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Contents

Original Article

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Antibody Immune Response Compared between Recombinant Viral Vector and mRNA Vaccines among Health Care Personnel Who Had Previously Received 2 Doses of Inactivated COVID-19 Vaccine

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Abstract:

Background: Currently, a booster dose of AstraZeneca or Pfizer vaccine was introduced to healthcare personnel who had previously received two doses of inactivated vaccine.

Objective: To compare immune response and rate of adverse reactions after the third dose of AstraZeneca or Pfizer vaccines among healthcare personnel who had already received two doses of inactivated vaccine. We also determined immune response in those who received 2 doses of AstraZeneca vaccine.

Methods: We conducted an observational study at the Mae Fah Luang University Medical Center Hospital. We recruited healthcare personnel who had received AstraZeneca or Pfizer vaccine after 2 doses of Sinovac or received 2 doses of AstraZeneca vaccine. Those who were immunocompromised or had a history of COVID-19 infection were excluded. Participants were divided into 3 groups: (1) Sinovac-Sinovac-AstraZeneca, (2) Sinovac-Sinovac-Pfizer and (3) AstraZeneca-AstraZeneca. Immunoassay for anti-SARS-CoV-2 Spike protein (S) were performed at 30-60 days after the last vaccination dose. Adverse reactions after the third dose of vaccination were collected by using a questionnaire. We performed Kruskal Wallis test to compare antibody titer levels among the 3 groups. Comparisons of adverse reactions between group 1 and group 2 were analyzed using Chi-square or Fisher Exact test for categorical data as appropriate.

Results: There were 50, 111 and 18 participants of group 1, 2 and 3, respectively. All exhibited high titer levels of anti-SARS-CoV-2-S. The average antibody titer levels were highest in group 2 ($p < 0.001$), following by group 1 and group 3, respectively. Participants of group 1 reported fever and headache more frequently than those of group 2 ($p < 0.001$).

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Conclusion: Current vaccination regimens, booster AstraZeneca or Pfizer vaccine after two doses of inactivated vaccine and 2 doses of AstraZeneca are efficacious in producing immunity against SARS-CoV-2.

Keywords: COVID-19 vaccine, AstraZeneca, Pfizer, anti-SARS-CoV-2-S

Introduction

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been declared an ongoing global pandemic since March 2020. In Thailand, the first case of COVID-19 was reported in January 2020. Since March 2020, the number of cases has continuously increased despite many control measures introduced by the government.[1] To date, the most effective way to control the COVID-19 pandemic is to administer vaccinations to most of the population.

In Thailand, the initial COVID-19 vaccine, CoronaVac (Sinovac) and ChAdOx1 nCoV-19 (AstraZeneca) were provided, starting in February-March 2021. However, due to problems of vaccine distribution and mutation of the coronavirus, the outbreak of the Delta strain continued with a rapidly increasing number of new cases and deaths.

In July 2021, The Ministry of Public Health issued a new guideline for vaccination to improve the efficacy of COVID-19 prevention and control.[2] Healthcare personnel who had received two doses of inactivated vaccine would be provided with a third dose of AstraZeneca or BNT162b2 (Pfizer) vaccine, at least 4 weeks after the second dose. Those who had received 2 doses of AstraZeneca were not applicable because immunity remained high for at least 6 months.

The Mae Fah Luang University Medical Center Hospital has provided the third dose of vaccine, AstraZeneca or Pfizer, to its personnel, according to the government guideline since July 2021. However, the efficacy of the third dose has not been reported. Therefore, we conducted a study to determine immune response and adverse reactions after the third dose compared between AstraZeneca and Pfizer vaccines among healthcare personnel who had previously received two doses of inactivated vaccine. In addition, we also determined the level of immunity in personnel who received two doses of the AstraZeneca vaccine.

Method

We conducted an observational study in healthcare personnel at the Mae Fah Luang University Medical Center Hospital. We recruited individuals aged more than 18 years old who received a third dose of AstraZeneca or Pfizer after 2 doses of CoronaVac (Sinovac) or received 2 doses of AstraZeneca. Those who were immuno-compromised or had a history of COVID-19 infection were excluded. Participants were divided into 3 groups, based on the type of vaccination: (1) Sinovac-Sinovac-Astra-Zeneca, (2) Sinovac-Sinovac-Pfizer and (3) AstraZeneca-AstraZeneca.

Participants were asked to answer a questionnaire including age, gender, weight, height, underlying diseases, date of COVID-19 vaccinations and adverse reactions within 7 days after the last dose.

Blood tests for antigen-specific humoral immune response were performed at 30-60 days after the last dose of COVID-19 vaccine, using a commercial immunoassay, Elecsys Anti-SARS-CoV-2 S assay (Roche Diagnostics).

The Elecsys Anti-SARS-CoV-2 S assay is an immunoassay used to detect antibodies

(including IgG) to the SARS-CoV-2 spike (S) protein receptor-binding domain (RBD). Quantification of the antibody response can measure the specific antibody titer, ranging from 0.4 U/mL to 250 U/mL (up to 2500 U/mL for 10-fold dilution, and up to 12,500 U/mL for 50-fold dilution).

As per the manufacturer's instructions, antibody titer 0.8 U/mL or higher was considered positive for anti-SARS-CoV-2 S. A result of 15 U/mL or higher had optimized correlation to surrogate virus neutralization method. A result of 132 U/mL or higher was considered as high titer for anti-SARS-CoV-2 S.[3]

The research protocol was approved by The Mae Fah Luang University Ethics Committee on Human Research (EC234/2021).

Statistical analysis

We performed Kruskal Wallis test to compare antibody titer levels among the 3 groups. Comparisons between group 1 and group 2 were analyzed using the Mann-Whitney U test for continuous data and Chi-square or Fisher Exact test for categorical data as appropriate. Factors associated with antibody titer levels and rate of adverse reactions between group 1 and 2 were analyzed using linear regression and logistic regression analysis, respectively. Statistically significant difference was considered at a p value of less than 0.05.

Result

There were 180 healthcare personnel who participated in the study. One case was excluded due to a 28-day interval between the 3rd dose and the time of the blood test. Fifty, 111 and 18 personnel were recruited to groups 1, 2 and 3, respectively. There were no differences in gender and underlying diseases among the groups. Regarding underlying diseases at risk, cardiovascular, chronic pulmonary and cerebrovascular diseases were reported in 1, 2 and 1 personnel of group 1, 2 and 3, respectively. Obesity was noted in 5, 3 and 2 personnel of group 1, 2 and 3, respectively. Participants of group 2 were younger than those of other groups. Body mass index (BMI) of group 1 was higher than that of group 2. All participants of group 1 and 2 received Sinovac vaccine for the first and second doses with no difference in the interval between doses. However, participants of group 2 received the third dose of vaccine significantly later than those of group 1. The interval between the last dose of vaccination and the time of blood test for immune response of group 2 was significantly longer than that of the other groups (Table 1).

All participants exhibited high titer levels of anti-SARS-CoV-2 S. The average antibody titer levels were highest in group 2, following by group 1 and group 3, respectively (Figure 1).

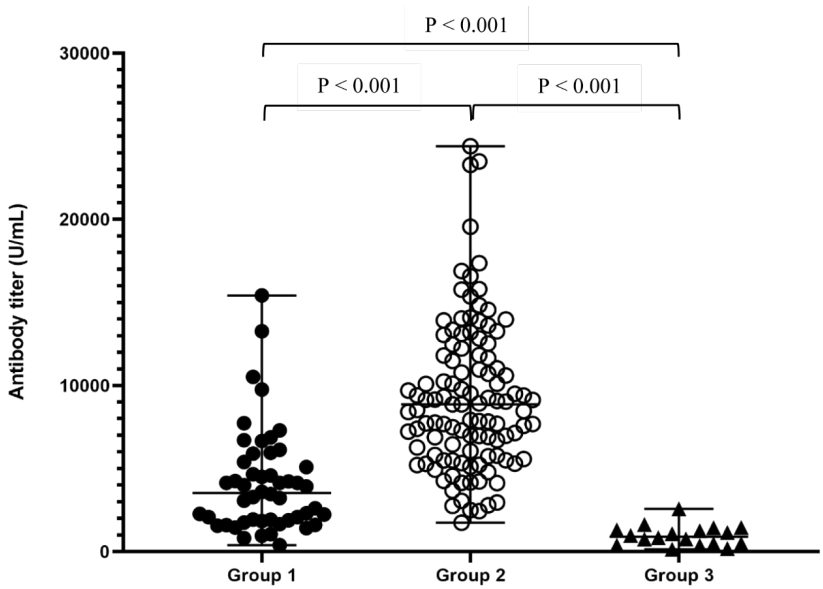
Besides the group, factors associated with antibody titer levels of group 1 and group 2 were the interval between the second and third doses of vaccination, the longer interval, and the higher antibody titer level (Table 2).

Overall adverse reactions reported after the 3rd dose of vaccination were not different between group 1 (78%) and group 2 (70.3%). However, participants of group 1 reported fever and headache more frequently than those of group 2 (Figure 2). Women reported symptoms nearly five-fold more frequently than men, related to adverse reactions after the 3rd dose of vaccination (Table 3).

Table 1 Demographic data of participants (N = 179)

Variables	Group 1	Group 2	Group 3	P value
n	50	111	18	
Male, n (%)	9 (18.0)	20 (18.0)	3 (16.7)	0.990
Age (year)	36.0 (21.0, 60.0)	31.0 (22.0, 57.0)	40.5 (21.0, 65.0)	0.001Ψ 0.436x 0.019δ
Body mass index (kg/m^2)	23.7 (17.9, 33.9)	21.6 (16.6, 33.2)	24.7 (18.7, 30.8)	0.026Ψ 0.739x 0.071δ
Underlying disease, n (%)	11 (22.0)	12 (10.8)	4 (22.2)	0.125
Interval (day) 1^{st} - 2^{nd} dose	21.0 (18.0, 26.0)	21.0 (21.0, 34.0)	84.0 (79.0, 102.0)	0.785Ψ
2^{nd} - 3^{rd} dose	63.0 (24.0, 103.0)	89.0 (13.0, 104)	NA	< 0.001Ψ
Last dose - blood test	53.0 (38.0, 56.0)	54.0 (52.0, 58.0)	52.0 (49.0, 58.0)	< 0.001Ψ 0.948x < 0.001δ

Ψ Comparison between group 1 and 2
x comparison between group 1 and 3
δ comparison between group 2 and 3



Median (U/mL)	3527.50	8859.00	903.00
Min (U/mL)	390.90	1740.00	134.10
Max (U/mL)	15425.00	24403.00	2574.00

Figure 1 Scatter plot of anti-SARS-CoV-2 S titer levels of 3 groups

Table 2 Factors associated to antibody titer compared between group 1 and group 2

Variables	Mean ± SD	Univariate		Multivariate	
		Standardized coefficient	P value	Standardized coefficient	P value
Group 2, n (%)	111 (68.9)	0.50 (3721.10, 6484.39)	<0.001	0.33 (1904.99, 4842.59)	<0.001
Male, n (%)	29 (18.0)	0.02 (-1685.22, 2157.92)	0.808	0.10 (-465.60, 2936.42)	0.153
Age (year),	33.39 ± 8.58	-0.24 (-214.13, -46.30)	0.003	-0.11 (-136.83, 11.27)	0.096
BMI (kg/m^2),	23.09 ± 3.88	-0.10 (-316.65, 63.30)	0.190	-0.002 (-169.95, 165.70)	0.980
Interval 2^{nd} to 3^{rd} dose (day)	75.29 ± 19.06	0.48 (83.56, 151.99)	<0.001	0.37 (57.18, 126.13)	<0.001

BMI: body mass index

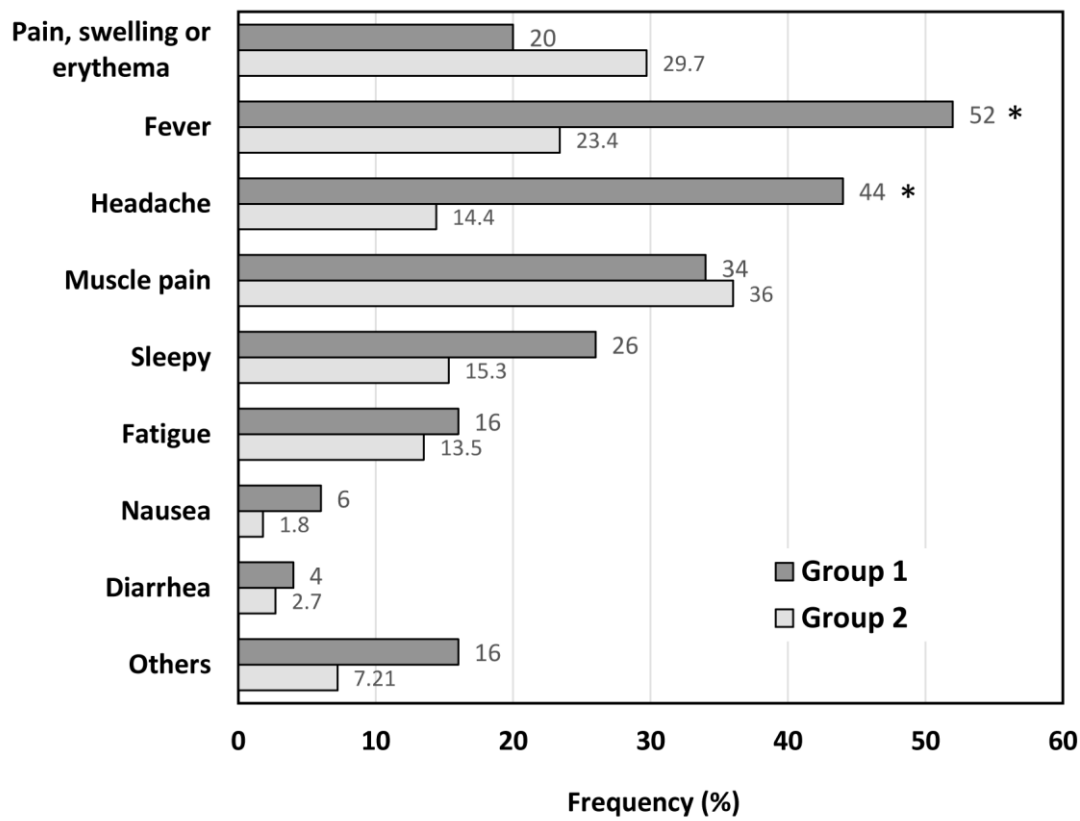


Figure 2 Comparison of adverse reaction rate (%) between group 1 and group 2
* $p < 0.001$

Table 3 Factors associated to adverse reaction after the 3rd dose of vaccination

Variables	N	Adverse reaction, n (%)	Odds ratio	95% CI	P value
Group 1	50	39 (78.0)	1.89	0.79, 4.50	0.153
Group 2	111	78 (70.3)	1		
Female	132	104 (78.8)	4.90	2.07, 11.62	< 0.001
Male	29	13 (44.8)	1		
Age (year), mean ± SD	33.39 ± 8.58	0.97	0.93, 1.01	0.155	

Discussion

To date, no study on the effect of a third boosting heterogenous vaccine after two doses of inactivated vaccine has been published. Corresponding to a previous study,[4] we demonstrated that boosting with either virus vectored (AstraZeneca) or mRNA (Pfizer) vaccine resulted in higher titer of anti-SARS-CoV-2 S in participants who had received two doses of inactivated vaccine. Participants who received Pfizer vaccine yielded higher antibody titers than those who received AstraZeneca vaccine as a booster dose. This is a similar finding with other studies on the immunogenicity of Pfizer vaccine as the prime or booster dose.[5,6] In addition to boosting the heterogenous vaccine, the study revealed that after receiving 2 doses of AstraZeneca, the antibody levels to SARS-CoV-2 were adequately high. The result supported the Thai guideline which stated that the third dose was not required for at least 6 months in persons receiving 2 doses of AstraZeneca vaccine.[7] Currently, there are no studies to determine what antibody titer level detected is necessary to prevent SARS-CoV-2 infection.

We revealed that the interval between the second and the third doses of vaccines was associated to antibody titer level. This finding was consistent with previous data showing that the longer the interval between the first and second doses of AstraZeneca vaccine, the higher the antibody titer.[7,8] Although the younger age group seemed to have higher antibody titers than those of older age, it was not significant in the adjusted model.

This study used immunoassay to detect antibody to SARS-CoV-2 spike protein. We did not perform a neutralizing antibody test which determines the functional ability of antibodies to prevent infection by SARS-CoV-2 in vitro. However, the assay showed good agreement with virus neutralization assays.[9]

Regarding adverse reactions after the third dose of vaccination, there was no difference between group 1 and group 2. However, fever and headache were reported more frequently in participants of group 1. A similar finding was reported after the first dose of AstraZeneca compared to Pfizer vaccine.[5]

Limitations of this study are the absences of virus neutralization assays, cellular immunity assays and antibody titer levels before the third boosting dose of vaccine. In addition, the adverse reactions reported might be inaccurate since the data was collected retrospectively 2 months after the last dose of vaccination.

Conclusion

Although antibody testing is not recommended following vaccination,[10] our study supports the efficacy of current vaccination regimens for healthcare personnel in Thailand. Boosting AstraZeneca or Pfizer vaccine after two doses of inactivated vaccine and 2 doses of AstraZeneca are efficacious in producing immunity against SARS-CoV-2.

Acknowledgement

We acknowledge Mae Fah Luang University for supporting the study by waiving the cost of the immunoassay tests.

Conflict of Interest

The authors declare no potential conflict of interest in this study.

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Special Article

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NK Cell-based Immunotherapy for Acute Myeloid Leukemia: An Exciting Future

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Abstract:

Given that allogeneic hematopoietic transplantation has been achieved in the treatment of acute myeloid leukemia (AML) over the past decade by providing graft-versus leukemia effect. However, age at diagnosis, donor availability, the treatment-related toxicities of allogeneic hematopoietic cell transplantation, relapse/refractory disease and minimal residual disease (MRD) prior to transplantation remain major problems. Novel approaches in cellular immunotherapy have contributed to substantial improvement in the treatment of hematologic malignancies, while adoptive NK cells therapy has also emerged as a promising treatment option, to improve survival in AML patients in the context of transplantation and non-transplantation. In this review, we summarize biology of NK cells and current different strategies of adoptive NK therapy for treating AML in clinical studies, also discuss about future directions of NK cell-based immunotherapy for the treatment of AML.

Keywords: NK-cell, Immunotherapy, Acute myeloid leukemia

Introduction

Over the past decade, breakthroughs in genetic and molecular research, which provide new insights into how acute myeloid leukemia (AML) develops and is regulated by complex molecular networks, have resulted in more effective treatments. These include chemotherapy, targeted therapy, and allogeneic hematopoietic cell transplantation, which provide both chemo-ablative and immuno-ablative effects. While these therapeutic approaches have the potential to cure AML, the 5-year survival rate using these treatments is still around 50% for patients aged up to 60 years, and 5%-15% for patients aged > 60 years. Although allogeneic hematopoietic cell transplantation is a standard treatment for patients with relapse, previous studies have indicated that relapse rates and non-relapse mortality remain high, with overall survival (OS) rates of 20% to 30%.[1-7] Minimal residual disease status (MRD) before allogeneic hematopoietic cell transplantation has a convincing impact on predicting relapse and survival after transplantation in all patient risk groups, including patients in complete remission (CR1) and CR2.[7] Apart from being a prognostic factor beforetransplantation, MRD status has been shown to provide additional prognostic clinical outcomes in AML after induction and consolidation treatments, and can be an efficient tool to

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establish risk-adapted treatment, such as allogeneic hematopoietic transplantation in patients with intermediate risk.[7-11]

Several novel approaches in cellular immunotherapy have revolutionized the treatment of hematologic malignancies, while adoptive ex-vivo expanded NK cell-therapy has also emerged as a promising treatment option, to improve survival rates among patients with AML who are not eligible for allogeneic hematopoietic cell transplantation, and to improve survival rates among patients who are MRD-positive before transplantation.[12-30] Due to our improved understanding of NK-cell biology and cell manipulation techniques, the application of NK-cell immunotherapy for treating malignancies, of both of hematologic and solid cancers, has progressed rapidly in the past few years; for example, ex-vivo adoptive transfer of NK cells with or without in-vivo cytokines, combinations with antibodies or drugs that enhance NK-cell cytotoxicity, or drugs that sensitize tumor cells to NK-cell lysis and chimeric antigen receptor NK cells.[17,20,26,31-41]

This review summarizes the biology, sources, and functions of NK cells in the AML tumor microenvironment. The historical clinical study of NK cell-based immunotherapy, and recent novel strategies to improve the efficacy of NK cell-based immunotherapy to treat acute myeloid leukemia in transplantation and non-transplantation settings, are also discussed

NK-cell biology and functions

NK cells are a subtype of innate lymphoid cells recognized by CD56+ve and CD3-ve. They are morphologically characterized as large granular lymphocytes, the granules of which contain both perforin and granzyme B, responsible for NK cell-mediated killing.[39,42,43] Human NK cells derive from common lymphoid progenitor cells in the bone marrow, as B cells and T cells, and develop in the secondary lymphoid organs as well as the liver and spleen. NK cells do not express antigen-specific antigen receptors; however, with the integration of signals delivered by activating and inhibitory receptors show as Figure 1 that bind with ligands on target cells, NK cells can kill malignantly transformed cells and virus-infected cells without prior antigen recognition directly. They do this by: 1) using perforin and granzyme B; 2) inducing apoptosis by tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) pathway; and 3) indirectly by antibody-mediated cellular cytotoxicity (ADCC), where antibodies bind target cells to low affinity IgG Fc receptor III on NK cells (CD16), known as the most potent activating receptor.[20,35,37,39] In addition to their cytotoxic capacity, NK cells can enhance the function and increase the number of other immune cells in the tumor microenvironment, including dendritic cells, T-helper cells, by secreting multiple cytokines and growth factors, such as interferon-γ, IL-13, TNF, XCL1, CCL4, and CCL5.[42,44-47]

Since the inhibitory signals from diverse inhibitory receptors provide immunological self-tolerance that has negative-feedback against the stimulating signal from activating receptors and prevent the killing of normal self-cells, the most important inhibitory receptors are killer-cell immunoglobulin-like receptors (KIRs); their ligands are generally major histocompatibility (MHC) class I. During development, an essential process for NK-cell tolerance, termed licensing, is the engagement between inhibitory KIR or NKG2A expressed on the surface of NK cells and several MHC class I (HLA-A, HLA-B, HLA-C, HLA-E) expressed on the surface of healthy cells, resulting in the maturation and function competency of NK cells[18,39,48-50,51,52-54] as show in Figure 2.

