)	P
Male (n = 43)	14,45 ± 6,68	<0,05
Female (n = 24)	10,5 ± 3,68	

Comment: homocysteine concentration in men with type 2 diabetes is 14.45 ± 6.68 µmol / l, higher than that of women with type 2 diabetes which is 10.5 ± 3.68 µmol / l, the difference is statistically significant with p <0.05.

Table 5. Homocystein characteristics in type 2 diabetes group with early kidney complications

Type 2 diabetes group	Homocystein (µmol/l)	p
MAU (-)	9,89 ± 3,46	<0,01
MAU (+)	16,4 ± 6,45	

Comment: In type 2 diabetic patients with MAU (+), the average homocysteine is 16.4 ± 6.45 µmol / l, higher than the type 2 diabetes group with MAU (-) of 9.89 ± 3.46 µmol / l, the difference is statistically significant with p <0.01.

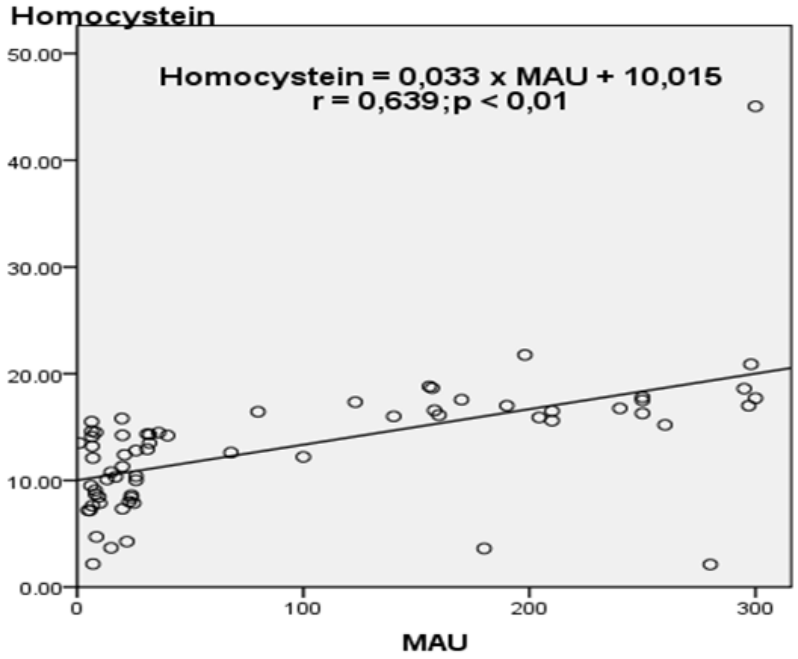

Figure 1. Correlation between homocysteine level and MAU in patients with type 2 diabetes

Comment: There is a strong positive correlation between homocysteine and MAU (r = 0.639; p <0.01).

3.3 Relationship between homocysteine serum with some biochemical indices in patients with type 2 diabetes

Table 6. Relationship between serum homocysteine and some biochemical indices in patients with type 2 diabetes

Index	r	P
HbA1c	0,227	>0,05
Creatinin	0,127	>0,05
Cholesterol	0,129	>0,05
Triglycerid	0,3	<0,05
HDL-Cholesterol	-0,085	>0,05
LDL-Cholesterol	0,057	>0,05

Comment: There was a statistically significant positive correlation between homocysteine and blood triglycerides (r = 0.3; p <0.05).

IV. DISCUSSION

Characteristics of research group:

Type 2 diabetes is usually detected late after the age of 40, the frequency of the disease increases with age. Most studies in the world show that the older the age, the higher the prevalence of diabetes. This is a non-intervention factor. In addition, the higher the age of type 2 diabetic patients, the greater it affects the progression and complications of the disease. In this study, we have 67 patients with type 2 diabetes and 63 without diabetes as a control group. There was no difference in age, sex, BMI, or blood lipid components between these two groups. The average age in type 2 diabetic patients was 65.58 ± 10 years and in the group without this disease was 62.57 ± 9.2 years (Table 1). This result is similar to the study of author Nguyen Thi Thanh Thuy (2018) which is 65.0 ± 6.0 years old [2];that of Luong Huu Dung (2015) is 64,2 ± 11,5 years old, that of control group 63,1 ± 12,9 years old [3], but older than author Duong Thi Tuyet (2008) [5]. This age difference may be due to the characteristics of the patients admitting to the treatment at different hospitals or the different goals and designs of the studies. On the other hand, in terms of gender, there was no difference between the two study groups (Table 2). Therefore, these results ensure the objectivity when analyzing and comparing the variables in this study.

Homocysteine characteristics of the research group

Our study found that the average homocysteine concentration of the diabetic group was 13.09 ± 6.07 µmol / l, which was statistically significant higher ($p < 0.01$) than the normal control group with 8.57 ± 3.4 µmol / l (Table 3). This result is similar to national studies [2,5,6,7] or international studies [4,8,9,10]. Thus, the homocysteine concentration was significantly higher in type 2 diabetic patients than that of the no-diabetes group and this factor may increase complications in patients with type 2 diabetes.

Gender is the factor affecting homocysteine concentration in the blood. The difference between the sexes is explained by hormone concentrations, body mass, and gender-related lifestyle differences. In male patients with type 2 diabetes, the homocytein concentration of 14.45 ± 6.68 µmol / l was statistically significantly higher than in the female with type 2 diabetes with 10.5 ± 3.68 µmol / l (Table 4) and it is similar to national and international studies [2,3,4,7]. The explanation for this difference may be that men have certain habits that women rarely experience, such as alcohol abuse, tobacco addiction, and drinking a lot of coffee, which can cause hyperhomocysteinemia. On the other hand, women often adhere to the diet for diabetics (less fat, less animal protein, more vegetables), so the homocysteine level in the blood is lower than that of men. In addition, the reproductive hormone concentration affects homocysteine metabolism. Testosterones, androgens increase blood homocysteine levels whereas estrogen reduces it.

Relationship between homocysteine serum level and MAU

Increased homocysteine levels in type 2 diabetic patients with retinal complications have been reported. [1]. Another study showed that the increase in homocysteine was statistically significant in type 2 diabetic

patients with acute coronary artery disease. [2]. In patients with type 2 diabetes, over time the progression of metabolic disorders and hemodynamic disorders will cause kidney damage. [3,5,7]. The results of the same research showed that homocysteine concentrations in diabetic group with MAU (+) were significantly higher than the uncomplicated MAU group (-) (Table 5) and there was a strong positive correlation of MAU with serum homocysteine level in type 2 diabetes patients (r = 0.639; p <0.01) (figure 1) (r = 0.639; p <0.01) (figure 1). Research by Cho E.H et al [4] concludes that: High hyperhomocysteine is associated with an increased risk of MAU occurrence. Homocysteine plays a role in the pathogenesis of type 2 diabetic nephropathy. Analysis results show that the increased rate of hyperhomocysteine in the group of early kidney damage was statistically significantly higher than the control group, and it demonstrates that hyperhomocysteinemia levels are closely related to kidney damage in patients with type 2 diabetes.

Relationship between homocysteine and some characteristics in patients with type 2 diabetes

Our research as well as other research in the country [2, 3, 5, 7] has not found a relationship between homocysteine concentration and HbA1c index (Table 6). This may suggest that homocysteine serum levels are independent factors associated with type 2 diabetes regardless of the level of HbA1c blood glucose control.

Regarding the relationship between homocysteine and blood lipid components of the study, we are similar to the author Luong Huu Dung. [3] There was a significant correlation between plasma homocysteine and triglycerides (r = 0.3; p <0.05), no correlation with cholesterol, HDL-cholesterol, LDL-cholesterol (Table 6). In contrast, author Nguyen Thi Thanh Thuy [2] did not find a correlation between homocysteine with blood lipid components; Author Duong Thi Tuyet [5] discovered an inverse correlation between homocysteine and HDL-cholesterol (r = - 0.3; p <0.05) or research results of Pham Toan Trung [7] found out a positive correlation between plasma homocysteine concentrations with LDL-c (r = 0.28, p <0.05). The correlation between homocysteine levels and different blood lipid indices of these studies may be because type 2 diabetics who have been treated with diet and lipid-lowering drugs should improve the lipid profile. Significantly, at the same time, renal function is also heterogeneous in the studies that contribute to altering homocysteine levels.

V. CONCLUSION

Homocysteine concentration increases in diabetic patients compared with no-diabetes group. The homocysteine index can be an independent predictor of kidney complications in type 2 diabetes patients, regardless of the level of blood glucose control.

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THE STUDY OF THE ASSOCIATION BETWEEN MALOCCLUSION, ORTHODONTIC TREATMENT AND TEMPOROMANDIBULAR JOINT DISORDER

Nguyen The Dung*

ABSTRACT

The Temporomandibular disorder (TMD) is a syndrome that includes painful and discomfort symptoms due to muscular and nerve disorders related to temporomandibular joint (TMJ). The cause of TMD can be due to many direct or indirect factors such as trauma, anatomy, pathophysiology, psychosocial factors and local factors such as malocclusion, abnormality of the mastication muscles, alteration of morphological structure of the condyles or a combination thereof [2]. Mechanical forces in orthodontics altering the position of teeth and jaw bones in malocclusion treatment possibly associate with TMD. The association between TMD, malocclusion and orthodontic treatment is the controversial subject for many studies and researches. We conducted research on this topic in order to discover more clinical evidences to support for this association. ***Objective***: The study aimed to analyze and identify clinical evidences about the possible association between malocclusion, orthodontic treatment and TMD. ***Methods***: Prospective study. ***Results***: The data collecting from 168 patients whose ages from 12 to 40 and with Angle class I, II, and III occlusions was analyzed. Each patient was examined for clinical evidence of possible association between malocclusion, orthodontic treatment and TMD. The Fisher test used to analyze the data to determine the existence of this association. ***Conclusion***: The results showed that malocclusion is the major pathological factor causing the TMD meanwhile orthodontic treatment is not an etiology of TMD. Furthermore, orthodontic treatment indeed tends to mitigate or reduce the risk of TMD development during and post orthodontic treatment. Therefore, we suggested orthodontic treatment should be indicated as the initial therapy for patients having both malocclusion and TMD.

I. INTRODUCTION

According to The American Association of Dental Research (AADR): TMD is a syndrome that includes painful and discomfort symptoms due to muscular and nerve disorders related to TMJ. The etiology of TMD can be due to many direct or indirect factors such as trauma, anatomy, pathophysiology, psychosocial factors and local factors such as malocclusion, abnormality of the mastication muscles, alteration of morphological structure of the condyles or a combination thereof [2]. Mechanical forces resulting from orthodontic treatments of Angle class II, III occlusion, open bites, anterior or posterior crossbite possibly associate with TMD [3,8]. The association between the cause of TMD, malocclusion, orthodontic treatment and vice versa is the controversial subject of many studies.

During or after the average two years of orthodontic treatment, patients could complain about TMD symptoms or blame

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their orthodontists for the etiology of this syndrome. Indeed, the hypothetically cause and effect relationship has led to malpractice lawsuits to many orthodontics.

Due to these reasons, we conducted a research on this topic to discover additionally clinical evidences to support the association between malocclusion and TMD and whether if orthodontic treatment causes, alleviates or cures TMD. Therefore, the objective of this study was to analyze and identify clinical evidences for the possible association between malocclusion, orthodontic treatment and TMD.

II. PARTICIPANTS AND METHODS

Participants: 168 patients were orthodontically treated from 2008 - 2020. Each patient had a complete profile including panoramic X-ray, cephalometric, patient's information such as gender, age and 3 self-assessments of TMD (1^{st}: pre-treatment, 2^{nd}: 1 year since treatment starting, 3^{rd}: 1 year post treatment). We conducted clinical examination, patient's self-assessments of TMD according to the recommendation of American Academy of Orofacial Pain in 1993 and Likert scale for 10 questions with different levels: level 0 (none: 0 point), level 1 (rarely: 1 point), level 2 (occasionally: 2 points), level 3 (frequently: 3 points). The points are from 0 to 30 and divided to different ranges:

- 0 point: normal
- 1-10 points: mild TMD
- 11-20 points: moderate TMD
- 21-30 points: severe TMD

The TMD self-assessments have been tested and verified using Cronbach's alpha test (>0.5).

Methods: Prospective study.

Data analysis: Software R 3.0.2 was used to analyze data.