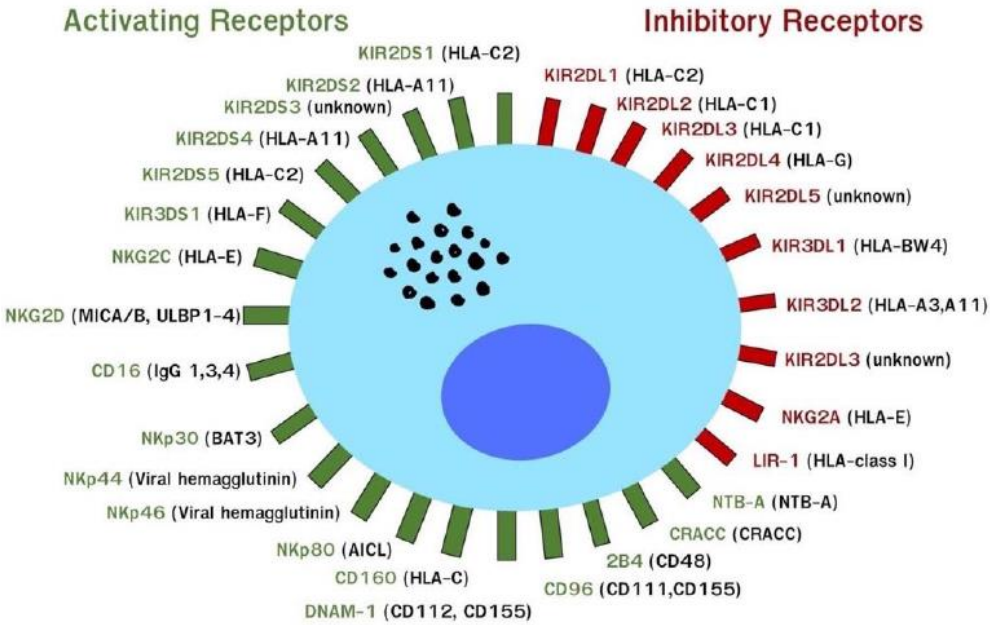

Figure 1 Summary of NK cell receptors and their ligands[14,16,20,42,49]

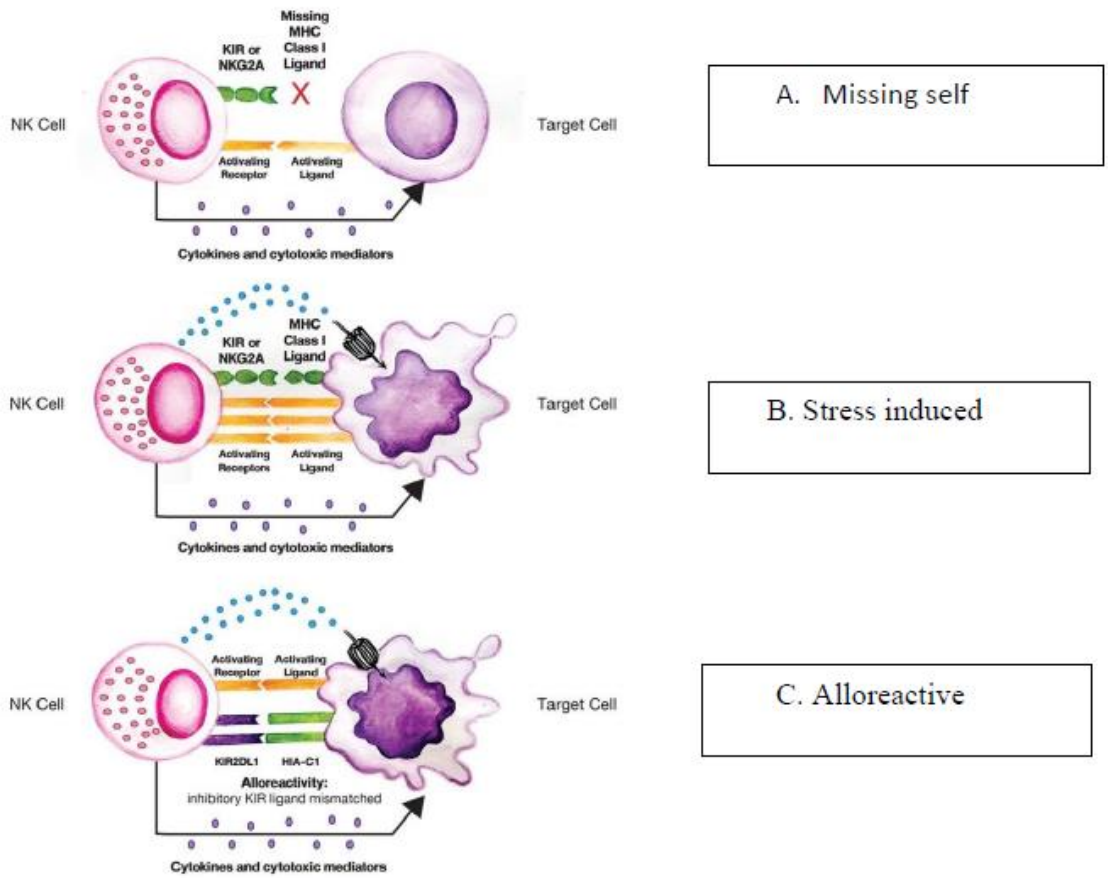

Figure 2 NK cells respond to transformed cell via (A) Transformed cells enhance target cells killing by decreased expression of MHC class I resulting in NK cells activation (B) Transformed cells upregulate ligand for NK cells activating receptor follow by enhancing the activating signal which promote cytokines release and cytotoxicity against the target cell (C) In the transplantation, NK cells alloreactivity can be promoted by selection of donor who will have inhibitory KIR ligand mismatched.[18,20,49,56,92]

Unlike T cells, NK cells in the allogeneic infusion do not result in graft-versus-host disease (GVHD). Importantly, several preclinical studies have noted that they may have the potential to protect GVHD by attacking host-antigen presenting cells.[51-54] Preclinical and clinical studies showed that NK cells play a key role in eliminating residual leukemia cells after haploidentical stem cell transplantation by KIR ligand mismatch, thus leading to further studies investigating the competence of adoptive immunotherapy with NK cells to eradicate leukemia.[48,55] While such a strategy can help improve survival and in-vivo proliferation of allogenic NK cells by using lymphodepleting treatment, many patients still experience serious side effects, such as pancytopenia and serious infection.[21,24] Moreover, the injection of IL-2 potentially causes a systemic side-effect from cytokine syndrome and the stimulation of regulatory T cells. [20,24,30]

Reduced NK-cell function in the AML tumor microenvironment

While preclinical studies showed that ex-vivo expanded NK cells can kill various types of cancer cells, only a small number of patients in clinical trials showed a response to adoptive NK-cell therapy because cancer cells can develop various mechanisms to escape NK-cell immune surveillance.[20,43,56]

In patients with AML, the function of NK cells in controlling AML-cell growth is impaired due to multiple immunosuppressive mechanisms in the AML tumor micro environment. Three major components are involved in functional defect of NK cells in the AML tumor microenvironment, resulting in the immune escape of AML cells.

First, aberrant epigenetic mutations of AML cells, resulting in the low expression or shedding of the natural killer group 2D (NKG2D) ligands that bind to NKG2D and natural cytotoxicity receptors (NCRs), which provide potent activating signals for NK cells. Moreover, the increased expression of inhibitory molecules, such as PD-L1and CD200, is also observed in AML cells. Coles et al. reported that increasing the expression of CD200 on AML cells may reduce NK-cell cytotoxic activity and promote AML-cell escape from NK-cell killing.[57-60]

Second, because the cytotoxicity function of NK cells is determined by the integration signals from activating and inhibitory receptors, the defective maturation of those receptors in AML patients results in NK-cell dysfunction. The increased expression of inhibitory receptors, such as KIR2DL2/DL3 and natural killer group2A (NKG2A), and the decreased expression of activating receptors, such as NKG2D, DNAX accessory molecule-1 (DNAM-1), natural cytotoxicity triggering 30 (NKp30), NKp46, natural cytotoxicity receptors (NCRs) can be found during AML development, leading to NK-cell dysfunction.61-64 Interestingly, the functional defect of NK cells persists, even in patients who achieve complete remission after chemotherapy. Moreover, upregulation of programmed cell death ligand-1 (PD-L1) and PD-L2 also observed in AML cells.[61-65]

In the AML tumor microenvironment, the complex interaction between the extracellular matrix, soluble factors, such as transforming growth factor (TGF)-B, IL-6, IL-10, prostaglandin E2, indolamine 2, 3-dioxygenase (IDO), and immunosuppressive cells, such as myeloid derived suppressor cells (MDSCs), regulatory T cells (Treg), and tumor-associated macrophages (TAMs) can suppress the function of NK cells, as well.[38,66-69]

Role of NK cells in allogeneic hematopoietic cell transplantation

NK cells are among the first immune cells to recover after the graft of stem cells into bone marrow, and play an important

role in preventing relapse by graft-versus-leukemia effect, to protect graft-versus-host disease by attacking recipient antigen-presenting cells and some infections, even where their functions are still impaired compared with the NK cells of healthy donors.[51,52,54,70-74] The beneficial effect of NK-cell alloreactivity in the context of allogeneic hematopoietic cell transplantation was first described in 2002. Ruggeri et al. found alloreactive NK-cell cytotoxicity against AML cells among AML patients who received donor inhibitory KIRs and host HLA ligand mismatches in bone-marrow graft haploidentical transplantation. This study demonstrated superior disease-free survival and a better 5-year survival rate (65%) for the inhibitory KIRs ligand mismatch group, compared with only 5% for the control group.[19] Since the family of 14 polymorphic KIR genes can be classified into 2 haplotypes, KIR A haplotypes comprises only one encoding activating KIR receptor gene (KIR2DS4) while KIR B haplotype comprises of 5 encoding activating KIR receptor genes (KIR2DS1, 2DS2, 2DS3, 2DS5, 3DS1) the effectiveness of alloreactive NK cells was also demonstrated in unrelated donor transplantation from donors with the KIR B haplotypes, which contain more activating KIRs gene.[55] Rapid NK-cell recovery after transplantation is also associated with better transplantation outcome.[18] Taken together, many investigators have explored the possibility of introducing the adoptive transfer of NK cells to treat acute myeloid leukemia patients in the clinical setting.

Role of adoptive NK-cell therapy in hematopoietic cell transplantation

Infusions of donor purified NK cells after haploidentical are feasible and well-tolerated. This treatment facilitates engraftment and provides graft-versus-leukemia alloreactivity without acute graft-versus-host disease.[74] Ciurea et al. conducted a phase-1 dose escalation study (1 x 10^5/kg to 1 x 10^8/kg) to determine the safety, feasibility, and maximum tolerated dose of membrane-bound interleukin 21 (mbIL21) expanded donor NK cells infused before and after haploidentical HSCT for high-risk myeloid malignancies. Thirteen patients aged 18-60 years were enrolled into the study. Ex-vivo expanded NK cells were infused on days -2, +7, and +28. No infusion-related reactions or dose-limiting toxicities were observed. All of the patients were engrafted with donor cells. Seven patients (54%) developed grade 1-2 acute GVHD, but no grade 3-4 acute GVHD was observed. Eleven of the 13 patients (85%) were still alive and in remission at last follow-up (median, 14.7 months). This trial demonstrated the efficacy and feasibility of infusing high doses of ex-vivo expanded NK cells by using feeder cells after haploidentical HSCT without serious adverse effects or increased grade 3-4 acute GVHD.[27] Another phase-I study aimed to investigate the effect of haploidentical donor NK-cell infusion early at day 6 and day 9 versus day 13 and day 20 after haploidentical transplantation in refractory acute myeloid leukemia. While the early infusion of adoptive NK cells was not associated with a reduction in the progression of leukemia, it was associated with increased toxicity from cytokine-release syndrome. In addition, a higher expression of NKp30 (> 90%) on donor NK cells was associated with higher complete remission.[75]

Interestingly, the adoptive transfer of haploidentical NK cells can be infused safely before hematopoietic-cell infusion as part of the conditioning regimen for transplantation. A phase I clinical study conducted by Lee et al. demonstrated that the infusion of IL-2 activated haploidentical donor NK cells at day 8 after busulfan, fludarabine and ATG, as part of the conditioning regimen for treating high-risk myeloid malignancies, was safe. Relapse-free survival was

associated with the number of infused NK cells.[76]

The adoptive transfer of haploidentical NK cells can also be used to clear residual leukemic cells in relapse/refractory AML patients who are eligible for transplantation. Bjorklund et al. recently published a phase I/II study aiming to evaluate the safety and efficacy of overnight IL-2 activated haploidentical NK-cell infusion in 16 patients with relapse refractory high-risk AML, MDS. The patients received a less-toxic lymphodepleting regimen with fludarabine at a dose of 25 mg/m^2 per day at days -7 to -4, cyclophosphamide at a dose of 25 mg/kg per day at days -3 and -2, and TLI (total lymphoid irradiation) at day -1 before infusion of haploidentical NK cells at a median infused dose of 6.7×10^6 cells/kg (range, 1.3–17.6×10^6 cells/kg). The NK-cell infusions were well-tolerated, and common adverse events were chills and nausea. Six of 16 patients achieved complete remission, and 5 proceeded to allogeneic hematopoietic cell transplantation. Five of 5 patients who achieved complete remission had detectable donor NK cells at day 7 and day 14 post-infusion.[26] In another clinical study, Vela et al. published a post-hoc analysis assessing the safety and efficacy of infusing expanded NK cells from haploidentical donors by using K562-mb15-41BBL as feeder cells in patients from two clinical trials. A total of 18 patients with relapsed or refractory ALL, AML, or bi-phenotypic acute leukemia were enrolled. Their mean age was 12 years (range 12.3 years). All patients received salvage chemotherapy prior to infusion of NK cells at a maximum dose of 1×10^8 /kg in a total of 2 cycles and injection of low-dose IL-2. All infusions were well-tolerated with no graft-versus-host disease or serious infusion-associated adverse events. Thirteen patients achieved complete remission, and 10 patients proceeded to allogeneic hematopoietic cell transplantation. Four patients were alive and leukemia-free > 750 days post-transplantation. These studies suggest that relapse/refractory AML and MDS cells were susceptible to adoptive NK-cell therapy and that the infusion of haploidentical NK cells may convert patients to being candidates for transplantation, so providing the opportunity for cure.[29] In addition, these findings may apply to developing a strategy to clear MRD before proceeding to transplantation, due to the negative impact of being MRD-positive on transplantation outcome.[11,77]

Clinical trials using adoptive NK-cell infusions

In light of the limitations: age at diagnosis, donor availability, and the treatment-related toxicities of allogeneic hematopoietic cell transplantation, the use of autologous NK-cell infusions was the first focus of adoptive NK-cell therapy to treat AML patients who are not candidates for allogeneic hematopoietic cell transplantation, due to the convenience of using the patient's peripheral blood and there being no requirement for lymphodepleting chemotherapy.[10] Wang et al. conducted a study to evaluate whether cytokine-induced killer (CIK) cells from patients with relapse or refractory AML can be expanded efficiently for clinical use, and to evaluate the efficacy of CIK in eradicating leukemic cells. Eleven patients with relapse/refractory AML were enrolled. The CD3+CD56+ cells in AML-derived CIK cells were expanded approximately 1,020-fold. The proportions of CD3+and CD3+CD56+ CIK cells from patients with AML were similar to those from healthy donors, and showed similarly high cytotoxicity against leukemic cell lines. Two patients had dramatically decreased blast cells in the peripheral blood by 2 weeks' post-infusion, then gradually increasing. No infusion-related adverse events were observed in all patients. This study demonstrated that the autologous CIK from the patients can be

expanded efficiently and comparable to the phenotypes of healthy donors.[28] However, subsequent clinical trials using this strategy in hematologic malignancy and solid cancers failed to achieve responses, even though infused autologous NK cells can expand in vivo.[78] The inhibitory effect of MHC class I expressed on cancer cells and the immunosuppressive status of patients due to their being heavily pretreated prior to cell collection resulted in poor NK-cell expansion and dysfunction. Thus, the investigators turned to developing clinical trials using allogeneic NK-cell therapy to treat hematologic malignancies.[56,57,79]

Given that KIR-HLA ligand mismatches had improved 5-year survival rates in haploidentical hematopoietic cell transplantation, NK cells form a haploidentical donor that may potentially have graft-versus-leukemia effect in non-hematopoietic cell transplantation, as well. Miller et al. tested haploidentical, related-donor NK-cell infusions in a non-transplantation setting to determine safety and in-vivo NK-cell expansion. Nineteen patients with poor AML prognoses were enrolled. All patients received lympho-depleting treatment with fludarabine and cyclophosphamide, and infusions of adoptive NK cells from haploidentical donors. All patients received subcutaneous IL-2 injections after the infusion of NK cells. Five patients achieved a hematological complete remission. Importantly, four patients were KIR ligand mismatched in the graft-versus-host direction and 3 of 4 (75%) achieved complete remission. This study demonstrated that the infusion of short-term IL-2-activated allogeneic haploidentical NK cells in patients with refractory leukemia can induce remission, while lympho-depleting treatment can cause serious adverse events, such as infections, but play an important role in the in-vivo expansion of infused adoptive NK cells.[24] The finding from this study highlighted lympho-depleting chemotherapy is necessary for adoptive allogeneic cellular therapy, including allogeneic NK cell-, CAR-T cell- and CAR-NK-cell therapy. Alloreactive NK-cell activity and clinical benefits have also been also observed in childhood AML. Rubnitz et al. conducted a pilot study to determine the safety, feasibility, and engraftment of haploidentical NK-cell infusions in 10 children with acute myeloid leukemia in first complete remission. The patients received fludarabine and cyclophosphamide as lymphodeplete treatment, followed by an infusion of KIR ligand mismatched NK cells and 6 doses of IL-2. All patients had engraftment for a median of 10 days. No nonhematologic toxicity and no graft-versus-host disease were observed. The 2-year event-free survival estimate was 100%, and median follow-up was 964 days.[22] The findings from this study indicated that the infusion of haploidentical NK cells is a promising consolidation therapy for pediatric patients with AML who are not eligible for transplantation. NK cell mediated cytotoxicity was also studied in elderly AML. Kottaridis et al. reported a phase-I clinical study illustrating the feasibility and toxicity of haploidentical NK-cell infusion in 7 patients with high-risk, elderly AML patients not eligible for allogeneic stem-cell transplantation. The median age for diagnosis was 65 years. The patients included in this clinical study received lympho-depleting treatment with fludarabine at a dose of 25mg/m^2 for 3 days and TBI as a single dose of 2 prior to infusion of NK cells, at a dose of 1 x 10^6/kg. At six months post-treatment, 3 patients treated in CR remained in remission (37.5%), while one patient who was infused at partial remission (PR)1 had achieved CR1 50 days post-infusion. All patients developed prolonged pancytopenia after lympho-depleting treatment. The infusion of NK cells was well-tolerated,

and no patient had infusion-related toxicity. The median time to relapse was 253.5 days post NK infusion (range, 58 to 845 days) and the median overall survival was 468.5 days (range, 148 to 1180 days). This study showed that the infusion of allogeneic NK cells could induce prolonged remission among high-risk elderly AML patients, but immunosuppressive treatment with fludarabine and TBI could include serious hematologic toxicity and serious infection, especially pneumonia and fungal infections due to prolonged neutropenia.[21]

While subcutaneous injection of IL-2 to stimulate in-vivo NK-cell proliferation and activation is associated with better outcomes in adoptive NK-cell therapy, the injection of IL-2 also stimulates regulatory T cells. Regulatory T cells (Treg), CD4+ CD25+ Foxp3+, expand rapidly after IL-2 is injected, and inhibit NK-cell proliferation and cytotoxicity. IL-2 diphtheria toxin (IL-2DT) is a recombinant cytotoxic fusion protein composed of the amino acid sequences for diphtheria toxin, followed by truncated amino-acid sequences for IL-2. IL2DT selectively deplete IL-2 receptor (CD25)-expressing cells, including regulatory T cells.[67] Bachanova et al[30] conducted a clinical study to determine the effect of regulatory T cells by using IL-2 diphtheria toxin (IL-2DT) for host Treg depletion. Fifty-seven refractory AML patients were enrolled. Patients were divided into 2 arms. All patients received cyclophosphamide and fludarabine, followed by NK-cell infusion, while one arm received IL-2DT injection. In the 15 patients who received IL-2DT, donor NK-cell expansion was detected in 27%, while in 42 patients who did not receive IL-2DT, the rate was only 10%. Regulatory T-cell depletion by IL2DT was associated with improved complete remission rates at day 28 (53% vs 21%; P = 0.02) and improved disease-free survival at 6 months (33% vs 5%; P < 0.01). Moreover, in the IL2DT cohort, NK-cell expansion correlated with peripheral blood regulatory T-cell depletion (< 5%) at day 7 (P < 0.01). This study demonstrated the efficacy of adoptive transferred NK cells to treat AML and highlighted the negative impact of host Treg.