III. RESULTS

Patient information: Through the data analysis of 168 patients, the results showed that: the average patient's age was 18.7 (SD 6.78), of which male accounted for 23% and female accounted for 77%. 74 patients had class I occlusion (44%), 54 patients had class II occlusion (32%) and 40 patients had class III occlusion (24%). The association between clinical symptoms and TMD was discovered according to each occlusion class as following:

Table 1: Scored TMJ self - assessment as beginning of orthodontic treatment

	QUESTIONS	ANSWER			
		NONE	YES		
			1	2	3
			Rarely	Occasionally	Frequently
1	Do you have any pain or difficulty when opening your mouth or yawning?	n=81	n=61	n=24	n=2
2	Do you ever have lockjaw or jaw luxation?	n=98	n=62	n=8	n=0
3	Do you feel any discomfort or muscle pain when chewing, speaking or moving your jaws?	n=95	n=1	n=10	n=0
4	Do you hear any TMJ sound?	n=109	n=57	n=2	n=0
5	Do you often have rigidity or fatigue in jaw area?	n=136	n=28	n=4	n=0

	QUESTIONS	ANSWER			
		NONE	YES		
			1	2	3
			Rarely	Occasionally	Frequently
6	Do you have pain in the TMJ in the area temporal or cheek areas?	n=128	n=36	n=4	n=0
7	Do you often have idiopathic headache, neck-ache or toothache?	n=139	n=28	n=1	n=0
8	Do you have any recent injury/trauma in head, neck and jaw areas?	n=163	n=5	n=0	n=0
9	Do you feel any recent abnormality when biting down?	n=90	n=54	n=23	n=1
10	Do you ever have treatment in the past for idiopathic facial or TMJ pain?	n=141	n=21	n=5	n=1

Table 2: Number of patients having TMD as the beginning of orthodontic treatment

Classification of Angle occlusion	Normal	TMD symptoms		
		Mild	Moderate	Severe
Angle class I	43	28	3	0
Angle class II	18	35	1	0
Angle class III	7	30	3	0
Total	68	93	7	0

The pre-treatment data indicated the total patients having TMD was 100 (59.6%), of which class I occlusion accounted for 18.5%, class II accounted for 21% and class III accounted for 20.1%.

Table 3: Scored TMD self-assessment as 1 year after the beginning of orthodontic treatment

	QUESTIONS	ANSWER			
		NONE	YES		
			1	2	3
			Rarely	Occasionally	Frequently
1	Do you have any pain or difficulty when opening your mouth or yawning?	n=113	n=54	n=1	n=0
2	Do you ever have lockjaw or jaw luxation?	n=146	n=22	n=0	n=0
3	Do you feel any discomfort or muscle pain when chewing, speaking or moving your jaws?	n=144	n=24	n=0	n=0
4	Do you hear any TMJ sound?	n=109	n=59	n=0	n=0
5	Do you often have rigidity or fatigue in jaw area?	n=162	n=6	n=0	n=0
6	Do you have pain in the TMJ in the area temporal or cheek areas?	n=156	n=12	n=0	n=0
7	Do you often have idiopathic headache, neck-ache or toothache?	n=162	n=6	n=0	n=0
8	Do you have any recent injury/trauma in head, neck and jaw areas?	n=168	n=0	n=0	n=0
9	Do you feel any recent abnormality when biting down?	n=95	n=73	n=0	n=0
10	Do you ever have treatment in the past for idiopathic facial or TMJ pain?	n=147	n=20	n=1	n=0

Table 4: Number of patients having TM as 1 year after the beginning of orthodontic treatment

Classification of Angle occlusion	Normal	TMD symptoms		
		Mild	Moderate	Severe
Angle class I	53	21	0	0
Angle class II	26	28	0	0
Angle class III	9	31	0	0
Total	88	80	0	0

The data of 1 year after the beginning of orthodontic treatment indicated the total patients having TMD was 80 (47.65%); of which class I accounted for 12.5%, class II accounted for 16.7% and class III accounted for 18.4%

Table 5: Scored TMD self-assessment as 1 year post orthodontic treatment

	QUESTIONS	ANSWER			
		NONE	YES		
			1	2	3
			Rarely	Occasionally	Frequently
1	Do you have any pain or difficulty when opening your mouth or yawning?	n=158	n=10	n=0	n=0
2	Do you ever have lockjaw or jaw luxation?	n=167	n=1	n=0	n=0
3	Do you feel any discomfort or muscle pain when chewing, speaking or moving your jaws?	n=166	n=2	n=0	n=0
4	Do you hear any TMJ sound?	n=113	n=55	n=0	n=0
5	Do you often have rigidity or fatigue in jaw area?	n=167	n=1	n=0	n=0
6	Do you have pain in the TMJ in the area temporal or cheek areas?	n=166	n=2	n=0	n=0
7	Do you often have idiopathic headache, neck-ache or toothache?	n=165	n=3	n=0	n=0
8	Do you have any recent injury/trauma in head, neck and jaw areas?	n=168	n=0	n=0	n=0
9	Do you feel any recent abnormality when biting down?	n=109	n=59	n=0	n=0
10	Do you ever have treatment in the past for idiopathic facial or TMJ pain?	n=157	n=11	n=0	n=0

Table 6: Number of patients having TMD as 1 year post orthodontic treatment

Classification of Angle occlusion	Normal	TMD symptoms		
		Mild	Moderate	Severe
Angle class I	59	15	0	0
Angle class II	41	13	0	0
Angle class III	17	23	0	0
Total	117	51	0	0

The data of 1 year post orthodontic treatment indicated the total patients having TMD was 52 (30.4%); of which class I accounted for 8.9%, class II accounted for 7.7% and class III accounted for 13.7%.

Table 7: The association between TMD symptoms with each Angle class of occlusion as beginning of orthodontic treatment

Angle class of occlusion	Normal	TMD symptoms	p-value
Angle class I	43	31	5.957e-05
Angle class II	18	36	
Angle class III	7	33	

Table 8: The association between TMD symptoms with each Angle class of occlusion as 1 year after beginning of orthodontic treatment

Angle class of occlusion	Normal	TMD symptoms	p-value
Angle class I	53	21	2.640332e-06
Angle class II	26	28	
Angle class III	9	31	

Table 9: The association between TMD symptoms with each Angle class of occlusion as 1 year post orthodontic treatment.

Angle class of occlusion	Normal	TMD symptoms	p-value
Angle class I	59	15	2.640332e-06
Angle class II	41	13	
Angle class III	17	23	

Table 10: The association between TMD symptoms with each period of orthodontic treatment.

TMD symptoms	Beginning of treatment	1 year after beginning of treatment	1 year post treatment	p-value
Normal	68	88	117	4.781e-07
Present of symptoms	100	80	51	

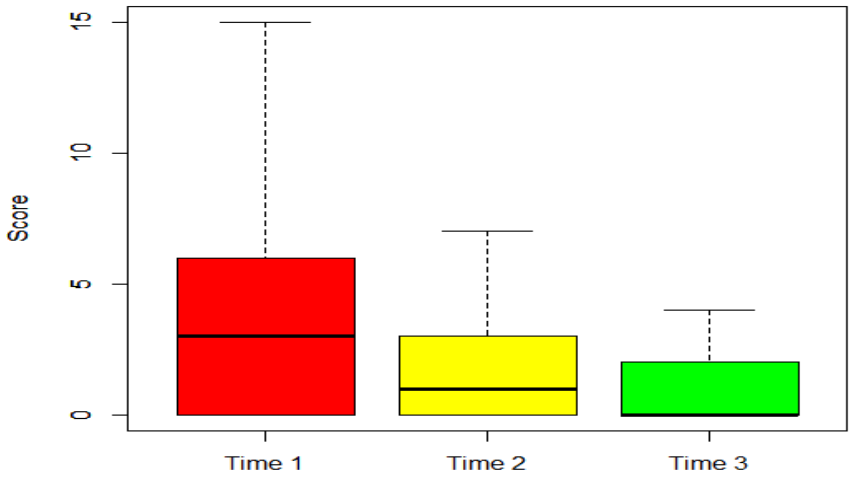

Figure 1: The severity of TMD at each period of orthodontic treatment

IV. DISCUSSION

Malocclusion and TMD

Results in table 7,8 and 9 demonstrated the proportion of class II occlusion patients manifested TMD was greater than that of class I occlusion. The greatest proportion was the patients having both class III occlusion and TMD. This variation was statistically significant according to Michelotti A, Idocice G. [6]. Among the factors causing TMD, malocclusion was frequently considered as one of the major factors causing TMD. Additionally, Thilander B, Rubio G, Pena L, De Mayorga C. [8] had demonstrated that TMD was significantly associated with anterior open bite, posterior crossbite, class III occlusion and severe overjet. Therefore, regardless the cause and effect relationship, it is important to screen for patients having TMD before beginning of orthodontic treatment because any TMD signs and symptoms occurring during or after the treatment can be attributed as the effect of orthodontic treatment [5,7]. T Henrikson et al indicated that it was essential to examine TMJ in patients with malocclusions in order to prevent signs and symptoms associated with TMD. Panoramic X-ray is valuable in the study of vertical height of condyles [4]. According to our other study, the association between TMD and the symmetry of the vertical height of condyles in groups of patients having different class of occlusion demonstrated the significant variation between TMD symptoms based on the symmetry of vertical height of condyles in class III malocclusion patients.[1]. In summary, malocclusion is a major factor causing TMD. Hence, the TMJ condition should be carefully examined in patients having malocclusion prior starting orthodontic treatment.

Modification of occlusion after orthodontic treatment can mitigate TMD

Through data analysis, before orthodontic treatment there were 7 patients exhibited moderate TMD (Table 2), and 1 year after beginning of treatment, there was no patient exhibited TMD (Table 4). Moreover, figure 1 demonstrated the median was significantly decreased through the orthodontic treatment, especially at 1 year post treatment. The median was 0 and 75% percentile was 2 indicated that 50% of patients no longer experienced TMD and 75% of patients experienced mild TMD (assessment score <2). Certainly, we could conclude that orthodontic treatment significantly reduced the severity of TMD in patients having malocclusions. Abrahamsson C, Henrikson et al analyzed the data of pre-and post-orthodontic treatment regarding the association between TMD with orthodontic treatments. The study concluded that orthodontic treatments could reduce the risks of developing TMD. [2]. Furthermore, many extensive evaluations and clinical studies have indicated that malocclusion is the major cause of developing TMD and occlusal modification resulting from orthodontic treatments tend to alleviate or reduce the risk of developing TMD [1,2,3]. According to Inger Egemark LDS et al, when analyzing studies on the results of orthodontic treatments related to TMD, it has concluded that orthodontic treatments did not increase but indeed reduces the risk of developing TMD. [7]. More recent studies have discovered that the signs and symptoms of TMD are less common in patients undergoing orthodontic treatments than those

not receiving the treatments. According to the future cohort research of MacFarlane TV et al, the results of over 20 years of orthodontic treatment follow-up showed that orthodontic treatments was not linked to the developing TMD even long time after the treatments. [5]. The results of Table 10 showed the effectiveness of orthodontic treatments in patients having TMD. The proportion of patients having TMD significantly decreased 1 year post treatments. The p-value of 4.781e-07 specified the effectiveness of orthodontic treatment and was statistically significant. This is a clinically scientific evidence that orthodontics does not increase the risk of developing TMD and in contrast orthodontic treatment significantly reduces the severity of TMD.

V. CONCLUSION

The Fisher test was applied to analyze the association between malocclusion and TMD. We concluded that malocclusion is one of the major pathological factors causing TMD, orthodontic treatments did not relate to developing TMD and lastly orthodontics tend to reduce the risk of developing TMD during or post treatments. Therefore, orthodontic treatment should be indicated as the initial therapy for patients having both malocclusion and TMD.

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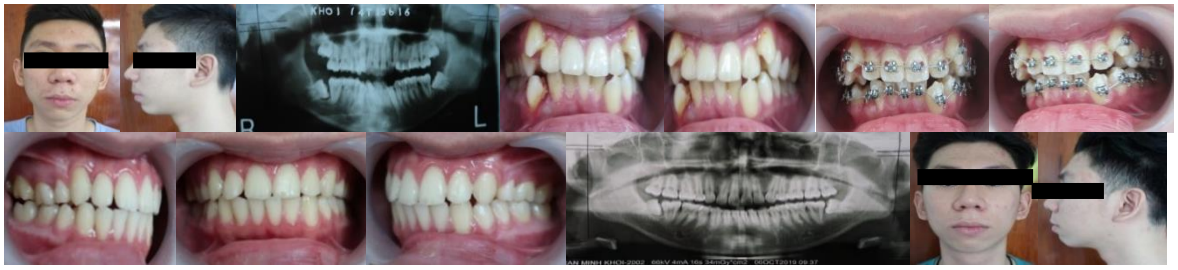
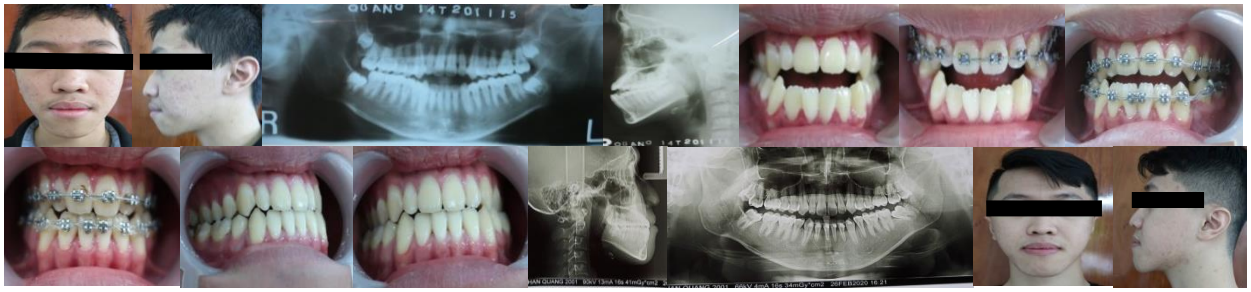
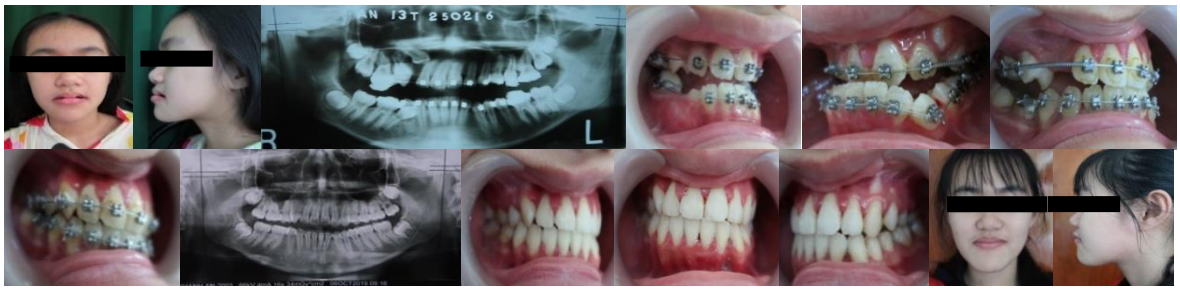
EXTRAORAL PHOTOGRAPHS OF PATIENTS UNDERGOING ORTHODONTIC TREATMENTS