Role of memory-like NK cells

NK cells function is involved in the innate immune response and can develop immunological memory, similar to the function of B cells and T cells in adaptive immunity. These adaptive NK cells are CD56 dim, CD57+, and CD94/NKG2C+ which is the activating HLA-E receptor that plays an important role in graft-versus-leukemia effects, and decreased expression of FeRγ, SYK, EAT-2, and PLZF. Moreover, this particular NK-cell subset has a DNA methylation pattern similar to cytotoxic T cells, and produces more IFN-γ and tumor necrosis factor-∝ that distinguish it from conventional NK cells.[80-83] Several clinical studies have reported an association between the activation and proliferation of these adaptive NK cells and lower relapse rates among HSCT patients experiencing human CMV reactivation after allogeneic hematopoietic cell transplantation. Cichocki et al. reported the outcome in 674 allogeneic HSCT recipients whose CMV reactivated with lower leukemia relapse (26%, P = 0.05) and superior disease-free survival (DFS) (55% P = 0.04) at 1 year compared with CMV-seronegative recipients who experienced higher relapse rates (35%) and lower DFS (46%).[84] The study of the reconstituting NK cells found that CMV reactivation is associated with higher frequencies and absolute numbers of CD56dimCD57+ NKG2C+ NK cells, particularly after RIC HCT. The expansion of these cells at 6 months' post-transplant was also associated with a lower risk of 2-year relapse.

Interestingly, this memory-like NK cell (CIML) can be generated by brief activation

with cytokines IL-12, IL-15, and IL-18, resulting in increased STAT5 signaling and CD 25 expression, and exhibiting enhanced cell proliferation, interferon gamma production, and cytotoxic functions for several weeks.[85] Romee et al[23] conducted a first-in-human phase I, dose-escalation clinical study to identify the maximum tolerated dose of memory-like NK cells administered to 9 patients with relapse refractory AML who were not candidates for hematopoietic cell transplantation (age 60-73 years). Patients were treated with fludarabine and cyclophosphamide, followed by allogeneic donor IL-12, IL-15, and IL-18 pre-activated NK cells in escalating doses: 0.5×10^6/kg (dose level 1), 1.0×10^6/kg (dose level 2). After the infusion of NK cells, the patients received 6 doses of low-dose rhIL-2 injections. Memory-like NK cells in the blood peaked at 7-14 days' post-infusion, and decreased in number after of 9 patients achieved CR. This study demonstrated that allogeneic human memory-like NK cells can potentially exhibit anti-leukemia functions after being transferred into relapse/refractory AML patients with active disease. In an ongoing study, NCT04354025, which is currently recruiting patients, the phase 2 clinical trial aims to investigate the effectiveness of cytokine-induced memory-like natural killer (CIML NK) cells in combination with chemotherapy as a treatment for refractory or relapsed pediatric AML. Fludarabine, cytarabine and filgrastim (FLAG) chemotherapy is used to lower leukemic burden and suppress the recipient's immune system to provide an environment for in-vivo CIML NK cell expansion. can potentially exhibit anti-leukemia functions after being transferred into relapse/refractory AML patients with active disease. In an ongoing study, NCT04354025, which is currently recruiting patients, the phase 2 clinical trial aims to investigate the effectiveness of cytokine-induced memory-like natural killer (CIML NK) cells in combination with chemotherapy as a treatment for refractory or relapsed pediatric AML. Fludarabine, cytarabine and filgrastim (FLAG) chemotherapy is used to lower leukemic burden and suppress the recipient's immune system to provide an environment for in-vivo CIML NK cell expansion. Overview of selected published clinical trials of NK cell infusion in AML are shown in Table 1.

Source and method of NK-cell isolation and activation

NK cells used for adoptive transfer in clinical trials can be collected from leukapheresis products. Peripheral blood mononuclear cells (PBMC) are enriched by centrifugation on a density gradient. T cells are removed using magnetic beads to remove CD3+ mononuclear cells with or without CD56+ selection, then highly purified NK cells can be isolated from PBMC. Some investigators also remove B cells using anti-CD19 beads.[24,43,86-88]

NK cells in peripheral blood are in a resting state. Grimm et al[89] generated lymphokine activated killer cells containing cytotoxic T cells and NK cells by incubating fresh lymphocytes with interleukin-2, resulting in the development of cytotoxic activity against autologous and solid tumor cells. Since then, activation with IL-2 has become a common method to stimulate NK cells; however, IL-2 also stimulate Treg cells, which can suppress in-vivo NK-cell expansion. For an alternative activation method, to avoid in-vivo NK-cell suppression, Miller and his group[30] demonstrated Treg depletion by introducing the use of IL-2DT instead of IL-12. Apart from IL-2, IL-15 is also used to activate NK cells. Preclinical studies in-vitro demonstrated that, compared to IL-2, IL-15 might enhance the expression of activating receptor and maintain cytotoxicity for longer. Pre-activation with IL-2, IL-15 and IL-18

Table 1 Overview of selected published clinical trials of NK cell infusion in AML

Ref	Indication	Source	Lympho-depletion	In vivo IL-2	N	Transplantation	Outcome
(74)	AML, CML	Haploidentical	HSCT conditioning	No	5	Post Haplo HSCT	CR in 4 patients, increased donor chimerism 2/5, median F/U 12 months
NCT00799799 (24)	AML	Haploidentical	Flu/Cy	Yes	19	No	CR in 5 patients,3/4 of patients with KIRL mismatch
(22)	AML	Haploidentical	Flu/Cy	Yes	10	No	2 years event-free survival of 100%
NCT00799799 (48)	AML	Haploidentical	Flu/CY	Yes	13	No	Three of 6 patients in CR, and disease-free after 34, 32, and 18 months
(23)	AML	Haploidentical	Flu/Cy	Yes	9	No	OR 55%, CR in 4 patients
(96)	AML	Partially HLA-matched cord	Flu/Cy	No	10	No	2/4 of MRD-positive patients became MRD-negative, which was sustained for 6 months
(26)	AML, MDS	Haploidentical	Flu/Cy/TLI	No	16	Preceded HSCT	CR in 5 patients, 1 in PR, 5 patients proceeded to HSCT
(27)	AML, CML	HSCT donor	HSCT conditioning	No	13	days 22, 17, and 128 post-transplant	CR in 7 of 8 AML patients. All were alive at median F/U 14.7 months
NCT01385423 and NCT02395822(108)	AML	Haploidentical	Flu/Cy	rhIL-15 IV or SQ	40	No	CR in 7 patients, CRS was observed in 56% of patients given SC rhIL-15
NCT00703820(109)	AML	Haploidentical	Flu/Cy	yes	21	No	Neither decreased relapse nor improved overall survival compared with chemotherapy alone
NCT00274846 and NCT01106950(30)	AML	Haploidentical	Flu/Cy	IL-2, IL-2DT	57	No	IL2DT associate with improved complete remission rates at day 28 (53% vs 21%; P 5 .02) and disease-free survival at 6 months (33% vs 5%; P < .01)
(75)	AML	Haploidentical	HSCT conditioning	No	91	First group day 6 and 9, second group day14 and 21 post-transplants	CR rate 57% in first month. Higher expression of NKp30 (>90%) on donor NK cells was an independent predictor of higher CR and reduced leukemia progression
NCT01520558 (25)	AML in CR1	Haploidentical	Flu/Cy CNDO-109-NK cells	No	12	No	RFS by dose level was 105 (3×10^5), 156 (1×10^6), and 337 (3×10^6) days. Exceeding 42.5 months in 2 patients

for short periods of time has also been used to generate cytokine-induced memory-like NK cells in a clinical study.[23,85] Other reagents have been used to activate NK cells include glycogen synthase kinase 3 inhibitor[90], and OKT3, which is an anti-CD3 antibody used to suppress T-cell expansion.[91]

Given that the clinical response of NK-cell therapy directly correlated with the number of infused cells, ex-vivo manipulation to increase the number of NK cells is required. Since NK cells can only be found in 10-15% of peripheral blood lymphocytes, it may be difficult to prepare enough NK cells for infusion at 1:1 effector- to targeted-cell ratio, or for multiple infusions by single apheresis.[18,20,43,86,87,92] Ex-vivo NK-cell expansion and cytotoxicity can be improved by adding feeder cells, such as autologous PBMC, Epstein-Barr virus-transformed lymphoblastoid cells (EBV-LCL), and K562-mbIL-15-4-1BBL cells, in the culture process. Subsequent culture protocols incorporating EBV-LCL as feeder cells with cytokine-containing media could augment NK cell expansion in the range 80–10,000-fold within 14–21 days.[86,93,94] Another protocol uses K562-mbIL-15-4-1BBL and stem cell growth tissue culture medium in GMP culture condition for 10 days can produce median 376-fold NK-cell expansion; as a result, enough expanded NK cells for 4 infusions can be obtained from one leukapheresis.[43,56,87]

Interestingly, NK cells can be generated from hematopoietic stem cells. CD34+ hematopoietic stem cells (HSPC) are isolated from umbilical cord blood and expanded for 14 days, then CD34+cells are exposed to stem cell factor, IL-2, IL-15, IL-17 and other growth factors for 21-28 days. NK cells can also be generated from induced pluripotent stem cells (iPSC) by promote differentiation into hematopoietic stem cell first, then differentiating CD34+ to be CD3-CD56+ cells.[95] Dolstra et al[96] reported the outcome of the first-in-human clinical study using NK cells generated from CD34 + isolated from partially HLA- matched umbilical cord to treat 10 elderly AML patients in morphological complete remission. The patients received HSPC-NK cells after lympho-depleting chemotherapy without in-vivo cytokine boosting. Neither GVHD nor infusion related toxicity was observed, and 2 of 4 MRD-positive patients became MRD-negative. Remarkably, NK cells derived from master clonal iPSC banking could provide greater benefit than primary NK cells from autologous or allogeneic donors in terms of being immediately available, with high numbers of infused cells, and donor selection for KIR B haplotype.

Conclusion and future directions

The results from preclinical studies and clinical trials of adoptive transfer NK cells in patients with AML suggest a promising treatment strategy. The treatment options for adoptive NK cells include induction remission in relapsed/refractory AML, consolidation treatment in patients who are not eligible for transplantation, eradication of MRD before proceeding to transplantation, and potential use as a strategy for early relapse treatment in patients who have mixed chimerism after transplantation. Compared with chimeric antigen receptor T-cells, adoptive NK-cell therapy provides better safety profiles, including a lower incidence of severe GVHD and cytokine-release syndrome. Moreover, adoptive NK cells are easy to prepare under good manufacturing practice standards, which may have an "off the shelf" benefit for treating patients in a short period of time.[97,98] However, a number of issues need be resolved for NK-cell immunotherapy. First, how to promote in-vivo expansion and proliferation because most patients have a short duration of response due to a short lifespan and the poor in-vivo expansion of infused NK cells.

Second, strategies to overcome various immune escape mechanisms in the AML tumor microenvironment. Current ongoing clinical trials will provide knowledge for developing and guiding future treatment strategies in NK cell-based immunotherapy.

Study design will be a key factor to determine efficacy and bring the best of NK cell-based immunotherapy to the treatment of AML. Importantly, the optimal number of infused cells must be evaluated; theoretically, the number of infused cells should reach an effector-target ratio that can control AML cells in different disease statuses, because even in patients who achieved morphological complete remission after induction chemotherapy may still have 10^9-10^{10} residual leukemic cells.[99] Since the incidence of severe GVHD or severe cytokine-release syndrome were very low compared to CAR-T cells, an effector-to-target cell ratio > 1:1 should be applied to study design. In order to improve in-vivo persistence and proliferation, genetic modification, such as HLA-knockdown or augmentation of in-vivo proliferation by transduction of membrane-bound receptor for cytokines, or hematopoietic growth factor, such as erythropoietin or thrombopoietin, should be evaluated.[13,20,31,56,93,94,100]

Given our knowledge of NK-cell biology and NK-cell dysfunction in the AML microenvironment, a combination of multiple treatment modalities may optimize outcomes for adoptive NK-cell therapy. By selecting donors with KIR ligand mismatches to improve NK-cell alloreactivity.[48,49,101,102] Using ex-vivo activation and genetic modification of NK cells to improve toxicity and in-vivo proliferation. Since NK-cell functions and activation depend on integration signals from activating and inhibitory receptors, monoclonal antibodies can be used to promote NK-cell toxicity and improve clinical outcomes. Preclinical findings targeting tumor-associated antigen, such as CD33, CD37, CLL-1, and FLT3[103–105], have shown promising outcomes, while monoclonal antibodies that bind to inhibitory receptors, such as anti-KIR antibodies and anti-PD1 antibody[106], are under investigation for the clinical treatment of AML. In addition, immunomodulatory drugs can be used to improve the outcomes of NK-cell therapy by using drugs that augment NK-cell function, such as lenalidomide, which indirectly augments NK-cell cytotoxicity and proliferation through the release of IL-2 and IFN-γ from surrounding T cells and dendritic cells[107], or using drugs that make AML cells more sensitive to NK cell-mediated lysis, such as bortezomib,[20,40,41] which could enhance the expression of HLA-E on the surfaces of cancer cells. In summary, NK cell-based immunotherapy is a new hope for treating acute myeloid leukemia, but key to the success of future clinical studies is the incorporation of modalities that enhance NK-cell cytotoxicity, promote in-vivo proliferation and survival, home in to the tumor site, and the development of novel drugs and expansion methods.

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Prevalence and Associated Factors of Stress upon Online Study among Mae Fah Luang University Preclinical Year Medical and Dental Students during COVID-19 Pandemic

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Abstract:

Background: Stress is common among medical and dental students in usual circumstance. As COVID-19 pandemic had great impact on higher education. Classes attending at the universities has also been altered, including introduction of online classes and examinations. Medical and dental students who regularly were stressed due to their exhausting curriculums might have been affected by this pandemic as well.

Objective: This study aimed to study stress in medical and dental students in preclinical year as a consequence of the lockdown from Covid-19 and its associated factors.

Methods: The study was conducted from 31st July to 15th August 2020 as a cross-sectional descriptive study which collected data via online questionnaires. The online questionnaires consist of 3 parts; demographic data, factors associated with stress from literature review and ST-5 (a stress assessment questionnaire). The measurement of association was analyzed by multivariable logistic regression.

Results: This study got data from 129 respondents in preclinical year of medical and dental students of Mae Fah Lung University with average age of 20.5 years. 53 (41.1%) of the respondents old are stressed. Prevalence of stress was found to be 41.5% in medical students and 58.5% in dental students. Factors associated with stress from multivariable logistic regression analysis were inappropriate or unfacilitated location for studying (p-value = 0.001), feeling isolated or lonely (p-value = 0.001) and daily life changes (p-value = 0.001).

Conclusion: Prevalence of Preclinical year stress is 41.5% in medical students and 58.5% in dental students after COVID-19 lockdown. Factors associated with stress from the study

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can be categorized into 3 categories: educational-related factors, learner-related factors and other physical-related factors.

Keywords: Stress, COVID-19, Medical and Dental students

Introduction

In the end of 2019, an outbreak of Coronavirus disease (SARS-CoV-2 or COVID-19) was reported in Wuhan, China. Not long later, COVID-19 was reported in Thailand and was specified as the 14th dangerous infective disease followed by the communicable diseases act, B.E.2558 (2015) by the Ministry of Public Health.[1] The Emergency Decree on Public Administration in Emergency situation had been declared to control the COVID-19 outbreak situation in Thailand.

Mae Fah Luang University (MFU) is a large educational institute located in Chiang Rai, Thailand. Students of this university comes from various provinces of Thailand as well as from other countries. Around 45.83% of the students from school of medicine and 37.5% of the students from school of dentistry are also from provinces other than Chiang Rai.[2] The COVID-19 lock down has greatly impact studying at the MFU.[3] Class attending at the university in the final semester of 2019 has been altered, including introduction of online classes and examinations. The online classes were conducted both synchronously via online applications such as Google hangouts meet, MFU Webex or Zoom application and asynchronously via Google classroom. The assessment and evaluation have also been altered to Google form via Google classroom. Mae Fah Luang University also declared remedial policy to help affected students from COVID-19 outbreak such as deduction of 10 % of the tuition fee and 50% of the dormitory fee, returning of public utility fee, inclusion of MFU free internet at 4 Mbps speed in all over 3 months without any payment, among others. Scholarships for the affected students have been altered as a consequence of the policy as well.

The declaration of the Ministry of Education followed by Thai qualifications framework for higher education; TQF: HED, B.E.2552 (2009) has specified the qualification framework for higher education in medical and dental study. The field of knowledge specified in medical and dental study must be the combination between interactive lectures and laboratory studies. The evaluation of the curriculum can be conducted as a subjective test, multiple choices or even synthesized-answer as designed by the curriculum.[4] This mean that all of the processes along the curriculum must be conducted by the university.

A study in prevalence of stress and its associated factors among Ramathibodi medical students during normal situation using Thai stress test,[5] found that 61.4% of medical students have moderate stress level and 2.4% have high stress level. The high stress level mainly comes from the exhausting of the curriculum, especially in the 4th – 6th year medical students of Ramathibodi hospital, Mahidol University.[6] The study also found that 218 medical students have no time to take a rest and also have a cumulative stress which can lead to mental health problems in the future. Several studies have also found that medical and dental students were regularly prone to stress even before the pandemic of COVID-19 due to their exhausting curriculum.[7] In light of the COVID-19 pandemic, this study of prevalence rate and associated factor in stress on online study among Mae Fah

Luang University preclinical year medical and dental students were conducted to further understand stress which affect in efficiency of study during COVID-19 lockdown especially in subject that need practical skills and also need to be on-site like Anatomy and Physiology class.

Objectives

This study aimed to study about prevalence rate and associated factor in stress on online study among Mae Fah Luang University Preclinical year Medical and Dental students during COVID-19 pandemic

Method

This study is a cross-sectional descriptive study which collects data using Google form online questionnaires. The data were collected from 31st July 2020 to 15th August 2020 from a preclinical year medical and dental students of Mae Fah Luang University. There are 3 inclusion criteria for preclinical year medical and dental students: (1) be at current student status in semester 1/2020; (2) Study online during COVID-19 situation followed by the announcement of Mae Fah Luang University; (2) Enroll in the online study session due to the COVID-19 situation announcement of Mae Fah Luang University in semester 1/2020; (3) 18 years and above age. We excluded (1) foreign students; (2) did not enroll in the online session in semester 1/2020. The target population of this research was 135 people which included 10% over the actual population.

Review of previous literatures was done as the first step of this study. The results of reviewing were used to design this study and the questionnaires. Google form was used to collect the data. The form consisted of 3 parts: (1) General demographic data; age, sex, native habitat, school, year; (2) Factors associated with stress from literature review; (3) ST-5 stress assessment questionnaires. ST-5 stress assessment questionnaires were stress evaluation of Department of Mental Health, Ministry of Public Health.[8] In ST-5 stress assessment questionnaires, we used the cut off score at 7 out of 15. The evaluation of 0-7 score was interpreted as no stress and 8-15 as having stress 8.

Descriptive statistics were used for demographic and other characteristics data. Binary logistic regression analyzer was used to analyze the COVID-19 stress factors. Factors with p-value < 0.05 were included into COVID-19 stress factors. Multivariable logistic regression was used to analyze factors associated factors related to stress in medical and dental students during COVID-19 lockdown. Factors with p-value < 0.01 were included into factors associated to stress during COVID-19 lock down. All statistics were done using STATA program to analyze the data.

This study was approved by Human Research Ethics Committee of Mae Fah Luang University on 11th August 2020. Funding for this study was granted by Research Administration Division, Mae Fah Luang University research and innovation institute.

Result

129 respondents responded to the questionnaires from 135 of target population. The overall data was shown in Table1.