1. TRAN MINH KH., year of birth: 2002, Diagnosis: Class II malocclusion (crowding and narrow maxillary and mandibular arches. *(Pre-treatment: TMD = 17 points: moderate TMD; Post-treatment TMD = 5 points: mild TMD).*



2. PHAN Q., year of birth: 2001, Diagnosis: Class III malocclusion, anterior open bite. *(Pre-treatment TMD = 16 points: moderate TMD; A Post-treatment TMD = 3 points: mild TMD).*



3. LE N. KH. A., year of birth: 2003, Diagnosis: Class III malocclusion (crowding and narrow maxillary and mandibular arches. *(Pre-treatment = 15 points: moderate TMD; Post-treatment TMD = 3 points: mild TMD).*

ABILITY TO REGULATE IMMUNITY FROM MESENCHYMAL STEM CELLS IN THE TREATMENT OF TRAUMATIC BRAIN INJURY

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ABSTRACT

Traumatic brain injury (TBI) is characterized that presents with a wide spectrum of clinical symptoms caused by an initial insult to the brain through an external mechanical force to the skull. TBI can potentially cause severe physical, cognitive and emotional impairment. The complex biochemical reactions of inflammatory processes in TBI significantly influence the overall severity of the brain damage and clinical sequelae. In the acute phase, brain tissue destroyed upon impact includes neurons, glia and endothelial cells, the latter of which makes up the blood-brain barrier. The use of mesenchymal stem cells (MSC) has been shown to be effective in treating traumatic brain injury, especially reduce inflammation in injured tissue. In this study, we aimed to investigate MSC's anti-inflammatory ability through regulating IL-6, IL-10, CRP, TNF-a factors, thereby reducing the trauma in the TBI. Biological effects of autologous MSC cell transplantation have been studied in 30 patients with molded TBI, after being filtered according to appropriate criteria. All patients received intravenous MSC and were monitored continuously during treatment with indicators of interest such as: Glasgow Coma Scale (GCS), Evaluation of Barthel index (BI), National Institute of Health Stroke Scale (NIHSS), leukocytes, IL-6, IL-10, CRP, TNF-a. We observed increased plasma level of inflammatory cytokines/molecules IL-6, CRP, TNF-a dramatically increased circulating leukocyte counts from TBI patients, which indicated an intense inflammatory response following TBI and significantly greater than that in control subjects with trauma. The results after transplantation MSC indicated that the majority of patients experienced improved health function in different degrees during the follow-up period. Lower serum levels of inflammatory factors IL-6, CRP, TNF-a and leukocytes population were detected following the transplantation along with an increase in IL-10, as compared with the levels prior to treatment. No serious adverse events were observed in any patient subsequent to transplantation. Overall, the present results suggest that transplantation of MSC is able to regulate inflammatory factors and appears to be safe for the treatment of TBI.

I. INTRODUCTION

Traumatic brain injury (TBI) is a leading cause of death and disability, affecting around 10 million people worldwide annually [1]. Report from the World Health Organization, in 2020 TBI continues to be a major health problem and a major cause of disability. Hospitalization rate related to TBI due to motor vehicle accident increased over the age of 44 - 64 years old and the leading cause of hospitalization related to TBI was due to falls [2]. Among all types of injuries,

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brain injuries are most likely to lead to death or permanent disability. Severe injuries or minor injuries cause deep and lasting damage to the patient, even minor injuries can lead to prolonged symptoms and serious long-term sequelae [3]. Despite modern technology and improved technology to manage TBI injuries, the overall death rate in severe TBI is still ~ 25% [4]. Patients who survive TBI trauma often experience headaches for weeks after the injury and potentially face long-lasting effects such as depression remains, Alzheimer or dementia in old age, which also affects the patients' families and communities [3]. Therefore, rehabilitation therapy is important for maximizing functional recovery following TBI. However, there are still limitations in current treatments because the heterogeneity of trauma based on injury location and severity, as well as patient age and associated comorbidities, has posed a significant challenge in the development of effective therapies for TBI [5].

Traumatic brain injury occurs either directly or indirectly from the outside, which is the result of an impact of the head with an object (bump, accident) or an indirect impact such as a whip or sudden acceleration. These forces damage the brain structure and change its function. The aforementioned factors, first cause basic injury to the brain and then secondary injury. Trauma is manifested by a cascade of many pathophysiological mechanisms, including inflammation, oxidation, gene activation, cytotoxicity and brain edema. The body initially responds to defense and repair mechanisms, but when the inflammatory response is excessive, it can lead to negative effects that prevent healing in the affected area [3]. A strong inflammatory response caused by trauma is a major component that exacerbates TBI damage. The signal at the site of the injury will activate the immune system and nerve cells as well as the accumulation of mononuclear and lymphocytes. When these factors are excessively activated, it can further stress localized lesions and contribute to secondary injury or delay at the site of injury. [1]. Inflammation is a reaction that protects the body from damage by external or internal factors such as tissue anemia, autoimmune or infectious reactions. At the site of injury, in tissues outside the brain, the primary underlying features of inflammation include swelling, redness, heat, and often pain. Factors triggered during the inflammatory process have been established, including invasion of circulating immune cells (lymphocytes and macrophages), and induction or activation of inflammatory mediators. such as kinin, cyclooxygenase and cytokine IL-1, IL-6, TNF-a [2], [6]. Inflammatory reactions when activated at a moderate level will have a clear benefit in the anti-infection state for a defined period of time. However, prolonged, excessive or inappropriate inflammation is the cause of the trauma worse. Many of these molecules, when overproduced and worked for a long time in damaged areas, have been shown to oxidative stress, and thus these factors are considered the main target for treatment interventions in all diseases [1],[5].

Research has shown that after acute TBI, the number of leukocytes increases in peripheral blood, since leukocytes are released from the marginal pool stores and bone marrow into the circulation increases the number of immune cells. The spike in macrophages, lymphocytes, and cytokines that cause inflammation leads to the accumulation of products of uncontrolled reactive oxygen chain reactions that can react

to traumatic reactions creating a group of endless reactive oxygen species (ROS) and consequently more traumatic injuries, damaged cells, massive neuronal death. However, the understanding of systemic oxidative changes after TBI is still limited. Oxidative stress and cytokines are involved in the onset of inflammatory processes, which play an important role in tissue and organ damage [1]. Stem cells isolated from bone marrow possess the capacity to self-renew and differentiate into mesenchymal cells when exposed to appropriate conditions and possess capacity to differentiate into numerous cell types, including neural cell [2], contribute to normal homeostasis, and exert therapeutic benefits either endogenously or following transplantation in injured organs, i.e., brain [7]. This suggests that mesenchymal stem cell (MSC) is a promising strategy for the treatment of TBI. Moreover, MSC has the ability to migrate to injured tissue sites and settle into niche drives [3]. This ability of Homing is a favorable mechanism for bringing stem cells to delicate sites like in the brain. In addition, injecting MSC has been shown to reduce the natural immune response and oxidative damage in injured tissue by releasing exosome and regulation of factor IL-10 produced by macrophages [3].

Significant advances in the application of MSC in regeneration have been implemented translated from laboratory studies into limited clinical trials for TBI [8], [9]. However, there is still much work remains to understand the repair mechanisms, regulate microenvironment as well as the specific mode of action of MSC (the way they target tissues, the role of paracrine factors, among others) in brain injury has limited the successful implementation of clinical practice. On another note, the immunogenic aspects of MSCs after transplantation are related to the nervous system and systemic inflammatory response is also among challenges not to be ignored.

The present study provides evidence of a novel therapeutic feature of stem cells involving their ability to regulate immune cells. there was an intimate relationship between MSC and IL-6, IL-10, CRP, TNF-a factors in the clinical traumatic brain injury (TBI).

II. METHODS

2.1.Patient

These studies were approved by the ethic committee of Hue Central Hospital in Vietnam. All the participants or the next of kin, caretakers, or guardians provided their written informed consent to participate in this study. Participating patients were divided into 2 groups including control group and MSC group. In MSC group, there are 15 patients, aged 22 - 56 years, weighing 45 - 83kg, hospitalized in a coma and treated with routine methods during and after the study of combining stem cell transplantation. The control group included 15 patients, aged 18-63, weighing 45-75kg, hospitalized in a coma and treated with routine methods. The patients were formed according to the Glasgow score (GCS), National Institutes of Health Sroke Scale (NHISS), and Barthel index (BI) less than 40 on admission. GCS includes only 3 tests response: eye, verbal, and best motor response. The lower GCS score indicates the more severe disease and the higher the risk of death. The lower GCS score indicates more severe disease and higher risk of death, which the lowest point is 1 while the highest point is 4–6 and the total

added score of all test ranges from 3 to 15. Patients with GCS ≤8 scores are considered to have severe brain damage and patients with GCS> 8 scores are considered to have moderate brain damage. patients were excluded if they met any of the following criteria: i) a participant in the study required a blood transfusion, no sample was taken from that patient to check hematological indicators; ii) TBI was combined with other internal organ injuries, history or family with peripheral neuropathy or autoimmune disease; iii) cognitive limitations, chronic inflammatory diseases, malignant neoplasms, chronic liver disease; iv) regular anti-inflammatory drug use, blood pathology or respiratory instability; v) pregnant or possibly pregnant; and vi) patients with cancer or malignant diseases

2.2. Methods

Preparation and transplantation of autologous MSC

- **Bone marrow collection**: Bone marrow collection procedure is carried out under sterile conditions. The bone marrow is drawn from the anterior pelvic position. Conduct 3-6 aspirate position on the pelvis to ensure increased myelosuppression and limit the mistaking of peripheral blood. Blood pressure, heart rate, oxygen saturation in the vessels, meningeal pressure are checked every 5 minutes within 1 hour [8], [9].

- **Mononuclear cell isolation**: Bone marrow mononuclear cells were then separated by density gradient centrifugation with Ficoll-Paque Plus 1.077g / mL (Miltenyi Biotec Inc.). Centrifugal fluid, receiving about 100ml of Buffy coat. The marrow fluid is diluted with PBS (Miltenyi BiotecInc.) And filtered with a 100µm filter to remove bone fragments. Add the diluted marrow fluid over the Ficoll-Paque layer, centrifuge at 400x g for 20 minutes at room temperature, collect the single-core ring. Mononuclear cells are removed with PBS and centrifuged.

- **Proliferation of mesenchymal stem cells**: Cells obtained after centrifugation with Ficoll-Paque were cultured in flask with StemMACS Cardiac Cultivation Medium XF, human (Miltenyi Biotec, Germany) containing 10% fetal bovine serum (FBS) (Miltenyi Biotec, Germany.) and 1% penicillin-streptomycin (Miltenyi Biotec, Germany) , density of 5x104 cells/ cm^2 at 37^oC and 5% CO_2, moisture 95%. After the first 24-36 hours, non-adherent cells are removed by replacing the new medium. Adherent cells develop into cell clusters in 3-7 days. When cell density reaches 80-90% of the area, cells are separated with Trypsin // EDTA 0.25% (Miltenyi BiotecInc, Germany) and washed three times with PBS.

All cell manipulation procedures were conducted in the Thermo Scientific™ 1300 Series Class II, Type A2 Biological Safety Cabinet Packages with sterile equipment. Only cells with a positive signal for the multipotential markers, octamer-binding transcription factor 4 (Oct4) and Nanog, were screened for further cloning, culturing and inducing. MSCs were fixed in 4% paraformaldehyde and then MSCs were blocked with bovine serum albumin (Sigma-Aldrich; Germany) at room temperature for 30 min. Cells were then incubated with primary antibodies against Nanog (1:1,000) and Oct4 (1:1,000) for 1 h at room temperature. Following this, cells were washed with phosphate-buffered saline. Subsequently, the cells were incubated with anti-rabbit secondary antibody (1:200) for 1 h at room temperature them visualized under a fluorescent microscope. All antibodies

were supplied by Miltenyi Biotec, Germany.