Table 1 Demographic characteristics of the respondents

Variable	frequency	Percentage
Medical students	58	44.96 %
Dental students	71	55.04 %
2nd Year	62	48.06 %
3rd Year	58	44.96 %
4th Year	9	6.98 %
Mean age ± SD (Year)	20.55 ± 1.13	
Male	31	24.03 %
Female	98	75.97 %
Northern region	78	60.47 %
Other part of Thailand	51	39.53 %

Table 2 Comparison of baseline characteristics

Demographic data	Stressed	not stressed	p-value
School of			
Medicine	22 (41.51%)	36 (47.37%)	0.590
Dentistry	31 (58.49%)	40 (52.63%)	
Year			
Year 2	28 (52.83%)	34 (44.74%)	0.593
Year 3	21 (39.62%)	37 (48.68%)	
Year 4	4 (7.55%)	5 (6.58%)	
Sex			
Male	11 (20.75%)	20 (26.32%)	0.534
Female	42 (79.25%)	56 (73.68%)	
Age*	20 (18-24%)	20 (18-24%)	0.993T
Native Habitat			
North region	33 (62.26%)	45 (59.21%)	0.855
Others	20 (37.74%)	31 (40.79%)	

* = median (min-max)
T = Mann-Whitney U test

Table 3 Comparison between factors affecting stress on online study during COVID-19

Factors	Stressed	not stressed	p-value
Changing in daily life	48 (90.57%)	5 (9.43%)	0.000
Inappropriate of place during online study or different of parent economic status	41 (77.36%)	12 (22.64%)	0.001
Feeling lonely or isolated from family	18 (33.96%)	35 (66.04%)	0.001
Cancelling study abroad	25 (47.17%)	28 (52.83%)	0.008
Exaggerated and missed news report	41 (77.36%)	12 (22.64%)	0.016
Having problem to open camera to communicate with teachers and classmates	12 (22.64%)	41 (77.36%)	0.016
Happening of accidental situation during online study or examination; black out, run out of battery	46 (86.79%)	7 (13.21%)	0.021
Unstable and unclear study schedule	51 (96.23%)	2 (3.77%)	0.025
Decreasing of studying or working efficiency	49 (92.45%)	4 (7.55%)	0.076
Expectation in grade	43 (81.13%)	10 (18.87%)	0.107
Insufficient to efficiency internet	39 (73.58%)	14 (26.42%)	0.133
Cancelling of BYE NIOR in preclinical years	19 (35.85%)	34 (64.15%)	0.167
Income or economic status of parents	37 (69.81%)	16 (30.19%)	0.351
Shortage of surgical mask, disinfectants or other product	31 (58.49%)	22 (41.51%)	0.581

The comparison from the demographic data between stress and no stress was shown in table 2. There was no significant difference of characteristics from any demographic data which associated to stress level. As shown in Table 3, binary logistic regression analysis of association between factors studied and stress with p-value < 0.05 were; exaggerated and missed news report; inappropriate of place during online study; cancelling of study abroad; unstable or unclear of studying schedule; feeling isolated or lonely from

family; changing in daily life; happening of accidental situation during online study or examination; and having problem to open the camera to communicate with teachers and classmates.

Table 4 Association between factors and stress

Variable	Univariable Odd ratio	95% CI	p-value	odd ratio	95% CI	p-value
Sex Female Male (Reference)	1.36	0.59-3.15	0.468	1.39	0.59-3.27	0.446
School of Dentistry Medicine (Reference)	1.27	0.62-2.57	0.511	1.34	0.65-2.78	0.429
Age	0.97	0.71-1.33	0.853	1.05	0.70-1.58	0.799
Native habitat Others North region (Reference)	0.88	0.42-1.81	0.727	0.94	0.45-1.97	0.876
Year Year 3 Year 4 Year 2 (Reference)	0.69 0.97	0.33-1.43 0.24-3.97	0.319 0.968	0.65 0.97	0.28-1.50 0.122-4.57	0.317 0.753
Changes in daily life	5.92	2.11-16.60	0.001	6.05	2.13-17.16	0.001
Inappropriate of place during online study or different of parent economic status	3.80	1.72-8.32	0.001	4.34	1.88-10.03	0.001
Feeling lonely or isolated from family	5.07	1.93-13.28	0.001	5.60	2.04-15.36	0.001
Cancelling study abroad	2.88	1.35-6.12	0.006	3.33	1.50-7.37	0.003
Exaggerated and missed news report	2.62	1.20-5.77	0.016	2.58	1.14-5.81	0.023
Having problem to open camera to communicate with teachers and classmates	0.38	0.17-0.84	0.016	0.31	0.13-0.72	0.006

Table 4 Association between factors and stress (continued)

Variable	Univariable Odd ratio	95% CI	p-value	odd ratio	95% CI	p-value
Happening of accidental situation during online study or examination; black out, run out of battery	3.03	1.20-7.69	0.019	3.31	1.23-8.89	0.017
Unstable and unclear study schedule	5.26	1.14-24.39	0.034	5.88	1.23-28.11	0.027
Decreasing of studying or working efficiency	3.01	0.94-9.66	0.064	3.62	1.03-12.72	0.045
Expectation in grade	2.11	0.91-4.87	0.081	2.10	0.88-4.99	0.092
Insufficient to efficiency internet	1.92	0.89-4.12	0.094	2.37	1.02-5.51	0.044
Cancelling of BYE NIOR in preclinical years	1.80	0.83-3.89	0.135	2.03	0.90-4.54	0.086
Lacking of communication between people ex: Doing PBL, group project	2.24	0.97-5.16	0.059	2.16	0.90-5.19	0.084
Income or economic status of parents	1.51	0.72-3.18	0.28	1.48	0.69-3.19	0.316
Shortage of surgical mask, disinfectants or other product	1.41	0.69-2.86	0.95	1.39	0.67-2.87	0.379
Being close to COVID-19 patients or people at risk	1.29	0.63-2.65	0.491	1.29	0.61-2.70	0.506

Result in table 4 found that association between factors and stress in an online study of Mae Fah Luang University preclinical year medical and dental students during COVID-19 lockdown were analyzed by multivariable logistic regression analysis and specify p-value < 0.01: inappropriate of place during online study; cancelling of study; feeling isolated or lonely from family; changing in daily life; having problem to open the camera to communicate with teachers and classmates.

Figure 1 Prevalence of stress

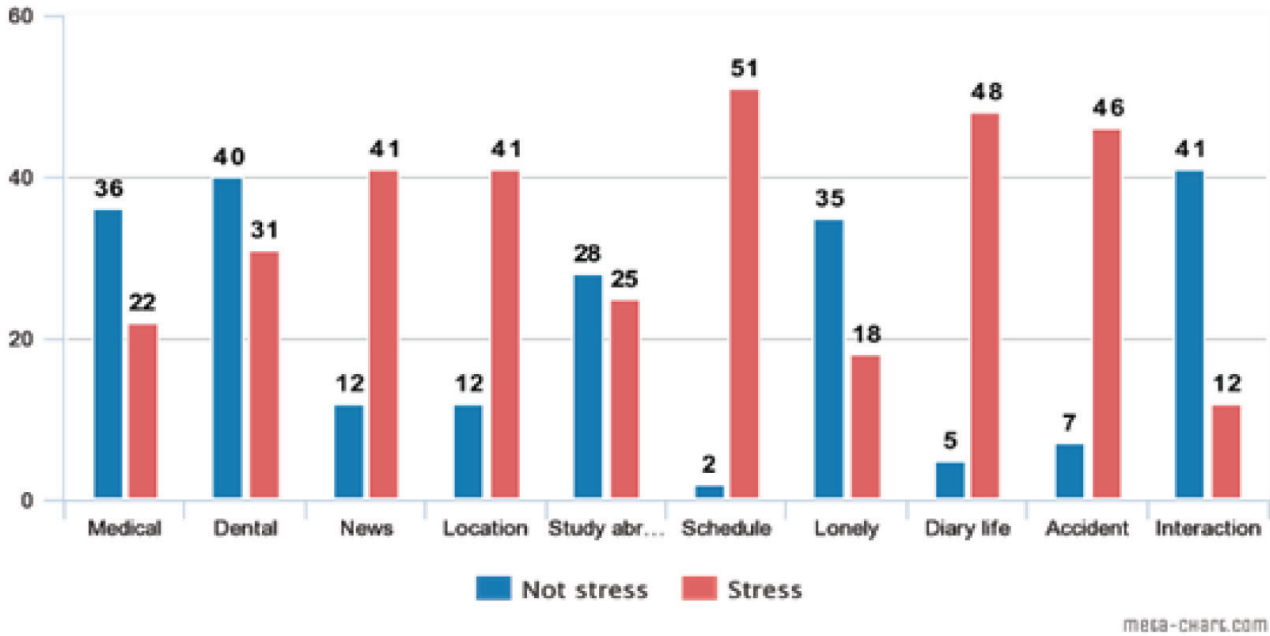

Figure 2 Factors affecting stress on online study during COVID-19

Figure 1 showed prevalence of stress from ST-5 questionnaires. 53 of preclinical year medical and dental students were stressed. 22 of them were medical students (41.51%) and 31 were dental students (58.49%). 76 people in preclinical year medical and dental students were not stressed. Figure 2 showed factors that affect to stress on an online study of Mae Fah Luang University preclinical year medical and dental students during COVID-19.

Discussion

Factors associated for stress in an online study during the COVID-19 situation among medical and dental students could be categorized into 3 categories; educational, learner and others physical-related factors.

Educational-related factors category were having problem to open the camera to communicate with teachers and classmates, happening of accidental situation during online study or examination (ex: black out,

run out of battery), unstable or unclear of studying schedule and insufficient of internet. Only factor of having problem to open the camera to communicate with teachers and classmates was found to be significantly associated with stress in our study. This finding was similar to the previous study from University of Galgotius in 2020 which describes 69.8% of students were having stress from an online study.[9] The study from Galgotius University was conducted as a cross-sectional observational study via google form questionnaires which collected 500 respondents from different schools and universities in India. Found that students were not comfortable with online studies which led to rising in their stress level.

Learner-related factors category included factor of changing in daily life, feeling isolated or lonely from family and decreasing of studying efficiency. The study was correlated with the previous study from Changzhi University in China.[10] The study was conducted in 2020 with 7143 college students using 7-item Generalized Anxiety Disorder Scale (GAD-7). As the result, found that 75.1% of the respondents had no symptoms of stress. And the proportions of students with mild, moderate, and severe stress were 21.3%, 2.7%, and 0.9%, respectively. Meanwhile, the correlation of Ali Abdullah Alomar research in 202011 also described as 44.1% of medical students in Saudi Arabia feel lonely and isolated from their family along with feeling of decreasing in their studying or working efficiency. They used a questionnaire with a Five-Point Likert Scale to collect the data. The questionnaire was distributed among 625 medical students through their emails. The cause of this factor could be from the cancelation and changing in flight policy declared by the government. This policy made medical students unable to go back homes to visit their friends or families like in normal situation. This category of factors might be alleviated by the use of social media technologies such as facetime-called, Line application or even emailing to each other. Factor of changing in daily life was another factor that was correlated with the study of Lee, Joyce[12] which was done in 2020 among 757 candidates via survey method. They described the online study could lead to stress problem due to the alteration of class attending which could now study anywhere they want. The problem in this category could be improved by the efficiency of planning and management from the education institutes like a government policy or a university support.

Other physical-related factors were inappropriate of place during online study or different of parent economic status. This factor was correlated with the study in stress of students in United states, USA during COVID-19 outbreak. A study of Lancker was correlated in this field.[13] They described that 5% of students living in Europe have a problem of no suitable place during online study along with 2.5% of students in United states who lived in rural were having problem with the inappropriate of climate changes led to their online study problem. The problem in this category might be improved by the efficiency of planning and management from the education institutes like a government policy. The government of the country must provide a good support and must support an online classroom which has the most likely condition to the normal classroom.

Conclusion

Prevalence rate and associated factors in stress on online study among Mae Fah Luang University preclinical year medical and dental students during COVID-19 pandemic is 41.5% in medical students and 58.5% in dental students.

Associated factors of stress in an online study of Mae Fah Luang University among preclinical year medical and dental students

during COVID-19 from this study can be categorized into 3 categories: educational, learner and other physical-related factors.

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Conflict of interest

There is no conflict of interest in this research.

Ethical approval

This study was approved by Human Research Ethics Committee of Mae Fah Luang University on 11th August 2020.

Informed consent

In this study, the informed consent had been done under the principle of research ethical standards in all of the data collected from target population.

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Risk Factors of Severe Hypoglycemia in Type 2 Diabetic Patients at District Hospital, Wiang Pa Pao Chiang Rai, Thailand

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Abstract:

Background: The incidence of hypoglycemia in diabetic patients in District Hospital Wiang Pa Pao, Chiang Rai increases every year. Hypoglycemia is very common in the emergency room, which potentially causes morbidity and mortality in these patients. Hypoglycemia causes limitation in the treatment of diabetes and subsequent glucose control. Therefore, this research attempted to identify the main factors associated with this hypoglycemic syndrome of type 2 diabetic patients in Wiang Pa Pao district, Chiang Rai province. It is anticipated that this information can be used to plan for treatment and prevention.

Objective: The objective of this study is to identify the main factors associated with hypoglycemic episodes in type 2 diabetic patients receiving health care at Wiang Pa Pao hospital, Chiang Rai during 2018 - 2020.

Methods: The design is a case control study in type 2 diabetic patients who received treatment at Wiang Pa Pao Hospital, Chiang Rai during 2018 - 2020. The number of cases and controls were 59 and 236 respectively. Data collection was obtained from the patient hospital information system including gender, occupation, weight, height, age, body mass index (BMI), HbA1c, eGFR, total cholesterol, diabetic medications, and comorbid diseases. Analytical statistics used in this study was univariable logistic regression described in odd ratio and statistical significance P-valve < 0.05.

Results: This study found that the factors associated with hypoglycemic syndrome in type 2 diabetic patients were BMI, HbA1C level and type of diabetic medications. In BMI of 18.5 - 22.9 kg/m^2, patients had a risk of developing hypoglycemia more than patients who had BMI 23 kg/m^2 (P-value 0.02, OR 2.51). In BMI below 18.5 kg/m^2, patients had a risk of developing hypoglycemia more than patients who had BMI 23 kg/m^2 (P-value < 0.0001,

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OR 7.14). This research also found that for the HbA1C 10.1% - 13% there was a risk for hypoglycemic episodes more than HbA1C 4 % - 7% (P-value 0.02, OR 2.14). Finally, this research found that the patients who used insulin treatment had a higher chance of developing hypoglycemic episodes compared to the patients who used oral anti-diabetic drugs alone (P-valve < 0.0001, OR 27.26) and patients who used both oral anti-diabetic agents and insulin, had a higher risk of developing hypoglycemic syndrome more than patients who used oral medications alone. (P-value < 0.0001, OR 3.95).

Conclusion: The study found that the level of HbA1c had an association with hypoglycemia in type 2 diabetic patients. The higher initial HbA1c level, the greater the chance of having the hypoglycemic episode. Additionally, patients who had low body mass index were at increased risk of hypoglycemia. The type of anti-diabetic medications also had an effect. Patients who used only insulin injection and patients who used combination of insulin and oral agents, had a higher risk of hypoglycemia than patients who used oral medication alone.

Keywords: Severe hypoglycemia, Type 2 Diabetes Mellitus

Introduction

Type 2 diabetes presents an important public health burden worldwide including Thailand. The number of people with diabetes in adults aged 20 years and over in Thailand will increase from 1,017,000 in 2000 to 1,923,000 in 2025.[1] Also, the incidence of hypoglycemia in type 2 diabetes increases every year. Approximately, 51 percent of hypoglycemic patients in western pacific region will increase from 2019-2045.[2] In 2017, Thailand was rated in fourth place of having large number of hypoglycemia in its region.[3] This issue become a problem of the country that need to be investigated. From the existing data, the incidence of hypoglycemia in diabetic patients who received healthcare in Wiang Pa Pao District Hospitals had been increasing dramatically since 2018. It is very commonly found in the emergency room, which potentially causes morbidity and mortality in these patients. The previous study found that factors related to hypoglycemia in type 2 diabetic patients including already known chronic complications of diabetes and glipizide uses[4] resulting in hospitalization and morbidity including accident, myocardial infarction, cardiac arrhythmia, seizure and coma.[5] Therefore, the research team attempted to identify the main factors associated with hypoglycemic episodes in type 2 diabetic patients in Wiang Pa Pao district, Chiang Rai province during 2018-2020. It is hoped that this information can be used to plan for the treatment and prevention regime.

Material and method

The study design is retrospective case control. Cases were type 2 diabetic patients who had a hypoglycemic episode. Controls were type 2 diabetic patients who never had a hypoglycemic episode.

The statistical parameters included age, body mass index, diabetic medication, HbA1c level, cholesterol level and comorbid diseases. Analytical statistics used in this study was univariable logistic regression described in odd ratio and statistical significance, P-valve < 0.05.

Population and sample

Type 2 diabetic patients aged over

15 years old who received treatment care at Wiang Pa Pao Hospital, Chiang Rai during 2018 - 2020. This hospital is a community hospital with 90 beds, and there are 3 internists with 10 - 15 interns which most of them are family medicine practitioner. Patient information was retrieved from the electronic hospital database and the research team received a permission to access an information from hospital director. Only first episode of hypoglycemia at emergency room was analyzed.

Inclusion criteria

Type 2 diabetic patients who had severe hypoglycemic episode according to Whipple's triad and for those who had multiple hypoglycemic episodes, the researcher select only the first one.

Exclusion criteria

Exclusion criteria are including incomplete patient information retrieved from the hospital data system which only from first episode of hypoglycemia at emergency room, patient who had hypoglycemic episode during admission from other medical illness such as sepsis, tumor, hormonal deficiency. Also, patient with pregnancy or critical ill such as shock, coma, and immunocompromised patients such as cancer, bedridden and HIV infection and patients who had underlying diseases including liver diseases, endocrine diseases and chronic kidney diseases are excluded from the study.

The research protocol was approved by the research ethical committee of Mae Fah Luang University.

Sample size

The total type 2 diabetic patient visits in 2018 - 2020 were 3,874. After divided into 2 groups based on hypoglycemic episode, the first group was patients who had a hypoglycemic episode of 674. After exclusion, the number of cases that were suitable for the study were 59. The second group was the patient who never had a hypoglycemic episode 2,873 visits. After simple random sampling, the number of controls were 236. Data collection was obtained from the patient information system of Wiang Pa Pao Hospital which were divided into 2 parts: Part 1, geographic data including gender, age, occupation, weight, height, body mass index, eGFR, HbA1c and total cholesterol and Part 2, study factors including age, occupation, body mass index, HbA1c, total cholesterol, co-morbid diseases and diabetic medications.

Statistical analysis

The data was analyzed by STATA version 16. Categorical variables were presented in form of frequency and percentage. Continuous variables were presented in mean and standard deviation. Using univariable logistic regression to analyzed the relationship of each factor and hypoglycemic episodes, described in odd ratio and statistical significance P-valve < 0.05.

Result

The number of cases was 59 and 37.29% were male and 62.71% were female. Majority of occupation was farmer (52.54%). The average body weight and height were 53.33 ± 10.71 kg and 155.56 ± 6.57 cm. The average body mass index of the case was 22.10 ± 4.61 kg/m2 which was lower than that of the control (24.90 ± 4.53 kg/m^2). On glycemic control, the average level of HbA1c of the case was higher than that of the control without statistical significance (10.32 mg% and 9.49 mg%). And for the kidney function, the eGFR of the case and control was not different (87.23 mL/min/1.73 m^2 and 92.47 mL/min/1.73 m^2) (Table 1).

For analysis of association of the risk factors including age, body mass index, HbA1c level, total cholesterol, diabetic medications and comorbid diseases with hypoglycemic episodes by using univariable logistic regression, this study found that patients with BMI of 18.5-22.9 kg/m^2 had risk of developing hypoglycemia more than patients who had BMI 23 kg/m^2 (P-value 0.02, OR 2.51). In BMI < 18.5 kg/m^2, patients had a risk of developing hypoglycemia more than patients who had BMI 23 kg/m^2 (P-value < 0.0001, OR 7.14). On the patients with HbA1C of 10.1% to 13% there was a risk for hypoglycemic episodes more than patients who had HbA1c of 4% to 7%

Table 1 Demographic data of hypoglycemic patients and control group.