- Assessment of differentiation potential of human pluripotent stem cells.

Adipogenic, chondrogenic and osteogenic lineages differentiation of MSCs. To evaluate MSC abilities, adipogenic and osteogenic differentiation assays were performed on isolated cells. Osteogenesis differentiation medium (Miltenyi Biotec) or adipogenesis differentiation medium (Miltenyi Biotec) was added into a culture when the fusion rate reached approximately 80%. The cells were cultured for 3 to 4 weeks before collection.The media were changed every 3 days. The cells were cultured at 37°C, 5% CO_2, in 95% humidified air. Adipogenic, osteogenic and chondrogenic differentiation assays were conducted three times. Alizarin Red S staining was used to analyze osteogenic lineages, whereas Oil Red O was used to analyze lipid droplets.

- **Graft cell criteria**: endotoxin concentration <5.0 EU / mL; negative with Gram stain and other microorganisms; ratio of living cells> 90%, negative of mycoplasma, ability to create CFU-F clusters, cell flow analysis.

- **CFU-F**: to test cell biology, so before proliferating or mesenchymal stem cell transplantation, cells must be assessed for their ability to form CFU-F. Cell culture in 25-bottle T-flask, density of 3x104 cells / cm^2 (37^oC, 5% CO_2, humidity of 95%). After 10-14 days, the cell clusters were stained with Giemsa. The CFU-F cluster is defined as a cluster with a diameter of about 1-8mm with more than 50 cells.

- **Flow cytometry**: To assess cell quality, test surface markers with FACS Canto II machine. Mononuclear cells are stained with monoclonal antibodies: CD34 and CD45. Post-culture mesenchymal stem cells were stained with monoclonal antibodies: CD45 (negative), CD73, CD90, and CD105 (positive).

- **Cell transplantation procedure**: after assessing cell quality, transplant into a patient by intravenous route. Transplantation of mononuclear cells for the first time about 2 hours after marrow aspiration. 2nd transplant with MSC after 9-13 days. Heparinization to avoid blood clots.

2.3. Patient evaluation:

All diseases are assessed according to a protocol including comprehensive physical and neurological examination, routine testing and hematological index before the procedure, and after transplantation.

- **Glasgow Coma Scale:** We have introduced, GCS is a method of assessing the patient's state of consciousness in a quantitative way. Set to assess the coma of a head injury victim. The lowest total GCS score is 3 (deep coma or death), and the highest is 15 (fully awake and awake), in general, the level of coma is assessed as: severe when GCS $\leq$ 8, average with GCS from 9 to 12, light when GCS $\geq$ 13.

- **Evaluation of Barthel index (BI):** Barthel index (BI-Barthel Index) is used to assess the severity of nerves. BI is used as a simple independent index to evaluate the functional index of patients with neuromuscular or musculoskeletal disorders, thereby assessing patient improvement. The BI index is determined at the time the patient is hospitalized and the time after cell transplantation.

- **National Institute of Health Stroke Scale (NIHSS):** to evaluate a patient's neurological status on the day of transplantation, and after cell infusion at 1, 3, 7, 30 and 60 days. The NIHSS scores are evaluated as 0 is no evident stroke

symptoms; 1 – 4 is mild stroke; 5 – 15 is moderate stroke; 16 – 20 is moderate to severe stroke; and 21 – 42 is a severe stroke.

- **Assessment of hematological index:** Hematological index including erythrocyte, leukocyte, hemoglobin concentration, and concentration of cytokines IL-6, IL-10, CRP are monitored daily. Respiratory index was assessed daily to monitor recovery levels.

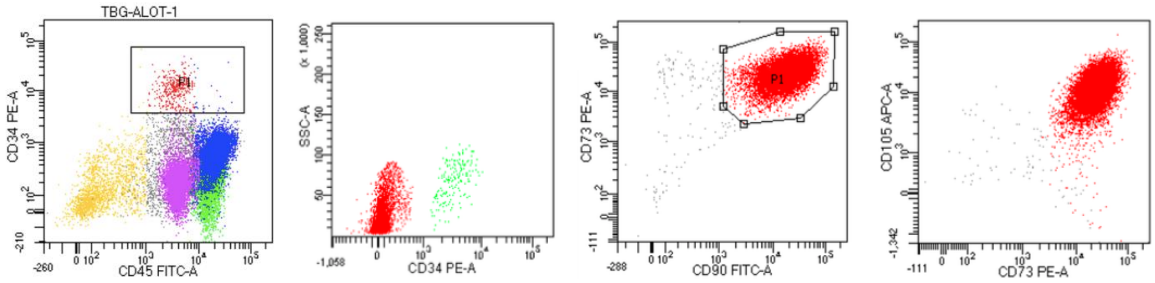
2.4. Statistical Analysis

Data are expressed as the mean ± standard deviation. The GraphPad Prism 8 software (GraphPad Software, LLC , San Diego, USA) was used for statistical analysis of the data. Differences among groups were established using one-way analysis of variance (ANOVA) and Fisher's protected t-tests or by a Student's t test when only two groups were compared. Statistical significance was established at P<0.05, P<0.01. # P<0.05, ##<0.01 for all the results in MSC group, and * P<0.05, **p<0.01 between two group.

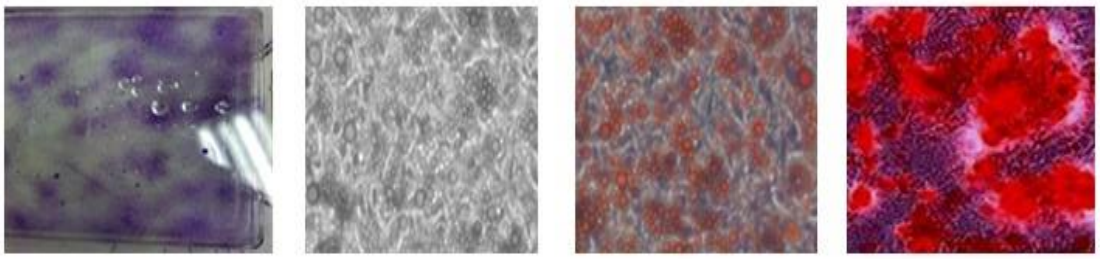
III. RESULTS

Quality and cell transplantation

The ultimate to potency and successful cell processing for stem cell products is prompt and reproducible engraftment and close monitoring is essential for safety and quality control. After being isolated cells from the bone marrow will be transplanted into the patient. surface marker profiling experiments by flow cytometry analysis were performed at 4 hours harvest. FACS test results before transplantation must ensure: CD34 positive mononuclear cells. Mesenchymal stem cells are CD73, CD90 and CD105 positive (about 98% positive).



A. Mononuclear cells B. Mesenchymal stem cells



C. CFU-F and differentiation along adipogenic, osteogenic, and chondrogenic lineages.

Figure 1: Quality control and assurance of cell processing for autologous stem cell transplantation.

Baseline characteristics of patients

The main characteristics of the 30 patients are presented in Table I. In summary, 8 female and 22 male patients with a mean age of 38.83 ± 11.65 years (range, 18–65 years) were enrolled and divided into 2 groups: Control group and MSC transplant group. Patients received intravenous (15 patients) MSC infusion at 2 hours after severe TBI (GCS score, 3–8). All patients were treated according to the standardized guidelines for the management of severe TBI in Hue Central Hospital.

Table 1: Baseline characteristics and clinical follow-up of patients

A. Control group

Characteristics	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient 7**	**Patient 8**	**Patient 9**	**Patient 10**
Sex	M	M	F	M	M	F	M	M	M	M
Age (years)	39	50	21	55	56	29	27	47	22	39
Etiology	traffic accident	traffic accident	traffic accident	traffic accident	traffic accident	traffic accident	traffic accident	traffic accident	fall accident	fall accident
athological features	SDH	EDH	SDH	SDH	SDH	SDH	SDH	EDH	EDH	SDH
GCS/NIHSS/BI scores										
Infusion day	**5/25**	**6/24**	**6/24**	**6/25**	**8/31**	**6/31**	**6/31**	**8/31**	**6/22**	**6/26**
Day 1	**5/25**	**6/24**	**6/24**	**3/25**	**5/31**	**7/31**	**8/31**	**8/31**	**7/22**	**6/26**
Day 3	**5/25**	**6/20**	**6/24**	**3/31**	**3/31**	**7/31**	**8/31**	**6/29**	**7/22**	**7/26**
Day 7	**6/25**	**8/20**	**8/20**		**3/31**	**7/25**	**11/31**	**6/25**	**8/22**	**10/20**
Day 30	**9/15/15**	**10/20/20**	**8/20/25**			**11/22/25**	**10/21/20**	**6/22/20**	**10/20/20**	**12/15/30**
Day 60	**10/15/35**	**10/20/40**	**10/15/35**			**11/15/30**	**10/21/30**		**12/17/35**	**12/15/45**

Characteristics	**Patient 11**	**Patient 12**	**Patient 13**	**Patient 14**	**Patient 15**
Sex	M	M	M	M	F
Age (years)	40	35	44	36	34
Etiology	traffic accident	traffic accident	traffic accident	traffic accident	traffic accident
athological features	EDH	SDH	SDH	SDH	SDH
GCS/NIHSS/BI scores					
Infusion day	**5/27**	**4/31**	**6/24**	**5/25**	**6/24**
Day 1	**5/27**	**4/31**	**6/24**	**5/25**	**6/24**
Day 3	**7/27**	**5/31**	**6/24**	**6/25**	**6/24**
Day 7	**8/25**	**6/30**	**8/20**	**6/25**	**7/24**
Day 30	**10/20/25**	**9/25/20**	**9/20/25**	**9/19/30**	**10/20/30**
Day 60	**11/13/35**	**10/17/40**	**12/15/35**	**11/15/40**	**11/20/45**

B. MSC transplant group

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Sex	F	M	M	F	M	F	M	M	M	M
Age (years)	27	48	23	63	38	18	60	49	39	28
Etiology	fall accident	traffic accident	traffic accident	fall accident	traffic accident	traffic accident	traffic accident	traffic accident	fall accident	fall accident
Athological features	ICH	EDH SDH	ICH SAH	SDH	SDH	EDH	EDH SDH	ICH	SDH	SDH
GCS/NIHSS/BI scores										
Infusion day	**6/25**	**5/24**	**5/22**	**5/23**	**6/31**	**6/31**	**6/22**	**6/29**	**5/24**	**6/26**
Day 1	**7/25**	**7/24**	**8/22**	**8/23**	**6/25**	**7/31**	**8/22**	**8/29**	**9/10**	**8/26**
Day 3	**7/25**	**9/15**	**10/22**	**8/23**	**6/25**	**7/31**	**8/22**	**8/29**	**10/10**	**8/26**
Day 7	**9/20**	**10/6**	**12/15**	**8/23**	**10/25**	**8/20**	**8/22**	**9/29**	**12/6**	**9/18**
Day 30	**14/15/25**	**14/6/20**	**15/10/25**	**9/21/30**	**13/25/30**	**10/15/15**	**12/18/25**	**13/20/20**	**12/3/20**	**12/15/15**
Day 60	**12/8/35**	**14/4/50**	**15/8/50**		**15/9/45**	**14/8/40**	**13/10/40**	**14/15/30**	**15/2/50**	**15/7/40**
Delivery route	intravenous	intravenous	intravenous	intravenous	intravenous	intravenous	intravenous	intravenous	intravenous	intravenous

Characteristics	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15
Sex	M	F	M	M	F
Age (years)	40	47	41	32	38
Etiology	traffic accident	traffic accident	traffic accident	traffic accident	traffic accident
Athological features	SDH	SDH	EDH	ICH	EDH
GCS/NIHSS/BI scores					
Infusion day	**5/31**	**5/22**	**5/29**	**5/24**	**6/24**
Day 1	**5/31**	**5/22**	**8/29**	**8/15**	**8/24**
Day 3	**7/25**	**8/22**	**8/29**	**10/15**	**8/20**
Day 7	**8/20**	**8/22**	**9/25**	**12/15**	**9/15**
Day 30	**12/15/25**	**12/18/25**	**13/15/30**	**14/10/25**	**12/15/15**
Day 60	**15/8/40**	**13/10/40**	**14/15/40**	**15/4/50**	**15/7/40**
Delivery route	intravenous	intravenous	intravenous	intravenous	intravenous

SDH, subdural hematoma; EDH, epidural hematoma; ICH, intracerebral hemorrhage; SAH, subarachnoid hemorrhage; GCS, Glasgow Coma Scale; NIHSS, National Institute of Health Stroke Scale; GOS, Glasgow Outcome Scale; TBI, traumatic brain injury.

Serial clinical and laboratory evaluations demonstrated no serious adverse events during the transplantation procedure and the follow-up period (60 days). Follow-up MRI evaluations did not demonstrate any tumor development or new lesions.