Geographic data	number (percentage)		P-value
	Diabetic patients with hypoglycemia (59)	Diabetic patients without hypoglycemia (236)	
Gender			
- Male	22 (37.29)	88 (37.29)	
- Female	37 (62.71)	148 (62.71)	
Occupation			
- Government officer	1 (1.69)	5 (2.12)	0.40
- Farmer	31 (52.54)	117 (49.58)	0.40
- Shopkeeper	2 (3.39)	22 (9.32)	0.40
- General employee	16 (27.12)	63 (26.69)	0.00
- Monk	1 (1.69)	0 (0)	0.49
- Elderly	8 (13.56)	14 (5.93)	0.28
- Housewife	0 (0)	12 (5.08)	
Weight (kg)			
- Mean ± SD	53.33 ± 10.71	63.20 ± 19.21	< 0.001
- Median	52 (34.6 - 88)	61 (33 - 274.9)	
Height (cm)			
- Mean ± SD	155.56 ± 6.57	157.91 ± 7.97	< 0.001
- Median	155 (140 - 168)	156 (138 - 185)	
Body mass index (kg/m^2)			
- Mean ± SD	22.10 ± 4.61	24.90 ± 4.53	< 0.001
- Median	21.33 (14.45 - 39.11)	24.27 (14.66 - 42.97)	
Hemoglobin A1C (mg%)			
- Mean ± SD	10.32 ± 2.68	9.49 ± 9.01	0.23
- Median	9.8 (5.2 - 14)	8.2 (5.4 - 14)	
eGFR (mL/min/1.73 m^2)			
- Mean ± SD	87.23 ± 19.03	92.47 ± 16.93	0.98
- Median	88.78 (60.05 - 137.47)	93.8 (60 - 172.8)	

(P-value 0.02, OR 2.14). In addition, insulin used patients had a higher chance of developing hypoglycemic episodes compared to the patients who used oral anti-diabetic medications (P-value < 0.0001, OR 27.26) and the patients who used both oral anti-diabetic drugs and insulin, had a higher risk of developing hypoglycemic episodes more than the patients who used oral anti-diabetic drugs alone (P-value < 0.0001, OR 3.95) as shown in Table 2

Table 2 Factors related with hypoglycemia

Risk factor	Number (Percentage)		OR	95% CI for OR		P-value
	Diabetic with hypoglycemia	Diabetic without hypoglycemia		Lower	Upper	
1. Age (year)						
- 21-50	11 (18.64)	49 (20.76)	1			
- 51-60	20 (33.90)	77 (36.63)	1.14	0.51	2.62	0.73
- 61-70	18 (30.51)	84 (35.59)	0.95	0.41	2.19	0.91
- 71-90	10 (16.95)	26 (11.02)	1.71	0.64	4.56	0.28
2. Body mass index (kg/m^2)						
- ≥ 23	21 (35.60)	150 (63.56)	1			
- 18.5-22.9	26 (44.07)	74 (31.36)	2.51	1.32	4.75	0.02*
- < 18.5	12 (20.34)	12 (20.34)	7.14	2.84	17.95	0.0001*
3. Hemoglobin A1C (mg%)						
- 4-7	12 (20.34)	55 (23.30)	1			
- 7.1-10	19 (32.20)	132 (55.93)	0.66	0.30	1.45	0.30
- 10.1-13	20 (33.90)	35 (14.83)	2.26	1.14	6.02	0.02*
- > 13	8 (13.56)	14 (5.93)	2.62	0.90	7.63	0.08
4. Total Cholesterol (mg/dL)						
- < 200	41 (69.50)	154 (65.25)	1			
- 200 – 239	15 (25.42)	61 (25.85)	0.97	0.50	1.88	0.92
- ≥ 240	3 (5.08)	21 (8.90)	0.54	0.15	1.91	0.34
5. Types of anti-diabetic drugs						
- Oral anti-diabetic drug	22 (37.29)	171 (72.46)	1			
- Insulin	11 (18.64)	3 (1.27)	27.26	7.07	105.02	0.0001*
- Combined insulin and oral anti-diabetic drugs	25 (42.37)	47 (19.91)	3.95	2.06	7.59	0.0001*

Table 2 Factors related with hypoglycemia (continued)

Risk factor	Number (Percentage)		OR	95% CI for OR		P-value
	Diabetic with hypoglycemia	Diabetic without hypoglycemia		Lower	Upper	
- Sulfonylurea group	21 (35.59)	116 (49.15)	1			
- Other group of oral anti-diabetic drug	38 (64.41)	120 (50.85)	1.75	0.97	3.16	0.06
6. Comorbid diseases						
- DM related diseases**	24 (40.68)	99 (41.95)	0.95	0.86	0. 53	1.69
- DM related Complications***	6 (10.17)	19 (8.05)	1.29	0.06	0.49	3.40
- Others	3 (5.08)	3 (1.27)	4.24	0.08	0.83	21.56

*P-valve < 0.05

** DM related diseases : hypertension, dyslipidemia

*** DM related complications : heart failure, peripheral arterial disease, DKA (diabetic ketoacidosis), HHS (hyperosmolar hyperglycemic state)

Discussion

Hypoglycemia is a preventable event that is commonly found in diabetic patients. In 2018 - 2020, there were 3,874 visits of type 2 diabetic patients in Wiang Pa Pao district and had 674 hypoglycemic events. Preventing hypoglycemia could help to reduce expense, morbidity and mortality. This study is a retrospective case - control that identifies the main factors associated with hypoglycemic episodes in type 2 diabetic patients at Wiang Pa Pao hospital during 2018-2020. The number of cases and controls are 59 and 236 respectively. The study factors are including age, body mass index, HbA1c, cholesterol level, anti-diabetic medications and comorbid diseases.

The research found that age was not associated with hypoglycemic events in type 2 diabetic patients which corresponded to the study from Chanakarn Chaitanakul[3]. However, body mass index was statistically associated with hypoglycemic episodes. In BMI of 18.5-22.9 kg/m^2, patients had a risk of developing hypoglycemia more than patients who have BMI 23 kg/m^2 (P-value 0.02, OR 2.51). In BMI < 18.5 kg/m^2, patients also had a risk of developing hypoglycemia more than patients who had BMI 23 kg/m^2 (P-value < 0.0001, OR 7.14). This result was opposite to the study from Chanakarn Chaitanakul that body mass index was not associated with hypoglycemia. In patient with low BMI, the capacity of insulin secretion was decrease then the body was used to the low level of insulin secretion. So, when the patient received the treatment with insulin, the body will loss glycemic balance and resulted in hypoglycemia. As for the HbA1C parameter, the result found that for the HbA1C of 10.1% - 13% there was a risk

for hypoglycemic episodes more than the patients with HbA1c of 4% - 7% (P-value 0.02, OR 2.14). This result was different from the study of Thuanjai Poosakaew[4], because of time collecting of HbA1c. One study shows that the patient with uncontrolled diabetes might experience hypoglycemic symptoms when blood glucose values were in normal range and might have blunted hypoglycemic awareness.[6] The researcher also found that total cholesterol level was not associated with hypoglycemia which opposite to the study from Thuanjai Poosakaew[4] because the different in categorized range of cholesterol level. Using insulin had a higher chance of developing hypoglycemic episodes compared to oral anti-diabetic medication. (P-value < 0.0001, OR 27.26) and the patients who used both oral anti-diabetic drugs and insulin, had a higher risk of developing hypoglycemic episode more than oral anti-diabetic drugs alone. (P-value < 0.0001, OR 3.95). Finally, comorbid diseases were not associated with hypoglycemia.

Because this is a case control study which received the information from electronic hospital database system, the researcher could not study other risk factors those were not recorded in the system. For example, the researcher could not evaluate the compliance of using anti-diabetic drugs, the educational level of patients and the duration of diabetes. Although those factors might be associated with hypoglycemic episode. Another limitation of this study is the number of cases which less than the calculated sample size.

Conclusion

This case control study found that factor associated with hypoglycemia in type 2 diabetic patients who received treatment at Wiang Pa Pao district hospital were level of HbA1c, body mass index and types of anti-diabetic medications. The higher initial HbA1c level, the greater the chance of having hypoglycemic episodes. Additionally, patients who had low body mass index were at increased risk of hypoglycemia. The type of diabetic medications also had an effect. Patients who used only insulin injection and patients who used combination of insulin and oral agents, had a higher risk of hypoglycemia than patients who used oral medication alone.

Finally, this research was created for studying the risk factor that associated with hypoglycemia in type 2 diabetic patient of Wiang Pa Pao hospital. The result is helpful for creating plan to prevent the hypoglycemic episode. It can reduce the cost of treatment and can help produce better quality of life for diabetic patient of the community.

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Conflict of Interest

The authors declare no potential conflict of interest in this study.

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Self-Administered Moxibustion of Dyslipidemia in Diabetic Patients: A Randomized, Double-Blind, Controlled Trial

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Abstract:

Background: Dyslipidemia is a common disease and a public health problem. Dyslipidemia is one of the leading risk factors of cardiovascular diseases that reduce quality of life, and cause premature deaths. Acupuncture and Moxibustion is a part of Traditional Chinese Medicine that has much research for their effectiveness in lowering blood lipid levels.

Objective: We aimed to study the effectiveness of Fenglong (ST 40) acupoint in lowering blood lipid level.

Methods: A randomized controlled clinical trial in 30 diabetes type II with dyslipidemia subjects was performed. Subjects were randomized into 2 group with 15 subjects in each group. The case group received box-moxibustion at Fenglong (ST 40) acupoint bilaterally and the control group received box-moxibustion at Shangjuxu (ST 37) bilaterally for 8 weeks under the instruction and supervision of a medical acupuncturist and a TCM practitioner for how to locate and apply the device. All subjects were prescribed to continue their individual antidiabetic drugs and take regular diet as usual. Subjects were assessed at the beginning and 8 weeks later. Fasting blood sugar (FBS) levels were also assessed.

Results: A significant reduction of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and FBS were observed only in cases.

Conclusion: Self-administration moxibustion in only Fenglong (ST 40) acupoint is effective in lowering TC and LDL-C.

Keywords: Acupuncture, Moxibustion, Dyslipidemia

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Background

Dyslipidemia is a common disease and a public health problem. Dyslipidemia is one of the leading risk factors of cardiovascular diseases that reduce quality of life, and cause premature deaths.[1] Statin drugs are needed to treat dyslipidemia if lifestyle changes do not work. Statins are prescribed for such treatment in order to reduce LDL-C levels, but statins can cause some adverse effects, which are intolerable by some patients such as elevated liver enzymes, rhabdomyolysis, etc.[2] Serious side effects such as intracerebral hemorrhage, cancer can eventually develop.[2,3] Moreover, in observational studies by Unai et al., clinical trials and meta-analyses indicated an increased risk of developing new-onset type 2 diabetes mellitus (T2DM) after long-term statin treatment. It has been shown that statins can impair insulin sensitivity and secretion by pancreatic β-cells and increase insulin resistance in peripheral tissues.[4]

Acupuncture & Moxibustion is a part of Traditional Chinese Medicine (TCM) that has much research for their effectiveness in lowering blood lipid levels.[5,6] Liu et al. analyzed the frequency of acupoints employed in 65 research articles by retrieving the main database of Chinese and English version clinical research literature on the acupoints for hyperlipidemia treated with acupuncture and moxibustion. It was found out that the first five top acupoints with high frequency use include Fenglong (ST 40), Zusanli (ST 36), Sanyinjiao (SP 6), Neiguan (PC 6) and Tianshu (ST 25). No any article included Shangjuxu (ST 37) within the composition of the acupoints.[7] It would be desirable to control dyslipidemia by safe and effective treatment modality. Among different methods, acupuncture & moxibustion is one of the most popular complementary treatments. Acupuncture & moxibustion is performed by stimulating particular points on the skin called acupoints. Thus, we aimed to perform a randomized controlled clinical trial in diabetic patients who suffer from dyslipidemia in order to examine its effectiveness in changing the lipid profile by moxibustion.

Figure 1 Warm-needling acupuncture

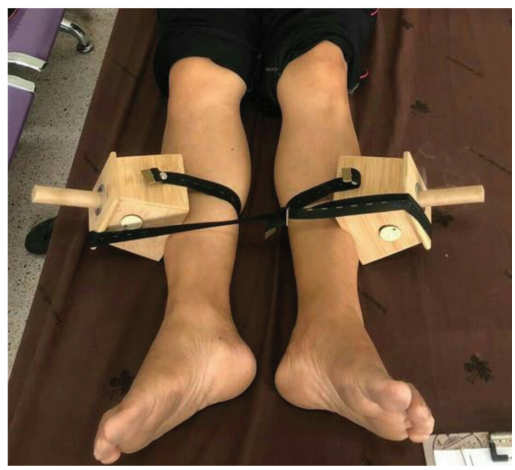

Figure 2 Box-moxibustion

Material and Methods

This was a prospective double-blind randomized controlled trial that was conducted between case and control group. Diabetic patients at DM clinic, Lansak hospital, Uthai Thani, Thailand, that was diagnosed dyslipidemia from blood test screening. Dyslipidemia in diabetic patient is defined as LDL-C level from 100 mg/dL and up.

Patients who had a history of bleeding disorders or were receiving anticoagulant or anti-platelet medications, had epilepsy, uncontrolled hypertension, diabetic neuropathy and active dermatological lesions at the area of moxibustion, as well as pregnant women or those patients whose mental disabilities made their participation in the study difficult were excluded from the study.

Written informed consents were obtained from all participants before their enrollment into the study. Participants were informed that they would be randomly assigned to one of the 2 study groups below by a statistician who was not involved in the implementation phase of the study using a Block of Two Randomization.

The TCM practitioners in Lansak hospital did not have any idea about using acupuncture & moxibustion to treat dyslipidemia before that, because they were new graduate and no lesson was taught for this disease, they just practice in very common diseases that known to be of help, so both doctors and participants were concealed about which acupoint was belong to real or sham. The moxibustion boxes were the same model with the width of the base measured 7.8 cm from both outer walls.

The study was done according to the principles of the Declaration of Helsinki. The study protocol was approved by the ethics committee of the Medical Science Ethics Committee of Dhurakij Pundit University.

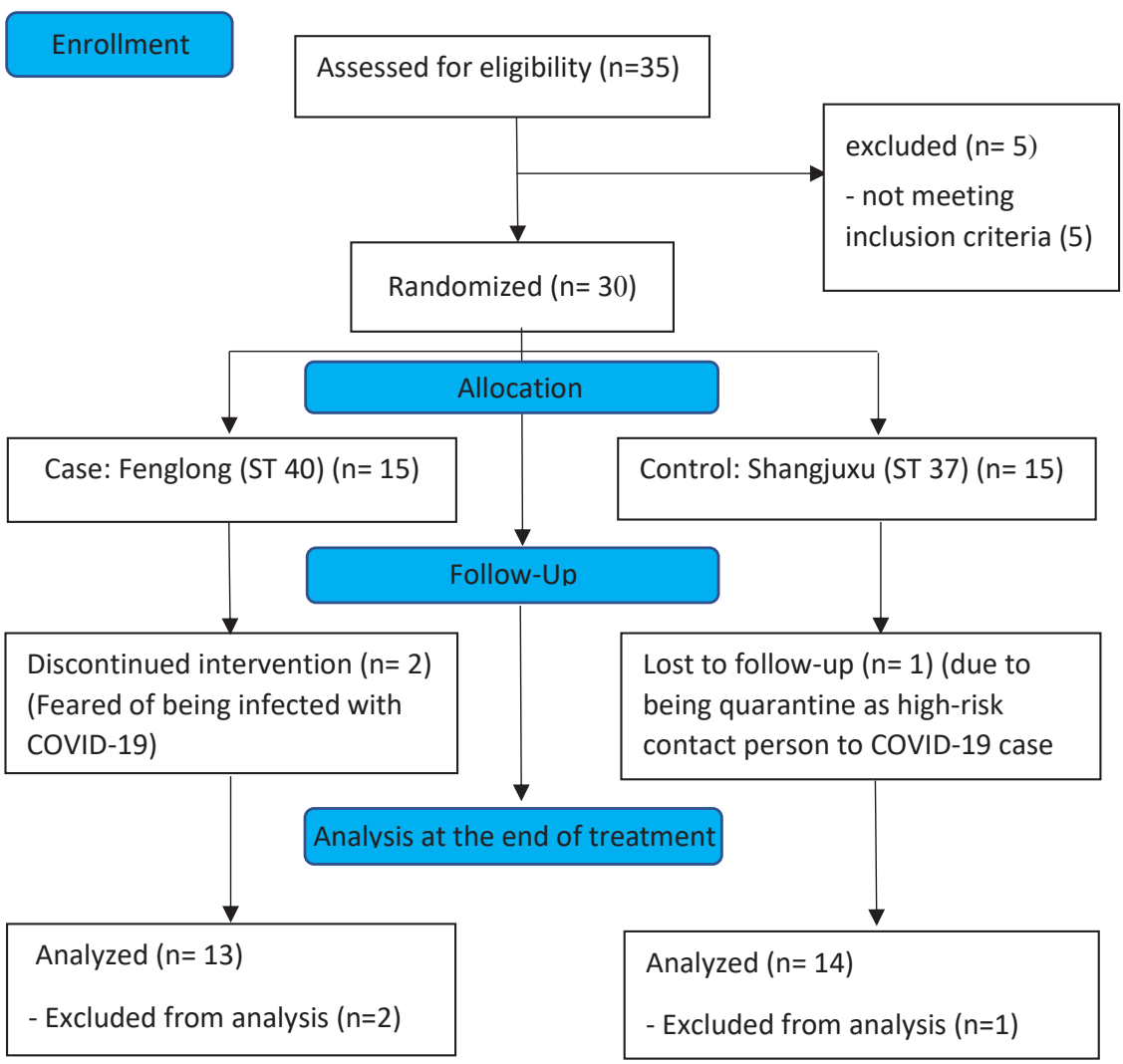

Figure 3 Flow diagram

Study Design and Subjects

Thirty subjects aged between 35-60 years old with LDL-C level 100-189 mg/dL, who denied to start with statin drugs firstly were recruited in this study, they were requested to visit twice a week for eight weeks of the study. Lipids profile and fasting blood sugar were measured before and after the experiment.

Sample Size Estimation and Statistical Analysis

The sample size was calculated based on a study of Rerksuppaphol & Rerksuppaphol.[6] Stata Software, Version 12.0 was used to calculate sample size, Estimated sample sizes for a two-sample means, based on the two-sided test, 5% α error and 90% power, the number of patients needed for the study was calculated to be 10 for each group. We considered p-value of less than 0.05 to establish the level of significance. All statistical analyses and graphics were performed using Stata Software, Version 15.0 (StataCorp). Data was presented as number, percentage and mean ± SD.

Moxibustion method

In the case group. Bilateral Fenlong (ST 40) on both lower legs were selected. The subjects were instructed by a medical acupuncturist and a TCM practitioner to locate the acupoints and strap the device correctly while they were sitting on bed and flexing the knees (Figure 1). After moxa stick (Han Yi) was lighted up, it was inserted into the hole of the moxibustion box (Figure 2). Subjects then placed the cap on the box body and adjusted the deepness needed to maintain the heat as much as they could tolerate. Each moxibustion treatment lasted for 20 minutes. After finishing the treatment, the device was released and the remaining moxa stick was pressed in a stainless-steel container to put out the flame of moxa stick and for further uses. All subjects were asked to receive two treatment sessions per week for a total of 8 weeks. During the treatment, subjects were allowed to stretch their knees or lie down for relaxation. In the control group, bilateral Shangjuxu (ST 37) on both lower legs were used and all the procedures were in the same manner as the case group. Adverse effects and satisfaction rate were evaluated. Although both Fenglong (ST 40) and Shangjuxu (ST 37) acupoints are in the Stomach Meridian and located between the groove of Tibia and Fibula (the passage of the meridian), they only have common effects on gastrointestinal tract disorder and local effects nearby. Fenglong (ST 40) is a special acupoint that can reduce all kinds of phlegm, but not for Shangjuxu (ST 37). Adverse effects and satisfaction rate were evaluated.

Anthropometric Measurements. Body weight (BW) and height were measured. The body mass index (BMI) was calculated as weight (kg) divided by height in meters squared (m^2). The distances between the two acupoints in each subject were measured in straight longitudinal line perpendicular to the foot with the range of 4.9 to 6.0 cm. (min.-max.). As a result, the two acupoints of each subject were not covered within a moxibustion box when applied to the individual of the two groups given the distance from the center of the moxibustion hole to the outer wall is 3.9 cm.

Collection of Blood Samples. Blood samples were taken from each patient for analysis after a 12-hour fasting, 2 times during the study (at the beginning and 8 weeks later). After venipuncture, blood samples were collected into Vacutainer tubes.

Biochemical Analysis. Lipid profile composed of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) including fasting blood sugar (FBS) level were measured at the laboratory of Lansak Hospital, which was certified by Department of Medical Science, Ministry of Public Health, Thailand.

Statistical Analysis. Data was checked for normality and homogeneity of variances. Values were expressed as percentage and mean ± SD. Independent t-test was analyzed for comparison between two groups. A two tailed P value of < 0.05 was considered statistically significant. Per protocol analysis was done for the better reflection to the effect of treatment with adequate statistical power.

Evaluation. The ability to locate acupoint and apply the device was correctly evaluated by our staffs.

Results

Demographic Data. Thirty participants fulfilled the inclusion criteria. Then, they were divided into 2 groups (cases and controls), by Block of Two Randomization, including 15 subjects in each group. By the end of the study, 3 subjects withdrew from the study; two subjects in case group declined to continue treatment for fear of COVID-19. One subject in control group was not available to get her blood sample after cessation at 8-week treatment given she was requested to self-quarantine as a high-risk contact person to a COVID-19 patient, so 27 participants completed the study (Figure 3). The analysis showed that sex, age, and BMI were not significantly different between cases and controls ($P > 0.05$) as shown in Table 1.

Table 1 Comparison of characteristics between case group and control group with Chi-square and Independent t-test

Group	Case	Control	Statistic used	p-value
Sex (M: F) n (%)	4:9 (30.8: 69.2)[a]	6:8 (42.9:57.1)[a]	0.695[c]	0.402
Age	52.77 (7.42)[b]	55.57 (5.45)[b]	-1.125[d]	0.271
Height	159.77 (7.34)[b]	160.07 (7.65)[b]	-0.105[d]	0.918
BW	74.10 (19.46)[b]	75.26 (16.45)[b]	-0.167[d]	0.868
BMI	29.04 (7.72)[b]	29.19 (4.86)[b]	-0.060[d]	0.953

M = Male; F = female; [a] Presented as n and percentage in proportion; [b] Presented as mean (SD); [c] Chi-square test; [d] Independent t-test

Comparison of lipid profile and FBS levels between the case and control Group at baseline. The analysis showed that lipid profile and FBS were not significantly different between cases and controls ($P>0.05$). Biochemical levels of participants were summarized in Table 2.

Table 2 Comparison of the mean (SD) of FBS, TC, TG, HDL-C and LDL-C between case group and control before study with independent t-test

Group	Case	Control	p-value
FBS	176.69 (66.81)	155.14 (59.92)	0.385
TC	209.08 (52.02)	222.57 (41.07)	0.460
TG	203.46 (103.70)	160.93 (73.62)	0.228
HDL-C	44.15 (9.13)	48.43 (7.53)	0.195
LDL-C	137.38 (36.20)	141.93 (38.66)	0.756

FBS: fasting blood sugar; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol

Comparison of the changes in different parameters between the case and control group. The analysis showed that TC, LDL-C, and FBS were significantly reduced ($P<0.05$). (Table 3)

Table 3 Comparison of the mean (SD) of FBS, TC, TG, HDL-C and LDL-C before and after in case group at the end of the study with Paired t-test

Group	Before	After	p-value
FBS	176.69 (66.81)	121.54 (31.65)	0.047
TC	209.08 (52.02)	174.54 (28.42)	0.018
TG	203.46 (103.70)	187.69 (119.04)	0.437
HDL-C	44.15 (9.13)	42.46 (13.54)	0.529
LDL-C	137.38 (36.20)	98.85 (21.69)	0.000

FBS: fasting blood sugar; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol

Comparison of the changes in parameters before and after the intervention within the control group. The analysis showed that there was no significant change in any of the parameters ($P > 0.05$). (Table 4)

Table 4 Comparison of the mean (SD) of FBS, TC, TG, HDL-C and LDL-C within control group at the end of the study with Paired t-test

Control	Before	After	p-value
FBS	155.14 (59.92)	164.50 (78.39)	0.515
TC	222.57 (41.07)	221.71 (44.95)	0.930
TG	160.93 (73.62)	155.07 (72.45)	0.746
HDL-C	48.43 (7.53)	46.36 (8.49)	0.234
LDL-C	141.93 (38.66)	144.36 (46.72)	0.795

FBS: fasting blood sugar; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol

Comparison of the changes in different parameters between the case and control group. In the case group, significant changes were found in TC (P = 0.003) and LDL-C (P = 0.004) but not in the control group. However, the other parameters namely TG, HDL-C, and FBS were not significantly changed between the case and control group (P > 0.05). (Table 5).

Table 5 Comparison of the mean (SD) of FBS, TC, TG, HDL-C and LDL-C between two groups at the end of the study with Independent t-test.

Group	Case	Control	p-value
FBS	121.54 (31.65)	164.50 (78.39)	0.078
TC	174.54 (28.42)	221.71 (44.95)	0.003
TG	187.69 (119.04)	155.07 (72.45)	0.394
HDL-C	42.46 (13.54)	46.36 (8.49)	0.375
LDL-C	98.85 (21.69)	144.36 (46.72)	0.004

FBS: fasting blood sugar; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol

The ability to locate acupoint and apply device. The subjects could locate acupoint and apply the device correctly at the range between 3 to 5 session of the experiment.

Discussion

The study showed that there is no significant difference in sex, age, BMI, and blood chemistry between the two groups (P > 0.05). When compared the result before and after intervention within the case group, a significant reduction in FBS (P = 0.047), TC (P = 0.018), and LDL-C (P = 0.000) were observed, whilst in the control group, no significant change in the blood chemistry levels (all P > 0.05) was observed. When compared the result after intervention between two groups, there were significant reductions in TC (P = 0.003) and LDL-C (P = 0.004) in cases, but no significant reduction in FBS level. Table 3-5 summarizes the levels of the parameters.

Consistent with our results, which indicated the efficacy of moxibustion therapy for reduction of lipid parameters in cases compared to controls, Liu et al. performed a meta-analysis on comparison of the effects of moxibustion and lipid-lowering drugs for primary hyperlipidemia. He concluded that in comparison with statins and fibrates, moxibustion had advantages in lowering TC and LDL-C.[7,8] Other studies had found the same result as ours regarding the reductions in TC and LDL-C. Rerksuppaphol and Rerksuppaphol performed a randomized controlled trial to compare the efficacy of electroacupuncture (EA) of body acupoints and Fenglong (ST 40) in controlling serum lipids in patients with dyslipidemia. Patients were randomized into two treatment groups (body acupuncture with 20 acupoints group and Fenglong group) and a control group. At the end of the treatment (week 8), TC and LDL-C in both active treatment groups were significantly lower than their baseline levels; meanwhile, in the control group, TC and LDL-C at the end of treatment were higher than baseline ($P < 0.01$). There was no statistically significant difference between TC and LDL-C levels between body acupuncture and Fenglong groups.[6] Li and Wang also reported significant changes in TC and LDL-C in acupuncture therapy compared with control subjects.[9]

Some studies reported the inability of acupuncture to increase HDL-C level and some other do not. M.T. Cabioglu and Ergene reported significant decreases of TG, TC, and LDL-C, but no changes in HDL-C in electroacupuncture group compared to controls. It had been suggested that these changes in lipid metabolism might be caused by increase in the serum beta-endorphin levels.[10]

While many studies showed favorable outcome for 4 major parameters of lipid profile (decrease of TG, TC, LDL-C, and increase of HDL-C). Abdi et al. performed a randomized controlled clinical trial in obese subjects to examine the effectiveness of body acupuncture on lipid profile and other parameters. Subjects received acupuncture for 6 weeks in combination with a low-calorie diet for a total of 12 weeks. After 6 weeks, significant reductions of TC ($P<0.001$), TG ($P<0.001$), HDL-C ($P<0.05$), and LDL-C ($P < 0.001$) were observed in authentic (cases) acupuncture subjects. In the sham (controls) acupuncture group, TC ($P < 0.01$), HDL-C ($P < 0.001$), and LDL-C ($P < 0.01$) were reduced significantly. At week twelfth, in the authentic group, there was significant reduction of TG ($P < 0.01$) and LDL-C ($P < 0.001$) but increased in HDL-C ($P<0.001$). In the control group, significant reduction was observed in TG ($P < 0.01$) and LDL-C ($P < 0.05$) but increase in HDL-C ($P < 0.001$). This meant both acupuncture and diet control played roles in significant changes in lipid profile and produced a sustained effect, which was more significant in cases compared to controls.[11] Yuan et al. reported a clinical study in obese adults with dyslipidemia, which participants were categorized by TCM syndrome differentiation into 6 different types. Acupoints were selected for acupuncture and moxibustion according to the syndrome differentiation, with additional warm-needling acupuncture in 2 acupoints being applied to the 3 of the 6 types (those with dampness or deficient syndrome). Treatment was performed every other day for 3 months to examine lipid profile before and after the study, it revealed that the mean difference of TC, TG, and LDL-C decreased significantly ($P<0.01$), and HDL-C increased significantly ($P < 0.01$).[12] The same result was obtained in other study, Huang et al. reported study in cases of severe obesity complicated with hyperlipidemia by categorized the subjects into 6 types of syndrome differentiation which was similar to Yuan et al. By performed warm-needling acupuncture

30 minutes every other day for 3 months.[13]

Chen et al. recruited 76 cases of hyperlipidemia patients to be treated with moxibustion at the same acupoints in different time, 3 times per week for 8 weeks. They were randomly divided into three groups: 10-minute group (group A, 25 cases), 20-minute group (group B, 25 cases) and 30-minute group (group C, 26 cases). Blood lipid and fasting blood glucose were observed before and after treatment. There was significant decrease in TC, TG, LDL-C, and FBS (all $P < 0.001$), but no significant difference of HDL-C ($P>0.05$) after treatment. The group C played more prominent role than group A in regulating TC ($P < 0.01$) and LDL-C ($P < 0.05$), there was no significant difference between group C and group B ($P > 0.05$). In conclusion, the degree of lowering lipid level varies with time of moxibustion, and moxibustion for 20-30 minutes was significantly better than that of 10 minutes.[14]

Zhang et al. studied the effect of electroacupuncture (EA) and its mechanism at "Fenglong" (ST 40) on rats with hyperlipidemia. After the treatment of EA at "Fenglong"(ST 40), the contents of TC, LDL-C significantly decreased (all $P < 0.01$), and the contents of TG, HDL-C did not change materially (all $P > 0.05$). So, EA at "Fenglong" (ST 40) had some therapeutic effects on decreasing the content of TC, LDL-C in rats of hyperlipemia and improve the gene expression of ABCA1, PPAR-alpha, LXR-alpha and RXR-alpha mRNA by promoting reverse cholesterol transport.[15] However, it should be noted that this could be a part of the mechanism of how acupuncture works and there could also be some other explanatory mechanism.

In our study, there was also significant reduction for TC ($P < 0.05$) and LDL-C ($P < 0.001$), but not TG and HDL-C. This may be explained by application of different acupoints in most studies whilst we employed only Fenglong (ST 40) acupoints in clinical trial regardless of syndrome differentiation, diet control program, duration of intervention, follow up visit to observe the sustained effects, etc. During the study, no side effect was detected. The satisfaction was rated with full scores of 5 by all participants. In subjects' point of view, these may be due to new intervention they experienced, without adverse effect, and the impression from the hospitality staffs.

In comparative experiments, Chen demonstrated that after acupuncture of "Sanyinjiao" (SP 6) for 2 - 4 hours, the blood sugar level of type-II DM patients decreased significantly, while in control group, acupuncture of non-acupoint had no obvious effect on blood sugar.[16] He also found that the effects of Sanyinjiao (SP 6), Diji (SP 8), and Yinlingquan (SP 9) used together can modulate blood sugar level via exiting vagal nerve-pancreatic islet system and reduce insulin resistances.[17] Chen et al. (2001) presented papers that compared the effects of simple acupuncture, simple herbal medicine recipe, and acupuncture plus herbal medicine recipe in type-II DM patients on serum glucagon, insulin sensitivity index, immune-cytokine, high coagulation state. The results showed that the best remedy was a combination of acupuncture and herbal medicine recipe.[18,19]

Hui et al. (2011) performed a study of 80 type-II DM, which patients were randomized into acupuncture and medication groups. Acupuncture was applied to Yishu (EX), Feishu (BL 13), Pishu (BL 20), and additional acupoints according to syndrome identification. The treatment was given once every other day for 12 weeks. For patients in the medication group, Glibenclamide (2.5-7.5 mg/time, 1-2 times/day according to blood sugar level) was given for 12 weeks. Fasting blood glucose (FBG), fasting insulin (FINS) and fasting leptin (FLP) were detected. Insulin sensitivity index (ISI) and homeostasis

model assessment-insulin resistance (HOMA-IR) were calculated. In comparison with pre-treatment, FBG levels and HOMA-IR in both acupuncture and medication groups, and FINS and FLP levels in the acupuncture group were decreased significantly ($P < 0.01$), while ISI in both acupuncture and medication groups, and FINS level in the medication group were increased remarkably after the treatment ($P < 0.01$). Comparison between two groups showed that after the treatment, FINS and FLP levels, and HOMA-IR of the acupuncture group were considerably lower than those of the medication group ($P < 0.01$), while ISI of the acupuncture group was significantly higher than that of the medication group ($P < 0.01$). It implied that acupuncture therapy is effective in lowering FLP level, which may contribute to its clinical effect in improving type-II DM.[20] In terms of traditional medicine, it is believed that acupuncture regulates the qi and blood in the body via meridian and acupoints to normalize the internal organs' functions, thus reversing the pathology induced by the imbalance of the body homeostasis.

We could not find any study that had evaluated the effects of moxibustion therapy on diabetic patients comorbid with dyslipidemia, by searching keywords in PubMed, Google scholars, China National Knowledge Infrastructure (CNKI), VIP database, Science Direct, Oxford Open, Springer Open, Cambridge Care, Hindawi Publishing Corporation, DOAJ, Thai JO, and CUJO.

Conclusion

Self-administration moxibustion is found to be effective for reduction of TC and LDL-C in diabetic patients with dyslipidemia. It is safe, convenient and economical. Moxibustion can be used as a proffered or synergic treatment option for lipid control. Moreover, moxibustion can be administered by patients themselves at home after practicing under supervision of acupuncturist for 4-5 times.

Abbreviations

BMI: body mass index; DM: diabetes mellitus; EA: electroacupuncture; FBS: fasting blood sugar; FINS: fasting insulin; FLP: fasting leptin; hr.: hour; ISI: Insulin sensitivity index; kg: kilogram; LDL-C: low-density lipoprotein cholesterol; m^2: square meter; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment-insulin resistance; SD: standard deviation; TCM: Traditional Chinese Medicine.

Conflict of interest statement

The authors have no conflict of interest to report.

Role of funding source

The study sponsor had no role in the planning, execution or analysis of the study

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Fort Pichaidaphak Hospital (FPCDH) COVID-19 System: The COVID-19 Screening and Monitoring Tools for Patients and Active Case Finding

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Abstract:

Background: The situation of the epidemic of COVID-19 from December 2019 until the present, the violence affected the service of the secondary care unit. The insufficient medical personnel caused the heavy workload. As a result, many service recipients have to wait more than a day for access to COVID-19 screening tests. Fort Pichaidaphak Hospital is a secondary care unit has performed screening duties for the SARS-CoV-2 since the risk screening process, specimen collection, laboratory analysis, reporting the results of the examination and repeating follow-up appointments in high-risk groups undergoing quarantine.

Objective: The researchers aimed to evaluate the efficacy of The FPCDH COVID-19 system, compared with conventional system using disease investigation form (Novelcorona2) and also the satisfaction of the medical personnel and the patients who are undergoing quarantine.

Methods: The FPCDH COVID-19 system was established by the Pathology Department in two platforms: a web application platform and a Line official account platform that is connected to the system of the Department of Disease Control, Ministry of Public Health and the National Health Security Office (NHSO) to reduce the turnaround time for both reactive and proactive COVID-19 screening services and reporting laboratory results. Moreover, it was used to monitor abnormal symptoms of those who were undergoing quarantine and assessed the medical personnel and patients' satisfaction.

Results: Conventional system using disease investigation form (Novelcorona2) took longer periods of time then putting the screening personnel at risk of contacting the patient's illness for an average of 2 minutes 52 seconds per patient. The examination reporting procedure was duplicated and delayed, taking an average of 7 minutes 56 seconds per sample, while proactive FPCDH COVID-19 system took 78 minutes per 100 patients. In addition, repeating follow-up appointments in high-risk contacts who had been quarantined was able to be followed up only 58.97% by using the conventional method. Thereby increasing the

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community infection rate compared to the FPCDH COVID-19 system which was able to increase the percentage of repeat follow-up appointments in high-risk contact group to 88.93%. FPCDH COVID-19 system was able to reduce the time in the reporting process by using an average time of 34 seconds per 1 sample and reduce the time for proactive COVID-19 screening by using an average duration of 31 minutes per 100 patients. Satisfaction rate in using the FPCDH COVID-19 system was more than 85.00%.

Conclusion: FPCDH COVID-19 system is highly efficacious screening and monitoring tools for COVID-19 patients.

Keywords: COVID-19, Home isolation, Community isolation, Screening, Report

Introduction

In the situation of the epidemic of COVID-19 from December 2019 until the present several waves of violence affected the service of the secondary care unit which has insufficient number of medical personnel to meet the workload which affects many parts of the service.[1,2] Both cause service delays. As a result, many service recipients have to wait more than a day for access to COVID-19 screening tests. Fort Pichaidaphak Hospital is a secondary care unit has performed screening duties for the COVID-19 virus since the risk screening process, collect specimens, laboratory analysis, reporting the results of the examination and repeat follow-up appointments in high-risk groups undergoing quarantine.

Because COVID-19 is a disease that is spread through droplets transmission, which can be transmitted through long talks or contact with secretions from patients. In addition, in order to test for COVID-19 infection, a history of screening must be taken using a disease investigation form (Novelcorona2) in all patients. Therefore, the screening officer must spend time only taking the history and recording the information in the disease investigation form, an average of 2 minutes 52 seconds per patient. This dis not include the length of time to measure vital signs and complete other records, which was the amount of time that if personal protective equipment was not properly worn. The screening officer had the opportunity to high risk contact group.

In addition to reactive activities at Fort Pichaidaphak Hospital, The RTA (Royal Thai Army) Biosafety mobile unit had given proactive COVID-19 screening services in numerous locations such as schools, marketplaces, and neighborhoods. In order to proactively screen for COVID-19, officers were required to register the patient's personal information. which was used to sequence numbers for receiving the sampling device. Examination and reporting at least one registration officer was required and using an average of 1 hour and 18 minutes per 100 patients, resulting in long waiting periods and high-risk grouping because each proactive screening had a large number of patients.

Reporting of laboratory results was a redundant process and multichannel reporting was required. There were four reporting channels: Co-lab system of the Department of Disease Control, Ministry of Public Health, Uttaradit COVID-19 online of Uttaradit Provincial Public Health Office, Fort Pichaidaphak Hospital system, and other hospitals that sent specimens for testing.

This process was redundant and results in an average reporting time of 7 minutes 56 seconds per sample, and could also result

in errors such as incomplete or delayed results, etc.

The COVID-19 screening test according to the guidelines of Uttaradit Province, a repeat follow-up appointment was scheduled for high-risk contacts who were quarantined on the 7th day after contact with confirmed cases or if found to have abnormal symptoms such as fever, cough, sore throat, stuffy nose, etc. when the number of high-risk exposures increased It was difficult and incomplete to follow up for repeat examinations or follow up on abnormalities. In the period from April 15, 2021 to November 16, 2021, a total of 78 high-risk exposures were quarantined, but only 46 were able to follow up for repeat examinations, or 58.97%. The high-risk exposures that were not re-examined which might be a source of further spread of infection in the community.

Methods

The FPCDH COVID-19 system was established by the Pathology Department in two platforms: a web application platform used to report test results, view COVID-19 test results, monitor abnormal symptoms of those in quarantine, and follow up for re-testing. Line official account (Line OA) platform was used to register for COVID-19 testing by connecting to the identity verification system (Authentication Code) of the NHSO and used to view laboratory results for patients, It was also used to report symptoms for high-risk contact groups who were in quarantine.

Registration system for COVID-19 test and Record the Novelcorona2 form

Patients could register for COVID-19 screening by filling out their personal information, symptoms and risk history. Including the ability to choose the date and time of the examination in advance via Line OA so that patients did not have to wait for long history taking and the system would automatically assess the risks, divided into PUI groups, high-risk groups. and low-risk groups Connected to the identity verification system of NHSO. The information that the patient filled in could be reviewed and edited by the staff later. As well as being able to print a disease investigation form (Novelcorona2) immediately without the screening staff having to record again and patients could check the results by themselves through Line OA.

Proactive COVID-19 screening system

Proactively register for COVID-19 screening by filling in the necessary information for quick convenience, which patients were able to register themselves via Line OA. Once registered, they would receive a sample code. For contacting the staff to pick up the device for collecting samples to receive the service of collecting specimens at the RTA Biosafety mobile unit immediately without having to wait in line to fill in for a long time.

Laboratory Reporting System

Reporting system from FPCDH COVID-19 system Synchronized the results of the examination to the Co-lab system to send the examination results and number of examinations per day to the database of the Ministry of Health for real-time national daily reports. It also integrated the reporting system with the Uttaradit Provincial Public Health Office to be able to send all test results into the UTTARADIT COVID19 ONLINE system, which was an overview system of the province. If a positive result was found or an infection was found, the notification would be immediately sent to the LINE group of the Uttaradit's Communicable Disease Control Committee, for rapid investigation of the disease. The patient information included the card identification number (CID), name, surname, gender, age, address, and result. For preventing and restricting the patient

information, they were concealed with X, which 3 of 13 numbers for CID and the last word of surname. The people who were authorized to access patient information consist of Situation Awareness Team (SAT) of Uttaradit Provincial Public Health Office. Moreover, a reporting system was developed for other hospitals but restrict accessing to patient information. The medical personnel must register and using their own username and password to access the FPCDH COVID-19system.

Home isolation system

This was the system for tracking high-risk contact groups during quarantine that did not detect the infection the first time to let the patient know the date of the next examination and could report abnormal symptoms on a daily basis so that officer could monitor abnormalities and their current location for quarantine. This was useful for the next repeat examination appointment.

Results

In the process of reporting the laboratory results after development, officers could report results only once through the FPCDH COVID-19 system. It could eliminate redundant process which took average reporting time of 7 minutes 56 seconds (476 seconds) per 1 sample. On the other hand, the FPCDH COVID-19 system could reduce average reporting time to 34 seconds per 1 sample, which was 14 times shorter than the original method as shown in figure 1.