Neurological evaluation

Patients were followed up with standard clinical examination with GCS, BI, and GCS scoring. In MSC group (Table I.B) shows a summary of all the values prior to and following transplantation of MSC. No significant hemodynamic or respiratory changes were observed subsequent to MSC treatment in any patient. In fact, 15 patients in the MSC transplant group presented degrees of improvement in neurological and motor function as compared with the values before transplantation and better than the control group. in Table I.B, the GSC scores improved (range, 6−10 points) during follow-up and the NHISS scores also increased correspondingly (range, −4 to −23 points), similar to the BI score also changed (range, 10−25 points). one patient died after more than 1 month of MSC treatment. In contrast, the control group (Table I.A) had up to 3 patients who died during treatment without using MSC (death after 3, 7, 30 days). The GCS, NHISS and BI indices do not have significant differences between time periods (GCS, 4−6 points; NHISS, −4 to −15 points; BI, 5−20 points).

Some special cases occurred in both groups. patient 9 in MSC group (Table I.B), whose scores of GCS and NIHSS were improved at 1 day post-infusion or patients 6, 7 was hospitalized in a critical condition (GCS/NHISS: 6/31) but gave saw remarkable improvement after 2 months of follow-up. In the control group (Table I.A), patients 4, 5 showed that the GCS and NHISS indices were alarming (6/25; 8/31) and showed no signs of decline. As a result, they died a few days after being admitted to the hospital (3/31; 3/31).

Inflammatory Cytokine/Molecule Concentrations in Plasma

Plasma concentrations of IL-6, IL-10, CRP and TNF-α in Control group and MSC group were determined in 6 hours (6 h), 12 hours (12 h), 24 hours (24 h), 72 hours (72 h), 1 week (1 w), 1 month (1 m), and 2 months (2m) following injury (Fig.A, B, C, D, E, F, G, H,). The concentrations of IL-6, IL-10, CRP and TNF-α in control subjects were significantly increased from 6 h until 1 w (IL-6: 1.13, 1.26, 1.36, 1.39, 1.1-fold compared to 0 hour; IL-10: 1.12, 1.23, 1.36, 1.44, 1.08 compared to 0 hour, CRP: 1.11, 1.21, 1.26, 1.2, 1.05-fold compared to 0 hour, and TNF-α:1.13, 1.24, 1.34, 1.4, 0.25 after trauma).

In MSC group, however, concentrations of three molecules were significantly decreased at all time points assessed (6 h – 2 m after injury). Decreases of IL-6 (0.05, 0.1, 0.2, 0.38, 0.66, 0.84, 0.95-fold compared to 0 hour after trauma), CRP (0.16, 0.2, 0.27, 0.38, 0.54, 0.85, 0.95-fold compared to 0 hour after trauma), and TNF-α (0.13, 0.14, 0.29, 0.42, 0.52, 0.80, 0.95 -fold compared to 0 hour after trauma) after TBI were significantly greater than those of control subjects at 6 h –2 m following injury (Fig. A, B, E, F, G, H) . In contrast, IL-10 levels increased significantly after 3 days of MSC transplant (1.39, 1.55, 1.65, 1.64 -fold) then subsequently decreased during treatment (0.28, 0.65, 0.87 -fold) (Fig. C, D). (compared using t-tests, $P<0.05$, and $P<0.01$ for all the results of IL-6, IL-10, CRP, TNF-α from 6 h to 2 m).

After 2 months, IL-6 concentration decreased by 6.65-fold compared to the control group; IL-10 concentration decreased 1.67-fold compared to the control group; CRP concentration decreased 2.97-fold compared to the control group and TNF-α concentration decreased 2.89-fold compared to the control group.

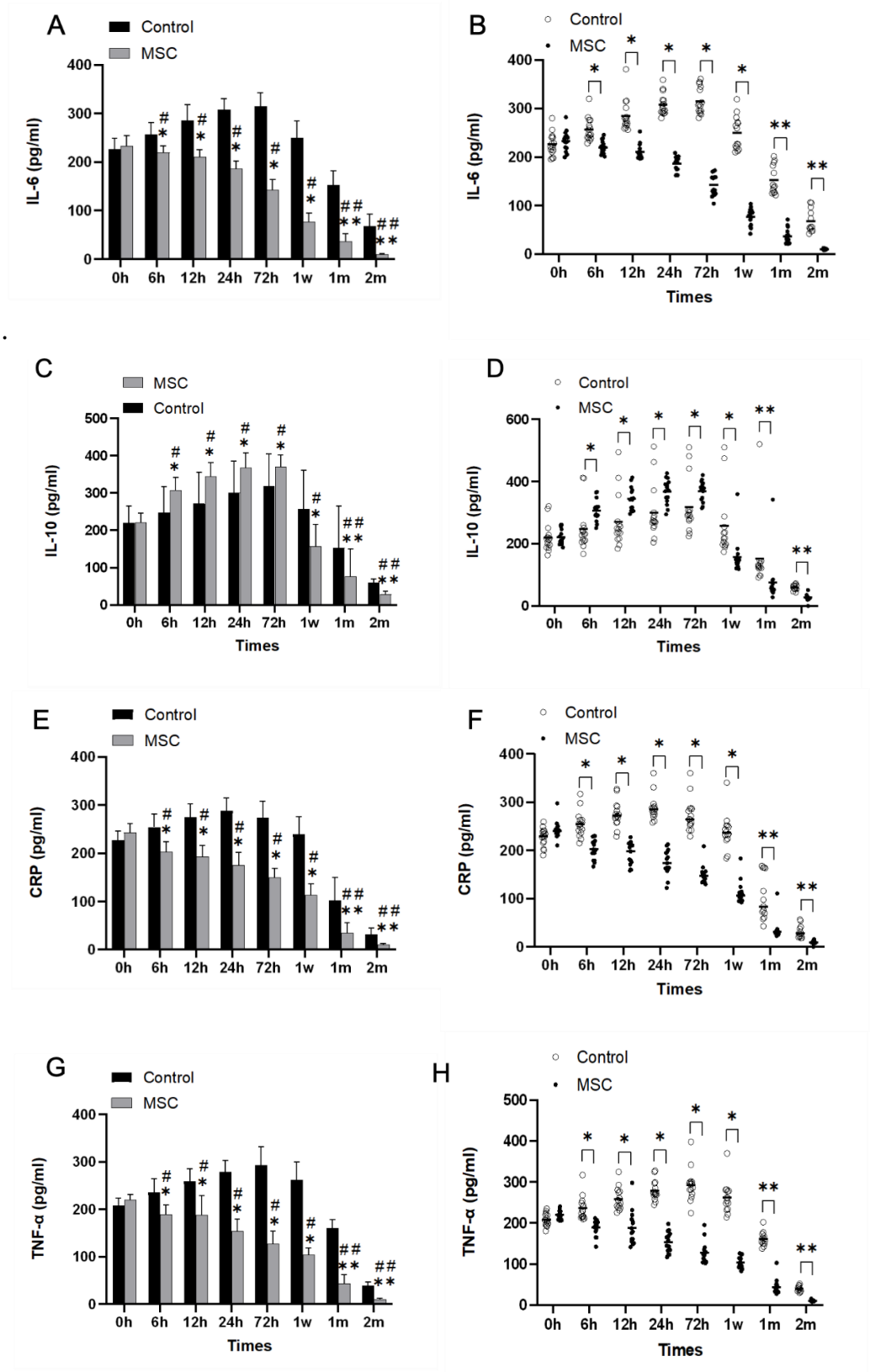

A
Control
MSC
IL-6 (pg/ml)
0h 6h 12h 24h 72h 1w 1m 2m
B
Control
MSC
IL-6 (pg/ml)
Times
C
MSC
Control
IL-10 (pg/ml)
Times
D
Control
MSC
IL-10 (pg/ml)
Times
E
Control
MSC
CRP (pg/ml)
F
Control
MSC
CRP (pg/ml)
Times
G
Control
MSC
TNF-α (pg/ml)
Times
H
Control
MSC
TNF-α (pg/ml)
Times

Figure 2: Inflammatory marker concentrations in plasma. The change in mean of IL-6 (A, B), IL-10 (C, D), CRP (E, F) and TNF-a (G, H) in plasma after injury in Control group subjects and MSC group subjects are presented. Concentration is expressed as mean ± SE values and the samples were shown as histograms at times including 0 h-2 months after the injury. The concentration of TNF-α, IL-6 and CRP in Control group (n=15) subject was significantly increased at 6 h-1 week. In contrast, the subjects in MSC (n=15) group had shown presenting favorable results after 6 hours of injecting MSC. ##P<0.01; #P<0.05, significantly different from original injury by Fisher's protected t tests. **P<0.01; *P<0.05, significantly different from Control group by Fisher's protected t tests.

Free Radical Production in Leukocyte Homogenates

The presence of free radicals in the leukocytes was estimated with the fluorescent DCF. The MSC group subjects had a low concentration of DCF and decreased of DCF concentration was significantly greater than those from control subjects (0 h: 24.87 ± 2.86; 6 h: 20.00 ± 2.88; 12 h: 15.99 ± 2.66; 24 h: 15.65 ± 2.12; 72 h: 13.87 ± 2.48; 1 w: 11.11 ± 1.75; 1 m: 8.43 ± 1.63; 2 m: 4.38 ± 0.75) (compared using t-tests, P<0.05 for all the time points). DCF concentrations increased significantly in control group at almost all the time points assessed. In control group, maximal increases were detected at 24 h after injury when DCF (36.37 ± 2.68) was increased by 1.56 -fold compared to the original and by 2.32-fold in the MSC group (15.13 ± 1.6). (compared using t-tests, P<0.05 for all these time points).

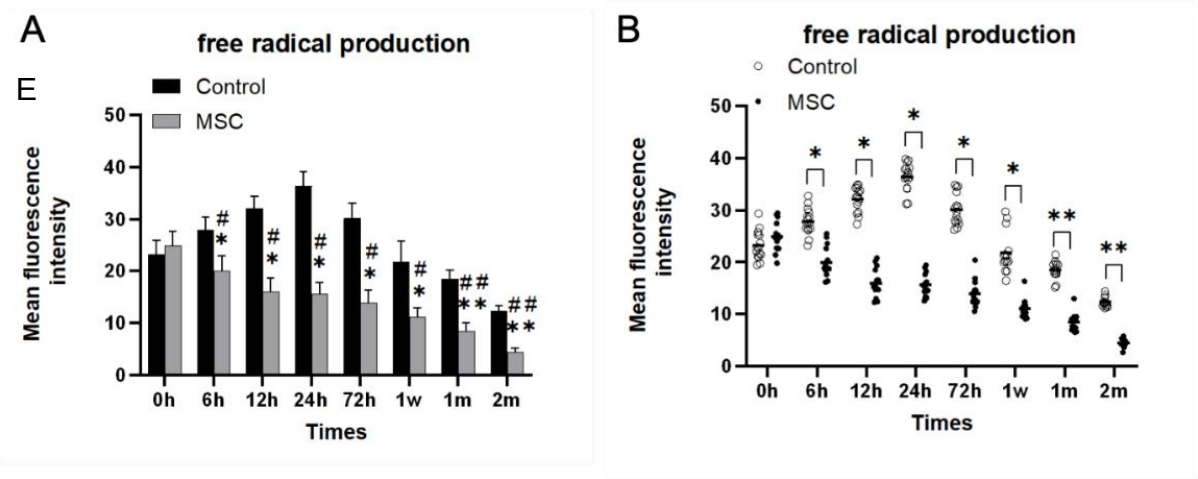


Figure 3: Free radical production in leukocyte homogenates. The presence of free radicals in the leukocytes was show by DCF in homogenates from subjects in both groups at times ranging from 6 h to 2 months after injury. (A) The concentration of DCF was decreased significantly in MSC group at most times assessed. Changes in leukocytes from MSC group were greater than those from Control group at 6 h, 12 h, 24 h, 72h, 1 week, 1 month and 2 months after injury. (B) Mean fluorescence intensity in leukocyte homogenates of Control group subjects (white circles) and MSC group subjects (black circles) were plotted as a scatter gram. constraining of free radical in leukocytes from MSC subjects were greater than those from Control subjects during the observation period.

Further determination of free radical productions between different severities of brain injury revealed significantly increases of free radical in leukocytes from control group subjects than those from MSC group subjects at all the time points assessed (STBI vs. MTBI: 1.4, 2.00, 2.32, 2.17, 1.96, 2.18, 2.79 -fold for 6 h to 2 m respectively) (compared using t-tests, P<0.05, P<0.01 for all time points from 6 h to 2 m after injury).

Magnetic resonance imaging (MRI)

All patients with traumatic brain injury were carefully examined to assess multiple system trauma and computerized tomography of the chest, abdomen and pelvis to consider other related injuries as well as build a correlation on the dose of cell transplantation.

Conducted cranial survey with Axial T1W, T2W, FLAIR, Sagittal T1W pulse sequences and did not inject magnetic resist drugs. The results showed the preservation of brain structure when the midline structure from deviation 7mm returned to normal. There were no significant changes in intracranial volume or cerebrospinal fluid.