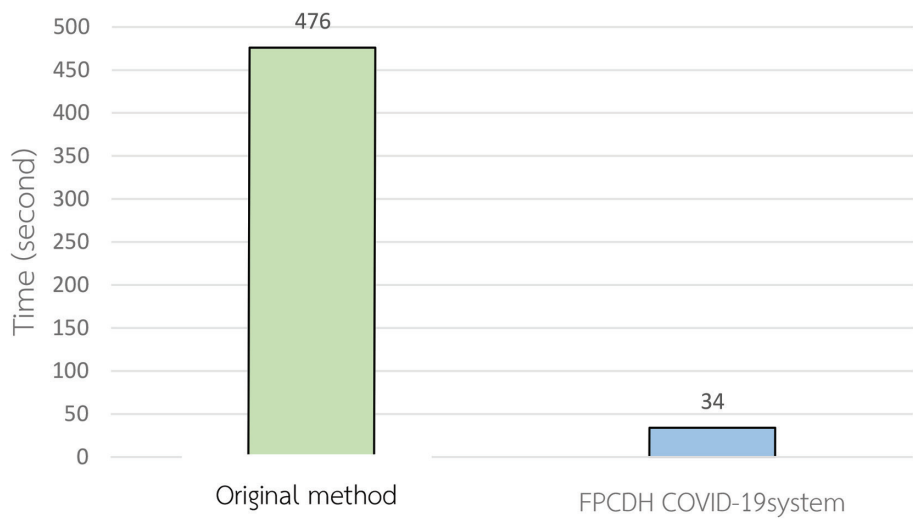

Figure 1 Time taken to report the laboratory results

The FPCDH COVID-19 system would synchronize the results of the examination to the Co-lab system, UTTARADIT COVID-19 ONLINE, Fort Pichaidaphak Hospital staff and other hospital staff who sent samples to be able to view and print the laboratory results. Currently, there were 12 hospitals registered to use the FPCDH COVID-19 system, which are: Fort Pichaidaphak Hospital (FPCDH), Uttaradit Provincial Public Health Office (UTT MOPH), Uttaradit Hospital, Laplae Hospital, Tha-Pla Hospital, Pichai Hospital, Fak Tha Hospital, Nampad Hospital, Bankhok Hospital, Thong Saen Khan Hospital, Tron Hospital and Phitsanuvej Uttaradit Hospital. The satisfaction levels of

medical personnel and patients were assessed by the questionnaire that consist of measuring the levels of difficulty and comfortability of using the application, registration process, exploring the laboratory report, and notifying symptoms in high-risk exposed groups. The satisfaction of FPCDH COVID-19 system was more than 85.00% for all hospitals as shown in figure 2.

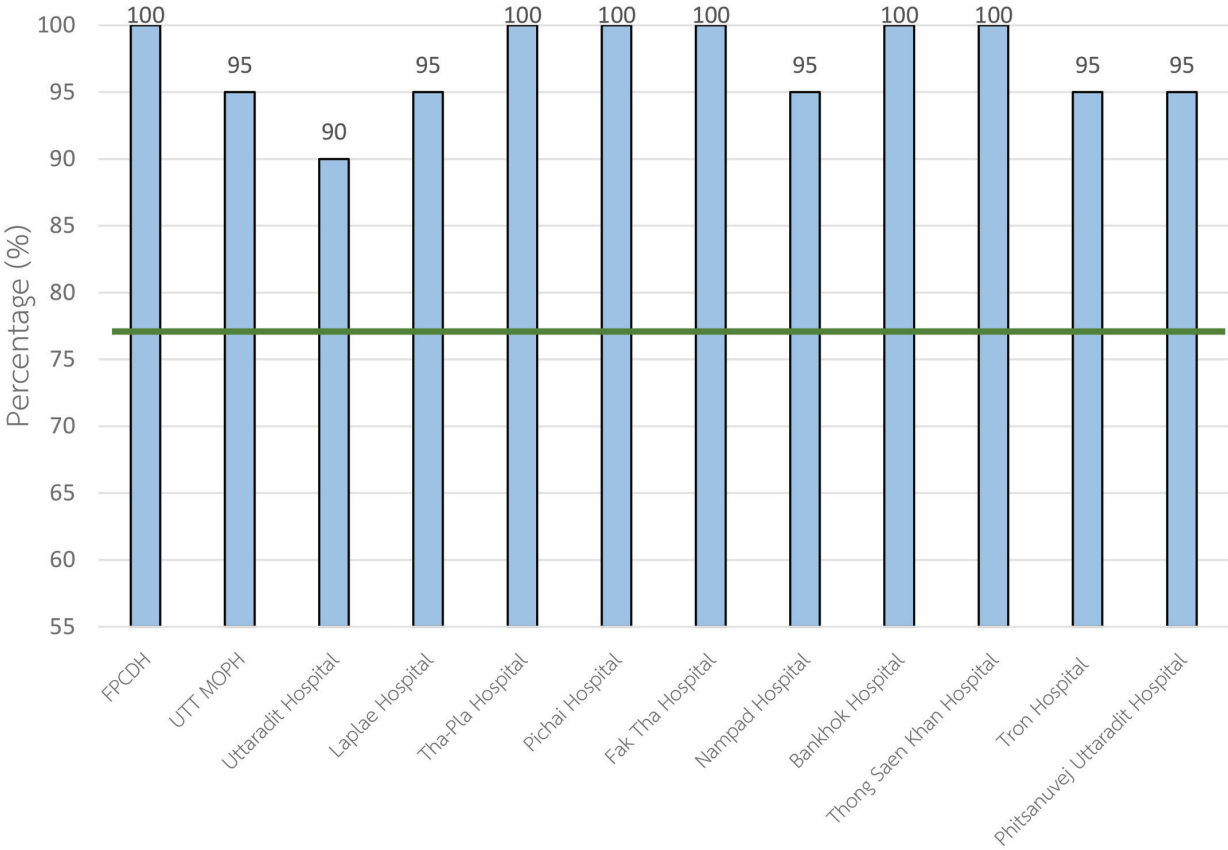

Figure 2 Percentage of satisfaction with using FPCDH COVID-19 system of community hospitals

After development, the FPCDH COVID-19 system could better facilitate patients. They could register for COVID-19 screening in advance and assess the risk accurately by themselves. In addition, officers did not need to take the history and record it in Novelcorona2 again. It could reduce the time of exposure of the personnel to screening patients or those with a history of high-risk contact group. And the patients had a level of satisfaction in using the FPCDH COVID-19 system via Line OA more than 85.00% as shown in figure 3.

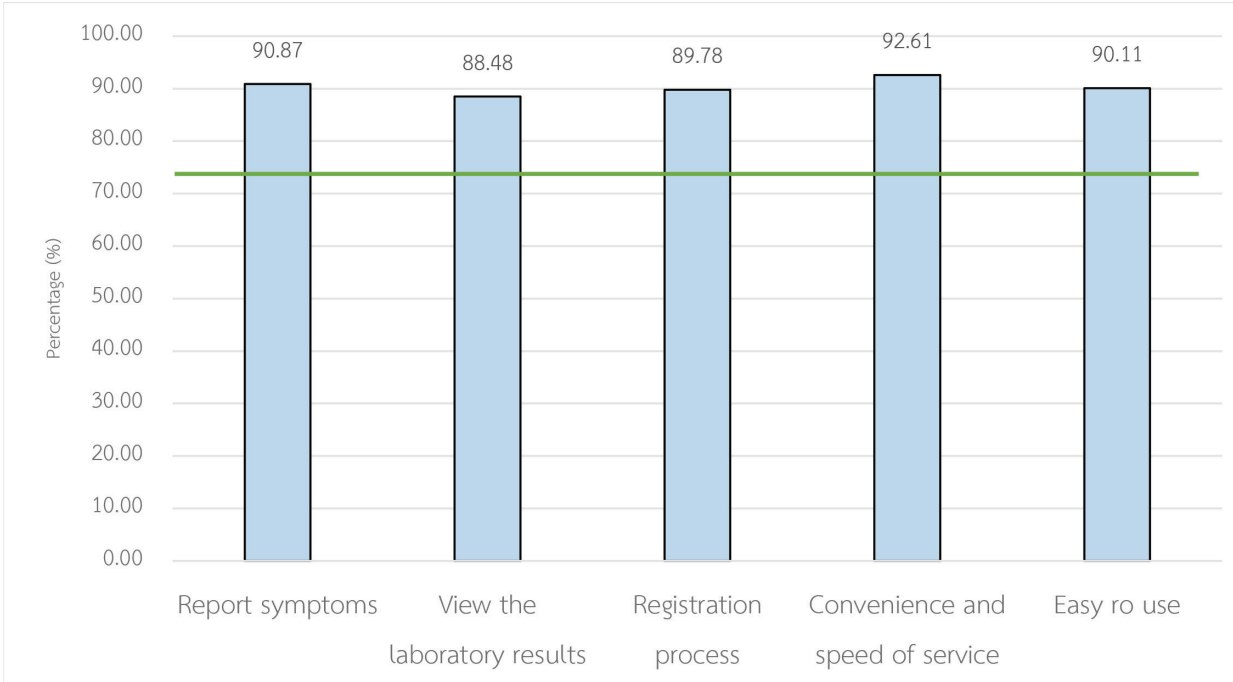

Figure 3 Percentage of users' satisfaction with using LINE OA

The RTA biosafety mobile unit was used in proactive screening. It had been developed that patients could register for screening by themselves through Line OA. Once registered, they received a sample code and brought it to contact the staff to receive the sample collection device and entered the process of collecting specimens immediately. For the original method, an average time of proactive COVID-19 screening service was 78 minutes per 100 patients. Thus, the FPCDH COVID-19 system could reduce the processing time to an average of 31 minutes per 100 patients, which was a reduction of 2.5 times from the original method, as shown in figure 4.

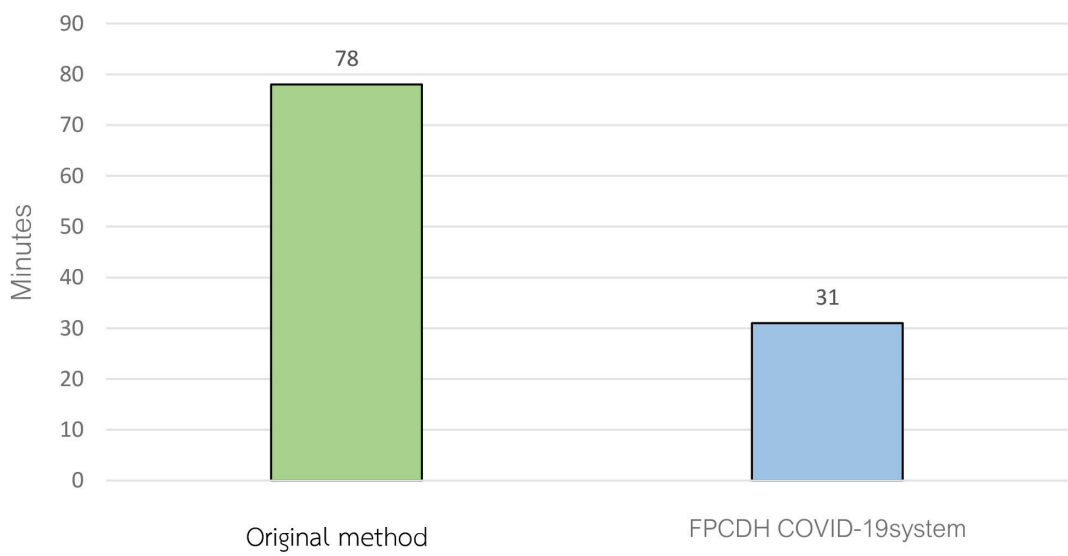

Figure 4 Duration of proactive COVID-19 screening service in 100 patients

After a home isolation system had been developed, staff could more easily monitor down symptoms of those in quarantine and had repeat follow-up examination appointments. From October 1, 2021 to October 31, 2021, a total of 614 high-risk contacts were quarantined, of which 546 were able to follow up for repeat examinations, representing 88.93%, as shown in figure 5.

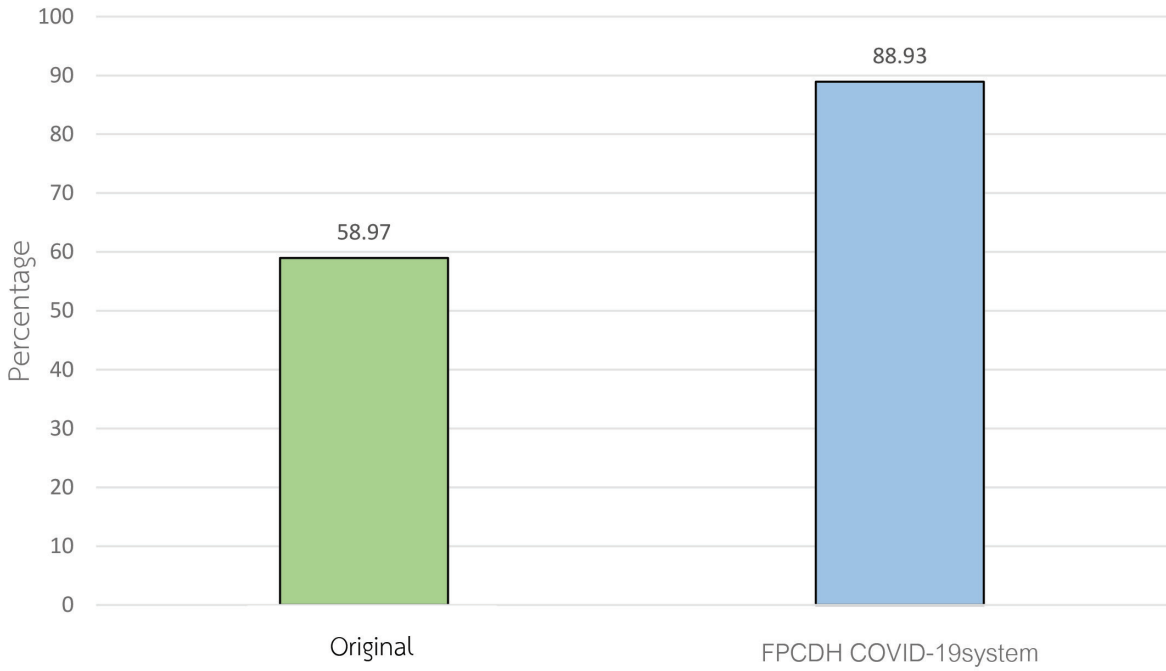

Figure 5 Percentage of repeat follow-up appointments

And patients were more than 85.00% satisfied with reporting abnormal symptoms with the FPCDH COVID-19 system via Line OA as shown in figure 3.

Conclusion

The FPCDH COVID-19 system was able to reduce the turnaround time for reactive, proactive COVID-19 screening services, and could eliminate a redundant process of reporting laboratory results. It could be used to monitor abnormal symptoms of those who are undergoing quarantine. Moreover, it could reduce the time of exposure of the personnel to screening patients or those with a history of high-risk contact group. The level of satisfaction in using the FPCDH COVID-19 system via Line OA in medical personnel and patients was more than 85.00%.

Discussion

The FPCDH COVID-19 system had many advantages such as faster screening, easy to access, reduced the contact time of the infected patient between service recipients and medical personnel. On the other hand, there were still limitations in patients who did not have smartphone or elderly patients that were unable to access FPCDH COVID-19 system.

Routine review of operating procedures for errors Look for opportunities to result in mistakes. and find redundant operational procedures until the workload was too much. Together with the use of the LEAN concept, the work process could be adjusted to be more efficient. by reducing the work process useless to provide secondary care units which had limited medical personnel who could

provide comprehensive medical services to patients. and support continuous development. As a result, it reduced operational errors and reduced unnecessary workload. In an era where digital technology and smartphones are covering more and more hospital users, applying technology to medical service systems can bring benefits in many ways such as reduce turnaround time, reduce direct contact with patients, store patient data, query or make use of databases, etc. The use of technology in the work has resulted in continuous improvement of the work.

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Physiologic Changes in The Elderly

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Abstract:

As the older population has been tremendously growing, the physiologic changes of all organ systems in increasing age should be extensively recognized. The changes include the increased risk of myocardial infarction or hypertension in cardiovascular system, respiratory failure in respiratory system, malignant melanoma in aging skin. The impaired coordination of muscles of swallowing also increases risk of aspiration. In addition, the immune system with altered function slows down the inflammation process of the body, leading to atypical presentation when the patient is acute illness. In this article, aging changes of organ systems and the potentially altered risk of diseases including the pharmacokinetic and pharmacodynamic changes in the elderly are described for the benefit of patient care.

Keywords: Physiology, Change, Elderly, Aging

Introduction

Nowadays, birth rate is declining in spite of increasing human life span. Trends of elderly populations growth occur in several countries especially in Europe and Asia.[1] Aging societies, according to United Nation, defined as the share of population aged more than 65 are exceed 7 percent of whole population in the country[2], going to pace all around the world. These global changes challenge social and family welfare system, economic systems and health care systems.[3] Aging is a natural process that occurs from accumulation of changes in any organ system, either function or number of cells and tissues. This changing process leads to progressive increasing the risk of disease and death.[3] These changes create the different perspectives of diseases between adults and elderly including epidemiology, characteristics, prognosis, complications and limitations of treatment. Therefore, physicians and healthcare workers need to know about changing in the organ system to increase their capability to treat and care elderly patients.

Theory of aging process

Currently, the process of aging is still questionable. Multiple theories were launched but were still controversial and unproved.[4,5] Generally, the process of aging is divided into intrinsic and extrinsic causes. The extrinsic causes or so-called "stochastic" are hypothesized that cumulative exposed

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of free radicals and radiation lead to cellular damage, error in protein synthesis and degradation of protein cross-linking. The intrinsic causes or "Developmental-genetic theories" are explained that there are preprogrammed, genetic controls which affect dynamic transformation of intracellular organ systems and erode homeostasis in adulthood after maturation is complete.[6,7]

Homeostenosis

Homeostenosis is the body phenomenon that decreases the ability to maintain balances of organ function under stress. Everybody is given excess capacity in organ and biologic systems called "physiologic reserve" at birth. This reserve is used to buffer any stress such as illness, trauma to maintain homeostasis. As time goes by, this reserve declines, and then aging body reduces capacity to endure any injury to organ systems. If the stress is over body reserve, it can cause disease, decompensation and death.[8,9] Figure 1 shows homeostenosis which means progressively reducing of body reserve according to increasing age and the attack of stress such as illness. At the end of the curve shows sharp decline, that means at the very end stages of life or very elderly, body reserves are easily reduced and lead to death when the stress comes out. This progressively body reserve declining definitely as "deconditioning" or "frail." [10]

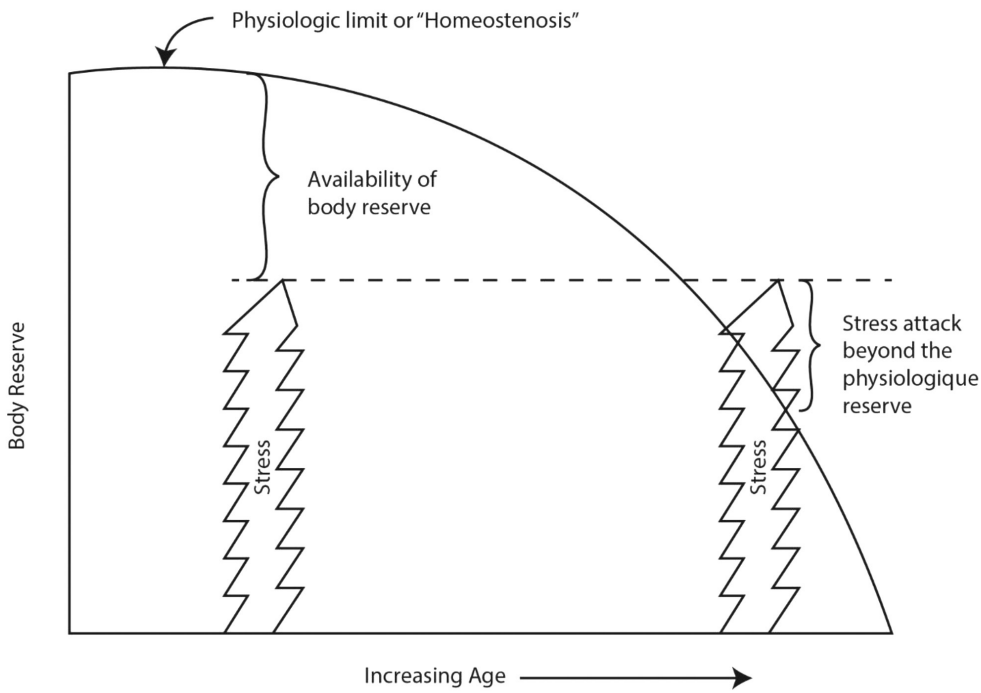

Figure1 Natural process of. homeostenosis Body composition

In the elderly, the body composition changes along aging process. Many studies show that body weight and body mass index (BMI) do not change significantly but fat mass is increased and muscle mass is decreased.[11,12] This changing process effects pharmacokinetics of the elderly then has a consequence of medical treatment of any disease in the elderly.

Theoretically, plasma protein binding and fat body mass affects drug volume distribution. Drugs with lower plasma protein binding capacities have higher volume of distribution, less plasma drug

concentration and drug toxicity. "Lipophilic drugs" refer to drugs that have high solubilities to fatty tissue, they have more capabilities to distribute drug level in extravascular tissues (Vd) than "Hydrophilic or water-soluble drugs". On the other hand, hydrophilic drugs have higher potency to concentrate in plasma volume.[13]

As aging changed, fatty mass is increased that effected the volume distribution of any drugs. Lipophilic drugs such as diazepam, thiopentone, fluoroquinolones, macrolides have more distribution of extravascular tissue (Vd) which leads to prolong half-life and accumulation of drugs. Therefore, the frequency of administration of these drugs should be monitored. In contrast, total body water in the elderly tends to be decreased, so highly water-soluble drugs or hydrophilic drugs have higher drug plasma concentrations. Hence, drug toxicity easily occurs in the elderly especially using hydrophilic drugs such as digoxin, aminoglycosides, penicillin, theophylline. All these phenomena explain why physicians should be aware when prescribing any drugs in elderly patients and have to "start low and go slow" during titration of drug dosages to achieve the most potency and least toxicity.