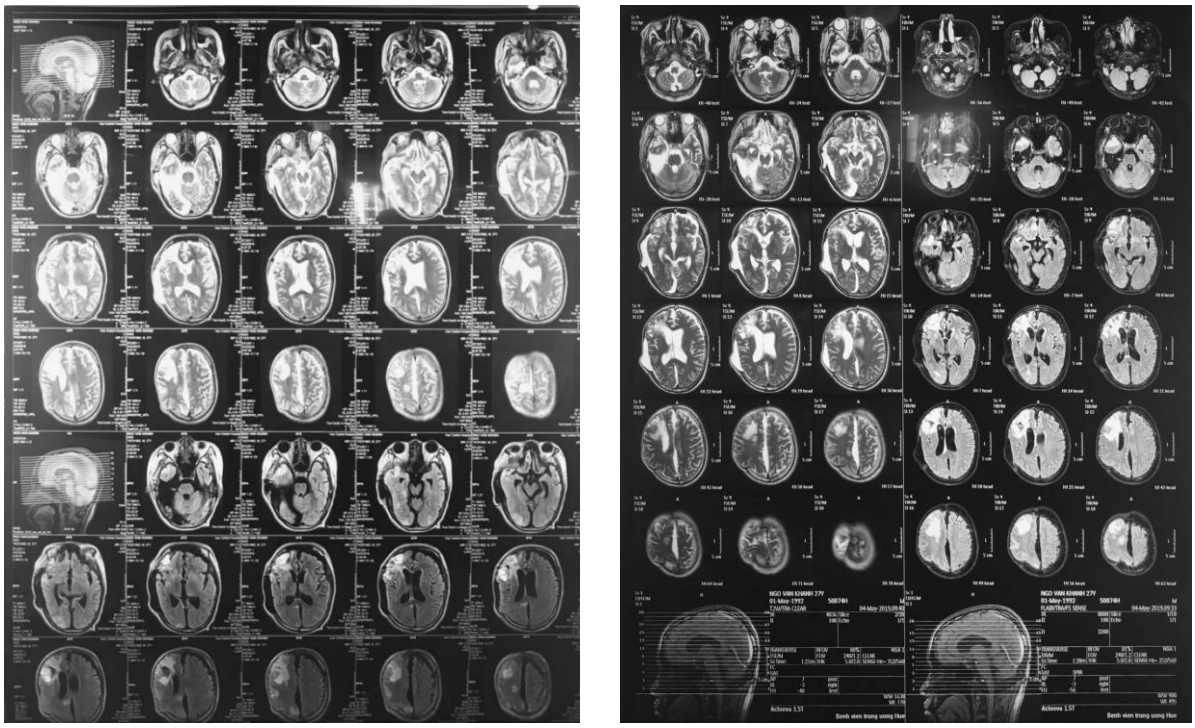


Figure 4. MRI results before and after treatment 6 months

Results of CT images of patients in both study groups were identified at the time of admission (before surgery) and MRI images in 1, 2 and 6 months after treatment.

IV. DISCUSSION

Our present clinical research suggesting that the delivery of autologous mesenchymal stem cells to patients with TBI has been shown the positive results in the treatment of traumatic brain injury compared to conventional methods. Transplant patients showed positive responses after 1 weeks of

treatment. Health, motor functions and consciousness assessed by Glasgow, HNISS, BI and hematological index showed a rapid recovery. In the control group, even after being discharged from the hospital, patients often complained of headaches for weeks after injury, showing that TBIs can lead to prolonged symptoms and serious sequelae for decades. Results revealed that this procedure was well tolerated, and there were no significant side effects in the presence of MSCs. Using intravenous infusion MSCs proves the safety and feasibility of this potential treatment in patients with severe TBI. Furthermore, there was no evidence of venous thromboembolism, tumor formation, intracranial infection or systemic infection in any of the patients following transplantation.

The cognitive scores (GCS, NIHSS, BI) of 15 patients improved with each follow-up period. The GCS and NIHSS scores improved significantly after 3 -7 day after MSC treatment, indicating a significant recovery of nerve function. While the control group improved only at the end of the follow-up period, but not significantly. MRI scan results provide positive feedback. Follow-up MRI evaluations did not demonstrate any new lesions or tumor development. In addition, no patients were newly diagnosed with arrhythmia or seizure during the follow-up period.

Our results are similar to study by Cox *et al.* 10 children that had a TBI injury with a Glasgow Coma Scale (GCS) score between 5 and 8 were grafted with MSC and monitored them over the course of 6 months [10]. Seven children showed positive improvement on the GCS. The other three children did not show a significant improvement to their quality of life. Subsequently, this study was conducted in adults in the treatment of brain stroke and presenting favorable results [11]. Tian *et al.* shows that the use of MSC has a marked effect after TBI [12]. A total of 97 patients (24 with persistent vegetative state and 73 with disturbance motor activity) received autologous bone marrow mesenchymal stem cell therapy voluntarily. 38 of 97 patients (39.2%) improved in the function of brain after transplant. 11 of 24 patients (45.8%) with persistent vegetative state showed posttherapeutic improvements in consciousness. Twenty-seven of 73 patients (37.0%) with a motor disorder began to show improvements in motor functions.

Although the rapid recovery of neurological function in 3 patients (6, 7, 9) as it demonstrates a promising treatment, it must be interpreted with caution. A certain degree of clinical recovery occurs in the majority of patients undergoing MSC cell transplantation in post-autonomous humans. However, it is emphasized that spontaneous resilience, without any other treatment, cannot be ruled out in these patients.

Our present study reported attenuated plasma level of inflammatory markers IL-6, TNF-α, CRP, and leukocyte from patients with MSC transplantation, suggesting systemic inflammatory response had been reduced following TBI. Intensively decreased production of immune cells and elevated expression of factors IL-6, TNF-α, CRP indicated an intense induction between MSC and immune responses originated from after TBI. MSCs enhances the activity of lymphocytes by increasing the function of interferon production in the body.

Inflammatory response following TBI often lead to multiple organ/tissue dysfunction, damage, or even death [1]. he entry of these inflammatory mediators to lesional site is characteristic of inflammatory

response [1], [2], [6]. Intensively increase, prolonged, excessive or inappropriate inflammation of local inflammatory mediators including cytokines eventually lead to complications of systemic hyperinflammation, followed by immunosuppression, multi-organ dysfunction syndrome and even death, days to weeks after trauma [1], [2], [5], [6], [13], [14].

In our current study, increased plasma levels of IL-6, IL-10, TNF-α and CRP were detected as early as 6 hours after TBI, their increasing levels remained until the end of our observation (2m) in TBI subjects, whereas MSC transplant patients showed a decreased level of the aforementioned factors was proportional to the increased level of IL-10 after transplant transplant. Previous studies also reported high levels of inflammatory markers in the blood after brain injury, including CRP, TNF- α, IL-1, IL-6, and furthers [2], [6], [15]. All of them are the dominant cytokines and protein in inflammation, produces the acute phase response and is involved in regulating the levels of other cytokines, can be created in lesional site of the brain and affect other organ/tissue functions [15], [16]. Leukocytes invasion has a significant impact on pathological processes of brain injury, which includes releasing inflammatory cytokines such as IL-1, IL-6, TNF-α [17]. This increase in oxidative activity may lead to systemic damage and exacerbate secondary local damage at the original TBI site [18]. In contrast, IL-10 acts as a counterbalancing factor, helps reduce systemic injury and is involved in regulating levels of other cytokines [19].

Our current study, emphasizes that MSC can reduce IL-6, TNF-α and CRP expression levels by its ability to regulate the activity of IL-10. In the control group there was a sharp increase in inflammatory factors and the long-term presence of leukocytes, while the MSC transplant group showed a steady decrease between inflammatory factors and immune cells. Our results can be explained by the study of Aggarwal *et al.* that MSC-derived PGE2 and related factors to up-regulate the anti-inflammatory cytokine interleukin IL-10 from immune cells and improved cell survival while reducing the secretion of pro-inflammatory tumor necrosis factor alpha (TNFα) and IL-12 [19]. This leads to shift in the ratio of T helper (Th) cells from a pro-inflammatory Th1 to an anti-inflammatory Th2 and increase an immunoregulatory regulatory T cell (T reg) phenotype [19], [20]. In addition to the actions of PGE2, MSC-derived TSG6 has also been demonstrated to have potent effects on macrophages [5]. When macrophages are challenged with inflammatory agents, they secrete inflammatory factors such as TNFα, IL1β, IL6, CRP and reactive oxygen species via the TLR2–nuclear factor kappa-B (NFkB). Activation of MSC increases the expression of TSG6 and participates a negative feedback loop by inhibiting NFkB via activation of the CD44 receptor [21], [22].

In conclusion, 15 severe TBI patients were successfully treated with intravenous of MSC in the present study. The transplantation of autologous MSC is feasible and appears to be safe for the treatment of TBI. The results indicated that all patients tolerated the procedure without major complications, Motor function and consciousness of patients have significantly recovered. Patients can communicate, laugh and talk to the doctor after 3 weeks of treatment, this presenting a favorable outlook

after the patients are discharged. Moreover, a decrease in the concentration of inflammatory factors in the serum was detected after MSC transplantation. Reducing the excretion of inflammatory factors of the immune system as well as limiting their accumulation at the site of injury, opening up a new research direction in the process of rehabilitation and limiting prolonged complications, improving improve the patient's later life.

V. ACKNOWLEDGMENTS:

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IMPACTS OF MOUTHWASH ON PREGNANT WOMEN AND PREGNANCY OUTCOMES: A SYSTEMATIC REVIEW

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ABSTRACT

Introduction: Periodontal disorders such as periodontitis, gingivitis, gum recession, have a greater or lesser effect on pregnant women's reproductive health. Pregnant women are using mouthwash to reduce the impact of oral disease. However, the result of mouthwash on pregnant women's reproductive outcomes is controversial. Therefore, to synthesize and offer a view of this issue, we proceed to write this review. **Methods:** We searched for baseline data on the following data platforms: PubMed, Google Scholar from 2009 to 2019. Only studies written entirely in English are selected. Two independent authors carried out an assessment of selected papers to provide the degree of consistency possible for papers to be included in the evaluation process. The case studies chosen have been tested rigorously. OR, RR rates were obtained from selected studies. Two authors who selected appropriate studies also extracted data and assessed the risk of bias. **Results:** Out of five articles found, two studies with a total of 692 participants met the selection criteria. Studies have focused on the effects of mouthwash on pregnant women's reproductive health. one study concluded a decrease in preterm birth incidence that less than 37 weeks, one showed a reduction in the rate of premature rupture of the membranes. Both of these studies agree there is an improvement in the periodontal health of pregnant women during pregnancy. **Conclusion:** The use of mouthwash could boost pregnant women 's fertility outcomes. However, to assess this relationship more precisely, experiments with more accurate randomized clinical trials require greater sample sizes with adequate follow-up time.

Keywords: *Mouthwash, pregnant women, pregnancy outcomes*

I. INTRODUCTION

Although there are several researches which disagree with the association between periodontal diseases (PD) and adverse pregnancy outcomes (APOs), most of the recent researches within 5 years confirm the original postulated belief that PD increases the risk of APOs.[1-4] Either the periodontal pathogens or the elevated levels of inflammatory mediators may pass through the placenta. They, then, cause tissue damage and trigure foetal inflammatory response, leading to miscarriage or premature birth.[5] Therefore, the most suitable methods of treating PD in expectant mothers are in demand.

The non-surgical treatment of periodontal diseases requires combination of various methods, which are named as follows: 1 - brushing and flossing, 2 - sub and supra-gingival irrigation, 3 - antibiotherapeutics, 4 - scaling and root planning, 5 - full mouth disinfection. [6] However, they raise concerns over cost-effectiveness. [7] In the light of this issue, it has come to our notice that the process of full mouth disinfection involves multiple times of mouth rinsing.

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Adding CPC mouthwash to the daily oral hygiene has shown greater efficiency in containing the microbial reservoir than the routine brushing and flossing. Moreover, mouthwash is economical, widely available and safe for expecting females. [8]

Nevertheless, whether it can improve periodontal health and pregnancy outcomes remains in questions as there has been no systematic review on the matter. The scientific knowledge on this subject, hence, is extremely necessary because of the development of oral health care products in the world. They can have undesirable effects on the health of consumers, especially pregnancy woman . We do this research with some of the objects: Gestational ages, some of index development of the infant that have been mentioned in two articles H Jiang et al. 2016 [9] and Marjorie Jeffcoat et al. 2011 [10].

The maximum severity of gingivitis was also seen during the third trimester of pregnancy.[15]

Pregnancy-related changes in the oral environment may have some untoward temporary or permanent effects on oral health. Most of these effects could be avoided by practising good oral hygiene. [16]

An increase in attachment loss may represent active periodontal infection accelerated by pregnancy [17].

II. METHODS

The requirements for Eligibility:

Only studies having all these requirements would be suitable for our review.