Skin and musculoskeletal system

Skin is the largest organ of the body that includes an area of about 20 square feet and is anatomically classified as 3 parts: epidermis, dermis and hypodermis. All parts have degenerative aging changes resulting in higher risk for skin disease than the normal population.

Epidermis and dermis become thinner. Dermoepidermal junction flattens and loss undulation. Vessel walls are also thinner. These changes increase the fragility of skin when applied with shearing stress. The skin becomes more transparent and easily bruising. Other that, thin skin is at risk of irritation and leading to wound such as skin that has been exposed to urine or feces.

Distribution of vascularity, fibroblast, fat tissue and collagen are also decreased. Hence, healing process is delayed in the elderly when they have trauma. Importantly, skin is one of protective barrier to any infections then elderly have higher susceptibility to skin infections than young adults.

The declining number of sweat glands makes skin drier and leads to higher risk of xerotic dermatitis, seborrheic dermatitis in the elderly. The number of Meissner's corpuscles and Pacinian corpuscles, which are sensory receptor, also decreased. Consequently, the elderly has less pain sensation and proprioception than young adults leading to higher risk of trauma, wound and falling.

The aging body has a change of keratinocytes, melanocytes, fibroblasts and endothelial cells. These changes cause the skin to grow when exposed to UV light and develop abnormal keratin and melanin growth, leading to skin malignancy such as actinic keratosis and melanoma.[14]

A decrease in the amount of keratinocytes and subdermal fat results in a wrinkle. The wrinkle itself affects UV exposure. An aged wrinkle is characterized by thin, finely and dry wrinkle, while photoaging or UV exposure skin appears. They are more likely to be deep, rough and lax wrinkle.[15]

According to musculoskeletal, the decreased amount of muscle mass leading to higher risk of sarcopenia. The definition of sarcopenia, according to The European Working Group on Sarcopenia in Older People (EWGSOP)[16] is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength and/or physical performance, associated with a risk of adverse outcomes such as physical disability, poor quality of life, and death. Muscle is a type of protein, therefore, when the amount of muscle is reduced, it may alter the pharmacodynamic effect of the drug-binding protein.

The aging body also reduces production of osteoblast, while osteoclast numbers remain unchanged.17 Meanwhile, 7-dehydrocholesterol production in the epidermal layer is decreased, the amount of vitamin D is increased, which attenuate calcium absorption[18] leading to osteopenia or osteoporosis and pathologic fracture in the elderly which subsequently increase morbidity and mortality.

Cardiovascular system

There are changing in endothelial wall; increased intimal thickness, vascular smooth muscle hypertrophy, fragmentation of the internal elastic membrane and an increase in the amount of collagen and collagen cross-linking in arterial walls, which may attribute to increasing afterload, systolic blood pressure and widening pulse pressure. Over the long term, ventricular hypertrophy can occur in the elderly.

Moreover, the thickening of the endothelium leads to impair endothelium dependent-vasodilatation. When the body needs more cardiac output, such as during exercising or acute illness, the body is unable to dilate the blood vessel to enhance blood flow for the increased demand of total organ system. This leads to increase risk of cardiac ischemia, coronary artery disease and peripheral vascular disease.[19]

Cardiovagal baroreflex sensitivity decreased which may induce several consequences including increased levels of BP variability, higher potency of orthostatic hypotension, an impaired ability to respond to acute challenges to the maintenance of BP, and increased risk of sudden cardiac death.[20]

From above, the arterial stiffness results in ventricular hypertrophy which make the ventricle thickening, but the ventricular mass is not clearly altered. Such age changes result in decreased end-diastolic volume, but the systolic volume remains unchanged. This leads to a decrease in myocardial relaxation and myocardial compliance, therefore the elderly is at higher risk of developing diastolic dysfunction or diastolic heart failure than young adult.[19]

In addition, myocardium has loss of myocytes, the cause of which myocytes loss is currently unknown. It was also found that there was a decrease in capillary density, both of which increased the likelihood of myocardial ischemia more easily with increasing age.[21]

Whilst both β adrenergic receptor density and the ratio of β1 to β2 receptors do not change with aging, senescent myocytes show a decreased responsiveness to β adrenergic stimulation.[22] As a result, the body is unable to drive maximum heart rate or maximum cardiac output according to stimuli or stress such as exercise, infection or shock. When the body fails to increase cardiac output, in case of increasing cardiac demand occur, this can lead to pumping failure or pulmonary edema. Other than that, the decreased responsiveness to β receptor can cause adverse effects easier than young adult when expose to beta-blocker drugs such as bradycardia or hypertension.

In addition to the change in the structure of the blood vessel and myocardium in the elderly, the autonomic regulation of heart also changes over time. According to Kuga K. et. al, the parasympathetic tone of sinus node functions is decreased, along with the conduction time of the sinus node. Study also shows that atrioventricular node is prolonged as the age increased.[23] This puts the elderly at higher risk of heart block such as sick sinus syndrome, AV block or bundle branch block than young adult.

Respiratory system

In the elderly, there are changes in the respiratory system, both in terms of structure and lung mechanics, which may not be noticeable change in normal conditions. But it results in the elderly having higher potency of respiratory failure when they have acute illness or respiratory infection. Therefore, the elderly has higher risk of intubation, prolong ventilator, longer hospitalization, morbidity and mortality outcome.

For structural changes, chest wall in elderly become stiffer. The anterior and posterior diameters of the thoracic cage change to be more likely round shape. Skeletal muscle of breathing has loose strength.[24,25] The lung parenchyma loses elasticity, causing the elastic recoil to deteriorate. In addition, the small airway has less elasticity and the airway caliber decreases. All of the changes result in decreased lung compliance and the elderly would require more effort to breathe and increase the chance of small airway obstruction more easily than young adult.

Cilia which function in the mucocilliary clearance of sweeping mucus and dirt out of the lungs[26] become slower with age. This aging change causes reduced capacity to get rid of secretion or germs like bacteria or virus from the lungs. Together with respiratory muscle atrophy with age, the elderly has decreased effort to cough to get rid of secretion. All of these put the elderly at higher risk of pneumonia and secretion obstruction.[25]

On the lung mechanics aspect of the elderly, decrease in elastic recoil and lung compliance causes reduction of the 1-second force expiratory volume (FEV1) and forced vital capacity (FVC). Study of B. Burrows et al. found that FEV1 decreased by approximately 35 mL/year, but this reduction was not directly proportional to the linear curve and found that men had a higher rate of reduction than women.[27]

Total lung capacity (TLC) in the elderly does not change with age and tidal volume remains the same. However, due to stiffer chest wall and loose respiratory muscle function, the elderly has decreased inspiratory reserve volume (IRV) and expiratory reserve volume (ERV), resulting in decreased vital capacity and increased lung residual volume. Force residual capacity (FRC) remains unchanged.[28]

Because of the increased airway resistance due to the smaller size of small airway caliber in aging change and decreasing elasticity of airway when the peak expiratory flow rate was examined, it was found to decrease with increasing age. [25,28]

Although, Force vital capacity and tidal volume decreases, minute ventilation of the elderly in resting state is not different from adulthood. Hence, it has compensation that respiratory rate should be increased. During physical exertion or stress that require an increased minute volume, these situations increase the likelihood shortness of breath and increase risk of respiratory failure especially when the elderly has acute illness.

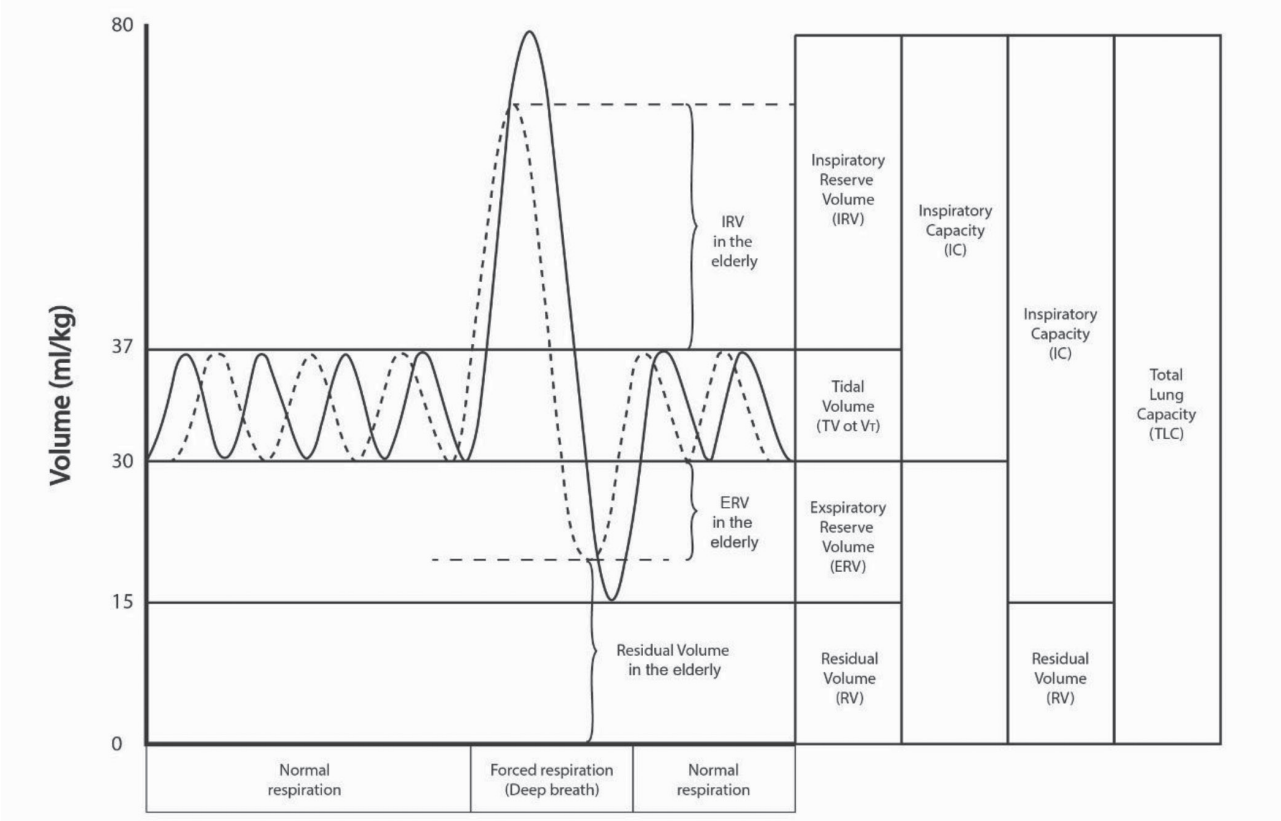

Figure 2 Schematic aging-response of lung mechanics[29]

Related to aging, the body's vessel thickens, and the lungs have more dead space and the alveolar-capillary membrane thickens. All these changes increase the chances of ventilation-perfusion imbalance.

According to H. Stam et.al study, the diffusion capacity for carbon monoxide (DLCO) was decreased with age by 0.2 mL CO/min/mmHg/year in men and by 0.15 mL CO/min/mmHg/year in women.[30]

Hypercapnic and hypoxic response were also declined with aging which resulted in no symptom or delayed response to hypoxemia or hypercapnia, presumably atypical presentation in the presence of pulmonary disease.

Gastrointestinal system

The changes are described according to anatomy as follows:

1. Oropharyngeal part

Oral epithelial mucosa is thinner and enamel production is reduced. Together with the gums are more receding. All these things make the teeth easily decayed and eroded. The function of orobuccal muscle is reduced. Some elderly who have so many dental caries or dental loss, that the number of teeth is less than 20, they are unable to close their mouth completely.

The coordination of the oropharyngeal muscles and the swallowing reflex are impaired, making it impossible to combine food into a food bolus before swallowing. Besides that, the coordination of swallowing muscle is not related to the amount of food eaten. This causes the higher risk of aspiration and leads to higher risk of aspiration pneumonia in the elderly.[30]

Additionally, the protein content of saliva changes, but the saliva flow rate remains the same. As a result of taking a variety of drugs, the elderly has higher potency to be xerostomia.[31]

According to Whelan et. Al. study, microbial population in the oropharynx in

elderly was changed in quantity and type of microbiome, therefore, immune defense from microbiome was different from adult, and led to higher risk for respiratory tract infection.[32]

2. Esophageal part

Loss of esophageal compliance, resulting in swallowing dysfunction, increases the risk of esophageal reflux and aspiration pneumonia.[33]

3. Stomach and intestinal part

In the stomach, there is a decrease in the amount of intestinal Cajal body. The Cajal body acts as a pacemaker, sending slow wave potential to the intestinal smooth muscle causing intestinal contraction. As the number of Cajal bodies decreases, the gastric emptying time and intestinal transit time are slowed down. Hence, the elderly eats less, causing anorexia due to indigestion and constipation. Moreover, the drug effect is slower due to longer transit times.[34,35]

Prostaglandins are unsaturated fatty acids and act as protective barrier of gastric mucosa from any irritants. In the elderly, there was a decrease in the amount of prostaglandins production. Therefore, the secretion of bicarbonate in the stomach is decreased, while parietal cells secretion of hydrochloric acid is the same, increasing the risk of gastritis or gastric ulcer.[36]

Due to the thickening of the vessel, the mesenteric, splanchnic and gastric blood flow is reduced, especially after meals. The body undergoes splanchnic blood pooling without compensation from the peripheral vascular system, resulting in postprandial hypotension which is one of the most common causes of syncope in the elderly.[37,38]

4. Hepatobiliary system

In the elderly, liver mass, liver perfusion, and liver blood flow are decreased while mitochondrial integrity and enzymatic activity remain unchanged.

Due to decreased hepatocyte, hepatic oxygen diffusion and liver blood flow, study of Anantharaju et.al. has shown that hepatic drug metabolism by cytochrome P450 is also reduced resulting in changes in pharmacokinetics, especially in the first part drug metabolism. Therefore, some drugs have a longer duration in the elderly and should be avoided such as benzodiazepines or some antihypertensive drugs.[39]

According to Le Coutour et.al., a decrease in hepatic perfusion affects hepatic drug clearance. If the elderly need to take the drugs with high clearance activity, the dose should be reduced by 40%, while with the low clearance drug should be reduced by 30%. Otherwise, drug toxicity may occur.[40]

Genitourinary system

With increasing age, there are structural, hemodynamic and physiologic changes of the kidney. Total nephron size and number are decreased. There are thickening of basement membrane, glomerulosclerosis, tubular atrophy and interstitial fibrosis.[41]

A decrease in the number of functional nephrons is associated with a decrease in glomerular filtration rate. The glomerular filtration rate begins decreasing 1mL/min/1.73 m^2/year, starting at 30 years of age. But the decreasing rate can change if other factors are affected, such as hypertension or drug toxicity, etc.[42,43]

Glomerulosclerosis contributing to hypertension in the elderly, but in various studies, there was no association with age-related declines in glomerular filtration rate.[40]

From these changes, when people get older, the number of function-preserved nephrons is reduced. So, the elderly has higher risk of acute kidney injury than young adults, especially when certain factors are affected, including acute illness, nephrotoxic drugs, atherosclerosis, etc.

For the lower urinary tract, a study by S. Madersbacher et.al. found that elderly

had significant decreased bladder capacity, average and peak urine flow rate, urinary voided volume but increased post-void residual volume.[44] All these changes make the elderly have frequency of urination and nocturia.

In elderly women, a significant decrease in functional urethral length and maximum urethral closing pressure puts them at an increasing risk of urinary incontinence and subsequent urinary tract infection.

On the contrary, elderly men are found that prostate volume increases which make the high likelihood of benign prostatic hypertrophy, urinary tract obstruction and urinary tract infection.

For female reproductive system, after menopause, there are changes of hypothalamic-pituitary-ovarian axis leading to atrophy of uterus, ovary, fallopian tube and vagina, reduction of cervical and vaginal secretion. All these changes increase risk of atrophic vaginitis in the elderly.

On the other hand, aging male reproductive system has effect of changing in hypothalamic-pituitary-gonadal axis too but their function is sufficient to maintain fertility in elderly men, except minimal change in sperm motility, quality and quantity.[45]

Nervous system

Related to peripheral nervous system, the β-receptor number is decreased and the response to the receptor is also decreased in the elderly. While the α adrenergic response remains normal, it does not change with age. Baroreceptor response also decreases resulting in a higher chance of beta-blocker side effects which is bradycardia and syncope than young adults.[46]

In the part of the autonomic nervous system, sympathetic overactivity is more common in the elderly while muscarinic parasympathetic activity was declined.[46]

All these changes affect the body as a whole, whether blood pressure, heart rate response to stress, cerebral blood flow or bladder activity. These increase potency of hypertension, coronary heart disease, syncope, incontinence or urinary tract obstruction, and also, change the efficacy of anticholinergic drug responsiveness.

In the aging brain, there are some losses of synaptic contacts and neuronal apoptosis that provoke age-dependent declines in sensory processing, motor performance and cognitive function. [47] The brain shrinks in volume, particularly in the frontal cortex.[48]

The shrinkage brain and synaptic loss cause memory decline in the elderly and increase risk of neurodegenerative diseases such as Alzheimer's disease, vascular dementia, etc. In normal elderly, their episodic memory function does not decline but it takes longer to recall. Multitasking and locomotive motor speeds are slower or disrupted when compared to young adult. Learning ability takes longer and more repetition than adult. Working memory or "skill" still intact when getting older. Finally, judgement and decision-making, known as "executive function", is still the same as always.[49]

Hematologic and immune system

In the elderly, bone marrow changes cellularity like every organ system. The percentage of hematopoietic cells that occupy bone marrow decreased from 40-60% in young adults to 20-40%. The remaining space is occupied by fat. However, sufficient stem cell proliferation and maturation is still preserved. Hence, the preserved transferred stem cells remain capable of normal hematopoiesis throughout entire life. Peripheral blood counts and parameters are kept unchanged.[50]

According to Davy Kevin P. et.al, total blood volume, plasma volume and

erythrocyte volume are significantly lower than in young adults.[51] These aging changes increase the tendency to anemia in the elderly, either from blood loss, nutritional anemia or myelodysplastic syndrome.

The immune response becomes altered as aging changes, called "immunosenescence". Immune response is classified as innate and adaptive immunity. The innate immune system has important components, phagocytes, which are the first barrier aggregated to eliminate aggression of bacteria and fungi, and neutrophils which have specific receptors (FMLP, GM-CSF, IL-8) to combat pathogen. Some studies show that phagocytosis and neutrophil function do not change with age but specific receptors decline signal function and chemotaxis leading to slower and less response to infection and inflammation in old age than young adults.[52]

Adaptive immunity has higher specificity to each particular pathogen than the innate immune system. Besides, adaptive immunity creates an immunological memory after an initial response to a specific pathogen and then produces an "antibody" which lasts long protection. This process also forms the basis of vaccination. T-cells and B-cells are the main components of adaptive immunity. Immunosenescence of aging reduces ability to accumulate T-cells, and decreases the output of naïve T-cells. Whereas, B-cells even decrease in number that leads to decreased secretion of immunoglobulin, they also shift antibody from foreign antigen to autologous antigen. All the changing process make elderly lower immunity to infection, lower expression of inflammation, higher risk of autoimmune disease and lower activity of vaccination.[53,54]

According to Wilkerson W.R. et.al study, the elderly was found to have increased fibrinogen, factor VIII, IX and coagulation proteins. This associates with dramatic increasing rate of arterial and venous thrombosis, like stroke, myocardial infarction and pulmonary embolism.[55]

Endocrine system

Like all other organ systems, the endocrine system undergoes age-related changes but the systems that are affected directly in the elderly and may need treatment are thyroid hormone and insulin secretion of the pancreas.

Aging changes of the thyroid involve thyroid hormone production, metabolism and action. There is an overall decrease secretion of TSH from the pituitary, T3 and T4 from thyroid gland but the distribution of TSH relatively tends to shift upward with aging. Therefore, prevalence of biochemical subclinical hypothyroidism is higher in the elderly.

Besides that, chronic diseases (such as chronic obstructive lung disease, arthritis) and drugs (such as lithium, amiodarone, glucocorticoids) can decrease T3 secretion, this phenomenon can represent like hypothyroid, even absence of thyroid disease, term "Non-thyroidal illness". Hence, thyroid function tests should be assessed carefully in the elderly.[56]

With aging change of the pancreas, apoptosis of β-cell occurs. Therefore, insulin production tends to decline with age. On the other hand, aging cell become less sensitive to insulin. The prevalence of insulin resistance is increasing with age, then the pancreas takes action with producing more insulin leading to pancreatic exhaustion. These changes make the elderly hyper-insulinemia and hyperglycemia. So, the prevalence of type 2 diabetes is higher in the elderly.[57,58]

Conclusion

There are aging changes in every organ system, resulting in the risk of developing some diseases such as atherosclerotic diseases, hypertension, diabetes

mellitus, osteoporosis, hypothyroidism or dementia more easily than young adults. It also affects the metabolism of drugs that will be used for treatment. Therefore, it is important to recognize about all these changes for the proper care of the elderly.

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