1.Clinical trials

2.Subject: pregnant woman

3.Used mouthwash no alcohol, contain CPCs

4.Language: English

5.Publish from 2009 to now

Criteria for exclusion: review study, case series, case report, case-control study, letters to the editor.

Strategy for Research

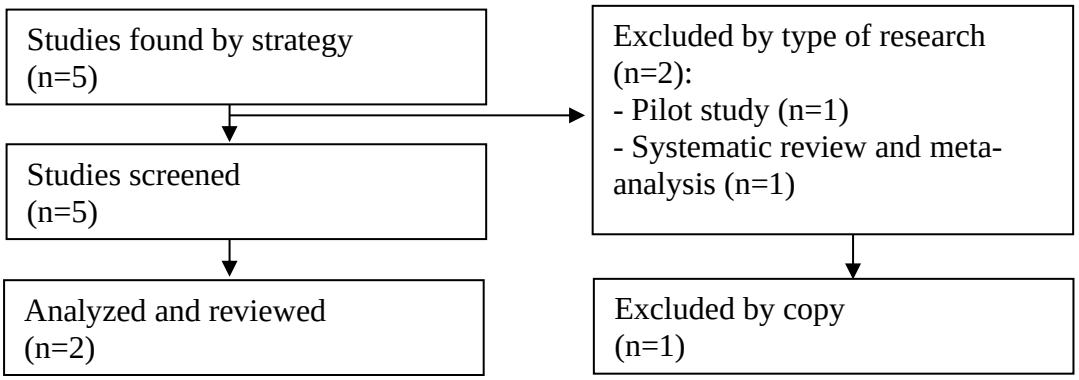
The authors searched on Pubmed library (http://www.ncbi.nlm.nih.gov/pubmed) independently. The time limitation was 10 years. Searching was done until 5/1/2020.

Strategy: (Mouthwash[MeSH Terms]) OR Mouth rinse[MeSH Terms]) OR Disinfection solution[Title]) AND (Pregnency outcomes[Title]) OR Preterm birth[MeSH Terms]) OR low birth weight[MeSH Terms])

All papers were read indepently by two authors, and all convergings between them were solved by the third author.

Study collection

We found a total of five studies from Pubmed library using our strategy. Three of them were excluded because of the type of research and copy. Finally, two studies were analyzed.



The data were extracted from studies are: birthweight, gestational week, rate of preterm birth (PTB)

III. RESULTS

We compare 2 studies in table 1, one study is randomized control trial research, and another is single-blinded control trial research.

This table describes the information of 2 studies. All pregnant women who attended the research were under 20 gestational week. Both studies show a better result in pregnancy outcomes.

Table 1. Describle some of main findings of the researchs

Name	Author/ publication year	Country	Sample size and control	Definition of PTB	Conclusions
Use of antiseptic mouth rinse during pregnancy and pregnancy outcomes: a randomized controlled clinical trial in rural China	2016 H Jiang,a,b,*,† X Xiong,c,* Y Su,d J Peng,e X Zhu,e J Wang,e M Chen,a,b X Qiana,f	China	Intervention: 232 Control: 234	PTB < 37 weeks	Improving in periodontal health and decreasing premature membrane rupture rate
Use of alcohol-free antimicrobial mouth rinse is associated with decreased incidence of preterm birth in a high-risk population	2011 Marjorie Jeffcoat, DMD; Samuel Parry, MD; Robert W. Gerlach, DDS; Matthew J. Doyle, PhD	USA	Intervention:71 Control: 155	PTB < 35 weeks	Decreasing the PTB rate

Table 2. Comparison between 2 studies

	Study	Intervention group	Control group	
Birthweight (g) (mean +_ SD)	(1)	3265.85 +_ 437.89	3300.05 +_ 442.55	
	(2)	3100.96 +_ 112.89	2625.30 +_ 62.75	
Gestational week (mean +_ SD)	(1)	39.54 +_ 1.52	39.54 +_ 1.28	
	(2)	38.4 +_ 0.52	36.8 +_ 0.29	
Preterm birth (%)	(1)	3.7%	2.4%	OR=1.59 (0.51–4.92) P= 0.43
	(2)	5.60%	21.90%	RR=0.26(0.096-0.7)

(1) H Jiang et al [9]

(2) Marjorie Jeffcoat et al [10]

All other variables have P<0.01

Table 2 compares 2 researches in terms of pregnancy outcomes. There are 3 factors were appraised in both research: birthweight, gestational week and preterm birth.

The statuses of infants were assessed in Birthweight while Pregnant women were evaluated by gestational week and preterm birth.

Birthweight:

The Birthweight increase significantly in the study (2) (approximately 500g), while Study 1 shows us no change.

Gestational weeks:

There is a difference between the 2 studies. The study (2) showed a significant difference in gestational weeks and birthweight while the other shows nothing change. In the study (1), there was no statistical difference between the test and control group. In the study (2), they recognized the significant difference between the intervention and control group. The mean gestational weeks in intervention group is 38,4 ±0,52, higher than control group (36,8 ±0,29) with P< 0,0001b.

Preterm Birth:

There are differences in the result of 2 studies. In the study (2), the rating of preterm birth in the Intervention group was dramatically lower than the control group. (5.6% versus 21.6%). In the study (1), preterm birth rates in the Intervention group were higher (3,7% versus 2,4%). However, it shows low reliability because its OR was 1.59 (0.51–4.92), and P was 0.43.

IV. DISCUSSION

There is an agreement in published researches and reviews about the efficacy of mouthwash in periodontitis. The measurements showed the periodontal improvement index after intervention with ingredients containing in commercial mouthwash.

CPC is a popular ingredient in non-alcohol mouthwash. CPC showed a positive effect on both antibacterial and reducing severe periodontitis. In comparison between CPC and NaF, solutions contain both CPC and NaF are superior to NaF only in anti-plaque and gingivitis efficacy. CPC was assessed in impact on the mechanical procedure, provided conclusive anti-plaque and anti-gingivitis results as a systematic review.

Researches showed a bacteriostatic and bactericide effect of chlorhexidine in different concentrations ranging. For mouthwash, recommend concentrations is from 0,12% to 0,2%. CHX is a principle for supragingival plaque. Study results show the improvement in the periodontal index, such as bleeding index, probing depth, clinical attachment level in both single using or adjunct. In a trial, CPC and CHX efficacy in reduce levels of bacteria during scaling were shown equally.

The researcher evaluated the impact of mouthwash on periodontal diseases in adjunction with mechanical therapy. In a meta-analysis, they found the evidence of better results of CHX adjunct compared to treated only, especially in Probing depth and CAL. Some studies research on combination mouth rinse. A clinical trial experiment on three solutions: CPC and Zinc lactate as a test group, CPC, and NaF as a positive control group, non-CPC as a negative control group have the same conclusion. After 6 weeks, the test group shown a higher reduction than the positive and negative control group.

They found some anti-bacteria chemicals contained in herbal and used them in mouthwash. In 2016, A.R. Pradeep et al. [11] investigated and compared the impact of Triphala mouthwash. Triphala is considered traditional medicine in India. In conclusion, the authors consider Triphala mouthwash as decreasing inflammatory and improving gingivitis.

In 2017, Karina Basso Santiago et al. [12] researched on Propolis mouthwash- agents from bee products in vivo and in vitro. They established in four groups: Control group, CHX or Propolis only group, and combination of CHX and Propolis group. Consequently, propolis is conclusive as a potential therapy to induce plaque and gingivitis.

Researches proceeded in other herbal-agents such as tea-oil, bio-oil, peppermint-oil … showed the same result. Thus, there are suggestions in researching in mouthwash - preventive and treatment periodontitis therapy in pregnant women, since anxiety for the negative effect of anti-bacteria chemicals like CHX, CPC, H2O2 in gestation.

We found that both studies agree that mouthwash can improve periodontal status while only Study [10] conclude that PTB can be prevented. We supposed that this result confirmed the consensus about the relation between PD and APOs. PD is caused by local factors, but it was proved to have an effect on system health through 3 mechanisms: [13, 14] bacteria, systemic inflammation, an autoimmune reaction. These 3 mechanisms occur concurrently, leading to APOs.

Strength and Limitation:

- Strength: both are controlled clinical trials, which indicates a high level of evidence-based research. One is one-blinded (the periodontist who examined the patients was blinded to who gets which treatment to remain objective and unbiased. They also took possible impact factors of PD and APOs into consideration.
- Limits: The research [9] was claimed to randomly engage participants into 2 groups, but there are significant differences in the periodontal score and the percent of severe PD at baseline, which may lead to a risk of bias. Moreover, there are a lack of data on the interaction between Mouthwash and pregnancy outcomes

V. CONCLUSION

Periodontal disease affects really bad to patient's health, especially in pregnant women, and mouthwash is clearly improving periodontal health in the pregnant woman. However, mouthwash doesn't have a clear effect on the birth weight of infants and PTB rates. This study demonstrates that pregnant women should use mouthwash free alcohol, contain CPCs to prevent PD and APOs. These assumptions need to be viewed with caution. However, it is unclear whether these benefits of mouthwash effect on pregnancy outcomes.

This research proves to be a theory and does not offer clinical guidance; despite the established advantages of mouthwash, further studies evaluating the association between the use of mouthwash and the outcomes of pregnancy and this process should be carried out.

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EFFICACY OF AFATINIB, AN IRREVERSIBLE EGFR TKIs IN PATIENTS WITH ADVANCED LUNG SQUAMOUS CELL CARCINOMA

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ABSTRACT

In 2013, the ERBB family blocker, Afatinib, is approved by FDA for second line therapy of squamous cell carcinoma after failure of platinum–based chemotherapy. In Vietnam, Afatinib has been indicated for the treatment of patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) including squamous cell carcinoma with prior chemotherapy since 2019. In this article, we will describe two clinical cases of metastatic squamous cell lung cancer treated with Afatinib after failing a previous platinum or immunotherapy regimen. Both cases demonstrated improvement in both clinical and imaging assessment.

RÉSUMÉ

En 2013, l'inhibiteur de la famille ERBB, l'afatinib, a été approuvé par la FDA pour le traitement du carcinoma épidermoide en deuxieme ligne, après échec de chimiothérapie qui contiennent du platine, sur la base des résultats d'une etude de phase 3 LUX-Lung 8. Dans cette étude, il a été rapporté que l'afatinib était supérieur à l'erlotinib en termes de survie sans progression (PFS moyenne 2,4 contre 1,9 mois; (HR) 0,82; p = 0,043) et la survie globale (SG moyenne 7,9 vs 6,8 mois; HR 0,81; p = 0,008).

Au Vietnam, l'afatinib a été indiqué pour le traitement des patients atteints d'un cancer du poumon non à petites cellules (CPNPC), l'histopathologie du cancer du poumon épidermoide, progressif ou métastatique, est en train de recevoir ou a recu une chimiothérapie qui contiennent du platine. Dans cet article, nous décrirons deux cas de cancer du poumon épidermoide métastatique traités par l'afatinib, après échec de chimiothérapie qui contiennent du platine ou immunologique. Les deux cas ont des résultats positifs, s'améliorant des deux côtés: imagerie clinique et diagnostique.

I. BACKGROUND

Squamous-cell carcinoma (SCC) of the lung is the second most prevalent histologic type of non-small-cell lung cancer (NSCLC) after lung adenocarcinoma[1]. The majority of which is frequently diagnosed in advanced, metastatic, or recurrent stage with very limited treatment options. Cytotoxic chemotherapy, most commonly a platinum – based doublet regimen is often used for first-line treatment and Erlotinib with Docetaxel were the second – line of choices as the only approved options[2]. On the other hand, with the rapid development of new treatments of advanced squamous cell carcinoma of the lung, several emerging agents have been considered for patients with SCC lung cancer, including the immune checkpoint inhibitor such as Pembrolizumab[3], Atezolizumab[4] and Nivolumab[5] or the EGFR

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monoclonal antibody necitumumab[6]. Regarding the ERBB family blockers, Afatinib is gaining attention and has already been approved in second-line treatment for squamous cell carcinoma[7].

Afatinib, an irreversible Tyrosine kinase inhibitor, binds to and reversibly inhibits signaling from all homodimers and heterodimers of the ERBB family members including EGFR (ErB1), HER 2 (ErbB2)-, and ErbB4, acting through connected intracellular pathways to control cellular proliferation and survival of cancer cells[8].

There is strong molecular evidences that EGFR and the other ErbB family of receptors such as Her2, ErbB3 and ErbB4 are associated with the pathogenesis of squamous cell carcinoma of the lung[9]. Although EGFR mutations are rare, about 60-80% of lung squamous cell carcinoma patients have overexpression or gene amplification of EGFR[10] and approximately 10% of tumors have EGFR number alterations. Moreover, other members of the ERBB family, including HER2 and HER3 are also often amplified in 20% and 30% cases respectively[11]. Therefore, with the above information, it is hypothesized that Afatinib will be effective in the squamous cell carcinoma subgroup.

In this article, we will report 2 clinical cases of advanced stage squamous cell carcinoma treated with Afatinib as second and third line therapy after failing with platinum-based chemotherapy.

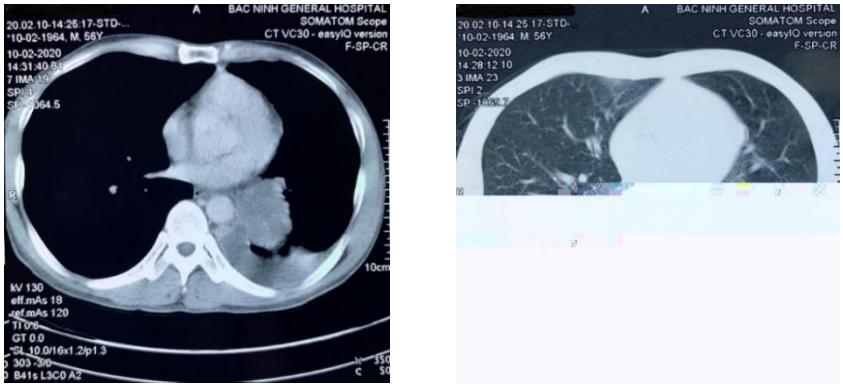
II. CASE PRESENTATIONS:

Case 1:

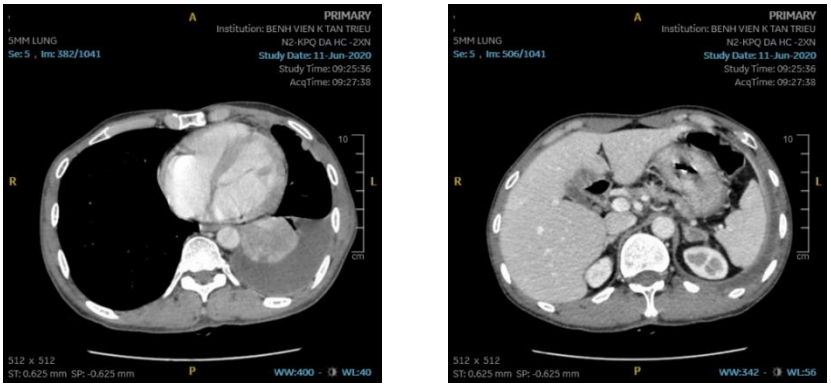
A 56-year-old male with long history of smoking and no comorbidity diseases came to our hospital after noticing a inguinal lymph node for about 3 months. He had no cough, no shortness of breath, and no chest pain. Upon admission, the patient had an inguinal lymph node biopsy performed with a result of metastatic squamous cell carcinoma. Chest CT showed a left lower lobe pulmonary tumor with a diameter of 4.3 x 4.9 cm with some mediastinal lymph nodes in the same side and left axillary lymph nodes. Abdominal CT was performed which demonstrated an enlarged left adrenal gland 4.8 x 3.8 cm in size. Brain MRI, bone scan, liver and kidney function were within normal limits. The patient was then diagnosed with left bronchial cancer cT2N3M1. Paclitaxel-Carboplatin chemotherapy regimen had been used to initially treat this patient. Unfortunately, after only one cycle, the disease has been confirmed to progress due to the appearance of new soft tissue lesions and enlarging inguinal area lesions. At that time, he also had pain as a result of progress.

We decided to switch to Afatinib as the second line for this patient. After 2 months, the patient condition was clinically improved. He no longer had pain, ECOG status index was 0, inguinal lymph node size significantly reduced. A CT scan of the chest showed a significant reduction in the size of the left lung tumor, achieving a partial response (image 3). Metastases of the left adrenal gland decreased in size to 1.7 x 2.7 cm (nearly 50% reduction compared to before treatment with Afatinib).

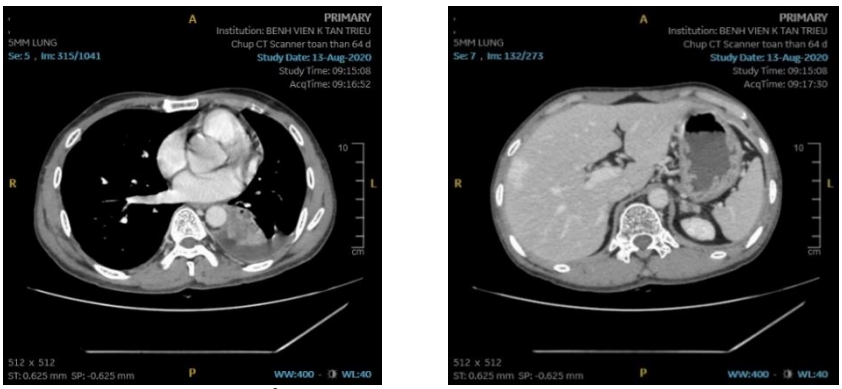
Currently, in the 4th month after treatment, the patient is healthy, in good condition, the inguinal lymph nodes continue to shrink. He has no cough, no chest pain and no dyspnea.



Before treatment with Afatinib



After 2 months of treatment with Afatinib



At current 4th month of treatment with Afatinib

Case 2:

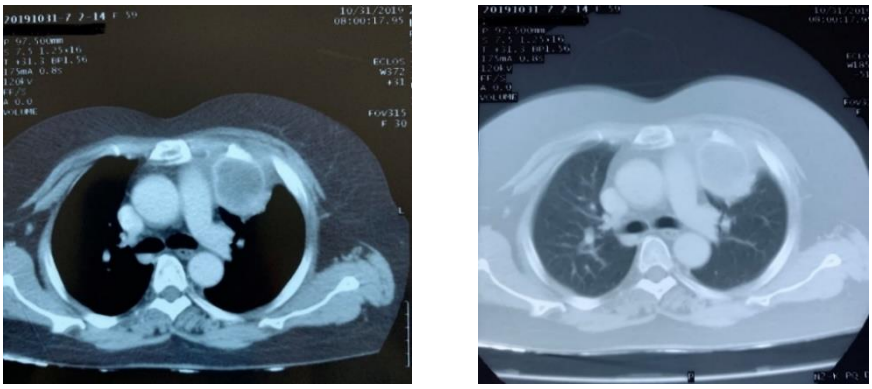
A 60-year-old female farmer started treatment in January 2018 with the diagnosis of left bronchial cancer T4N0M1 (metastases in bone, right adrenal gland) with histopathological result of squamous cell carcinoma. The patient has been treated with multiple lines of chemotherapy. Firstly, Gemcitabine - Cisplatin had been given for 6 cycles, which gave a partial response. We then had switched to Gemcitabine maintenance for 5 cycles until the disease progressed. Pembrolizumab was selected as subsequent systemic therapy after a 15% PD-

L1 positive result and a progression free survival of 8 months with 10 cycles with Pembrolizumab had been achieved.

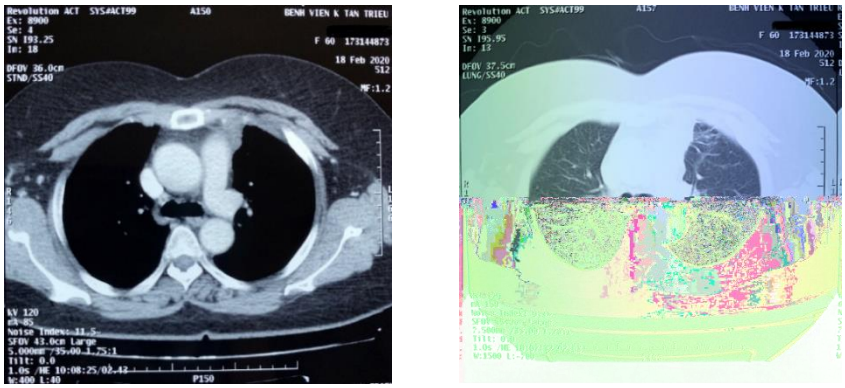
After that, the patient's left chest pain reappeared and increased, the ECOG index increased from 0 to 2. Chest CT showed a right lung tumor of 5.2 x 4.8 cm in size and mediastinal lymph nodes (station 5) of 1.7 cm in diameter. Abdominal CT showed right adrenal gland metastasis. With the results of the LUX LUNG 8 trial, we decided to choose Afatinib as the next therapy. The patient started taking Afatinib in November 2019 at an initial dose of 40 mg once daily. After 2 months and 4 months of treatment, the patient was clinically improved with the ECOG index decreased from 2 to 0, the patient had much better chest pressure, no cough, no shortness of breath. Chest CT and abdominal CT showed a partial response compared to before treatment (image 1 and 2).

However, after 8 months of treatment, the disease started progress, in which the lung tumor increased in size on chest CT. However, this progression was <30% (Figure 3) according to the RECIST 1.1 criteria and because the patient still (has) had clinical benefit, the physician decided to extend the duration of treatment with Afatinib for another 2 months before switching to the fourth line.

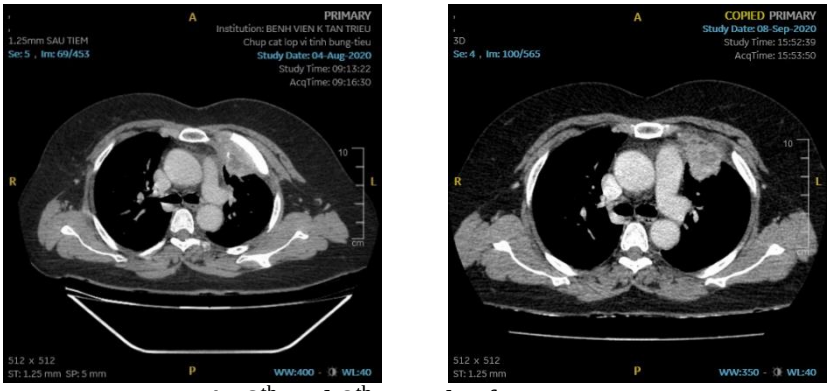
Thus, in this patient, a 8-month PFS was achieved, exceeding the doctor's expectation for therapy. In both clinical cases above, we did not record any serious side effects during treatment. The second case only had grade I diarrhea, which was well controlled by medication and did not delayed treatment.



Before treatment with Afatinib



After 4 months of treatment with Afatinib



At 8th and 9th month of treatment

III. DISCUSSION

Squamous cell carcinoma of the lung (SCC) is one of the most common type of lung cancers in Vietnam, accounting for about 20 to 30% of all cases. This type of cancer is also known as a difficult to treat disease with very few chemotherapy indications, especially after failure with Platinum-containing regimens. Recently with the development of targeted and immune therapies, many new factors have been approved for patients with squamous cell carcinoma in second line therapy, in which, afatinib, an ERBB family blocker has also been noted.

Afatinib was approved by the FDA in the treatment of second stage squamous cell carcinoma in 2013 based on the results of the LUX-LUNG 8 randomized phase 3 clinical trial, comparing the efficacy of Afatinib and Erlotinib after progression on chemotherapy. In 2019, Afatinib has been approved by the Vietnam Ministry of Health to treat patients with locally advanced and metastatic squamous cell carcinoma NSCLC who is currently or have prior chemotherapy with platinum derivatives. Both clinical cases described above were diagnosed with stage 4 squamous cell carcinoma with multiple metastases. Both failed first line chemotherapy with Platinum-containing regimens (Gemcitabine + Cisplatin, Paclitaxel + Carboplatin), followed by failure with immunotherapy. It must be said that, after the first and second line treatment, the patients were very tired with ECOG 2 and rapid progression on clinical and on radiologic assessment. At this time, switching to another chemotherapy regimen was no longer an option for clinicians to consider, but oral medications might be a good choice in these situations.

Afatinib was used in 2 cases with a starting dose of 40 mg, one tablet daily. In clinical case 1, although the duration of treatment was not long, but we initially achieved spectacular results with rapid clinical improvement with ECOG status index 0 only about 1 month after treatment. After 2 months of taking Afatinib, the patient was reassessed and showed a partial response in the lung and inguinal lymph nodes with a response rate up to 80%. And until now, the patient is still receiving treatment, giving a PFS of at least 6 months.

For clinical case 2, the patient was previously treated with multiple immunologic and chemotherapy regimens. Afatinib actually helped the patient achieve a response and stability for a long time with PFS of 8 months. The PFSs of both were generally superior to the results reported in

the LUX LUNG 8 trial in which afatinib was reported to be superior to erlotinib in terms of progression free survival [PFS; averagely 2.4 compared to 1.9 months; with HR 0.82; P = 0.043]. During the course of treatment, only patient No. 2 suffered from a side effect of grade I diarrhea, which was well controlled by Imodium x 2 tablets / day, without delaying the treatment.

In addition to its therapeutic effect and little adverse events, Afatinib could also help patients improve their quality of life due to its usability. Patients only need to take the pill daily without any infusion intervention.

IV. CONCLUSION

Squamous cell carcinoma is one of the common types of lung cancer and is often diagnosed at a later stage. There are still limited treatment options available for this group of patients, especially in second line. Both cases described had progression free survival of more than 4 months until now. Therefore, Afatinib has certainly been shown to be a good option for patients with squamous cell carcinoma after a failure with Platinum – based chemotherapy due to its effectiveness, convenience, and safety in clinical practice.

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