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The Effect Of Light Colour During Night Time To Sleep Quality

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Abstract

Introduction: Sleep is one of the basic human needs, where human uses one third of their time only for sleeping. Some contributing factors towards bad sleep quality are stress, depression, surrounding noise, and the use of a bright light. It is known that white lights cause the activation of melanopsin, causing circadian cycle shift, whereas using yellow light does not. The aim of this research was to analyze whether there is an effect of using either white or yellow light towards sleep quality

Method: This is RCT on 81 subjects. Sleep quality was measured using PSQI questionnaire on 3 measurements with each measurement taken every week. Result of the analyzed data using the General Linear Model – Repeated Measurement (GLM-RM) method

Result: There were significant differences on PSQI score between group that used white light during sleep (7.6 ± 2.3) in comparison to the control group (5.2 ± 1.8) ($p = 0.02$), but result also showed that there were no significant difference between the group that used yellow light (6.1 ± 1.7) during sleep in comparison with the control group ($p = 0.14$). Conclusion: There is a significant difference between the uses of white lights during sleep towards sleep quality, but there has not been any significant difference between the uses of yellow light during sleep towards sleep quality

Introduction

Human have several basic needs that needs to be fullfilled in order to survive. Maslow Hierarchy of needs is one the model that shows 5 basic needs of human, including eating, drinking, reproduction, and sleep.[1] Human use one third of their life to sleep. There are several benefits that is acquired during sleep, including memory consolidation, mood stability, the resting state of the organs, and promotes body growth.[2] A good sleep quality is not only measured by its duration, but also the quality of the sleep, the sleep-wake cycles, and daytime sleep dysfunction.[3]

A study conducted in China using the CPSQI (Chinese version of the PSQI) show that the prevalence of bad sleep quality was 41.5%.[4] Another study held in SLTP "X" Kelurahan Jati, East Jakarta show that bad sleep quality among students are 62.9%.[5] These data shows that bad sleep quality is still a common thing. Some factors affecting sleep quality are stress, depression, air quality, pain, and the use of light during sleep.[6,7] The common type of lights that are used these days are white LED (Light-Emitting Diode) light, which is a combination of blue light (450 – 470 nm) and yellow phosphor (580 nm). The use of white LED light could disrupt sleep quality because retina has a specialized cells called photosensitive retinal ganglion cells (pRGCs) which contains melanopsin. Melanopsin works as a regulator of the circadian rythm, and had peak absorption around 470 – 480 nm, which could cause increased sleep onset and reduced sleep duration.[8,9] On the other hand, yellow lights do not effect the circadian rhytm because of it's peak of emission was 580 nm and does not trigger the work of melanopsin.[10]

Objective
To observe whether there is an effect between the use of white colored light and yellow colored light towards sleep quality.

Methodology
Design
This research used numerical analytic comparative study and randomized trial with control method. Statistical analysis using method of General Linear Model – Repeated Measurement (GLM-RM).

Sample
The samples that are used in this study are medical student of Universitas Pelita Harapan who fulfills the inclusion criteria of aged 18 – 21 and have a normal BMI (18.5 – 22.9), and fulfill the exclusion criteria of consuming sleeping medication, caffeine near sleep time, using eye mask during sleep, and incomplete questionnaire answers and those who did not agree to participate in the research. They are then asked to sign an informed consent.

Method of Collecting Data
Data were collected using randomized trial control. Initially, subjects are divided into 3 groups by simple random allocation method using a table random. The three groups are in comparable condition at baseline; include a control group, a group that use white light (LED 5watt), and a group that use yellow light (LED 5watt). The control group are defined as subjects that sleep in their usual or regular sleeping environment. Afterwards, subjects will be asked to fill a PSQI questionnaire as an initial score before intervention. Afterwards, subjects will be given LED lights according to their group, which would be used for 2 weeks. PSQI score will be taken after a week of intervention and 2 weeks of intervention.

Data Analysis
Data obtained were analyzed and processed using *Microsoft Excell* 2007 and *Statistical Program for Social Science* (SPSS) 24.0.

Result
A total of 81 samples were collected from the target population and the characteristic as is shown on table 1.

Table 1. **Characteristic of subjects**

Characteristic	**n(%)**
Age	
18	3 (3.7%)
19	14 (17.3%)
20	36 (44.4%)
21	28 (34.6%)
Intervention	
White light	17
Yellow light	26
Control	38

Samples were then asked to fill the first PSQI questionnaire to see the baseline scores before each designated group was given their intervention. The scores were analyzed using ANOVA and showed that there were no significant differences ($p>0.05$) between groups before intervention, with score from *levene's statistic (*$p = 0.09$). Therefore, any difference in PSQI score between groups happen due to the intervention itself.

Table 2. ***Comparation Mean Score PSQI***

	PSQI_1	**PSQI_2**	**PSQI_3**
	(Mean ± SD)	(Mean ± SD)	(Mean ± SD**)**
White	4.8 ± 1.4	7.8 ± 2.1	7.6 ± 2.3
Yellow	5.9 ± 2.1	6.6 ± 2.3	6.1 ± 1.7
Control	5.7 ± 2.3	5.3 ± 1.9	5.2 ± 1.8

PSQI : Pittsburgh Sleep Quality Index. Good (score: ≤ 6), Bad (>6). PSQI 1: baseline; PSQI 2: a week intervention, PSQI 3: 2 weeks intervention

Table 3. ***Levene's Statistics PSQI_1(Baseline)***

PSQI_1	Levene statistic	Df1	Df2	Sig.
Based on mean	2.393	2	78	0.098
Based on median	2.117	2	78	0.127

Subjects were then given the intervention light according to their groups. PSQI score were taken after a week of intervention (PSQI 2) and 2 weeks after intervention (PSQI 3). The mean of PSQI score of each group after each week was shown in table 2.

The result showed, there are significant changes between the baseline PSQI score with the week1 score after the intervention. It could also be seen that a decline over all the group scores after 2 weeks of intervention.

Table 4. **Mean Deference a week of intervention**

Intervention group	Compared group	Mean Difference	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
White	Yellow	1.13	0.28	-0.5	2.76
	Control	2.42	0.01	0.9	3.96
Yellow	White	-1.13	0.28	-2.76	0.5
	Control	1.29	0.05	-0.03	2.63
Control	White	2.42	< 0.01	-3.96	-0.9
	Yellow	-1.29	0.05	-2.36	-0.03

PSQI score were then analyzed using ANOVA. method. Result in table 4 showed that after a week of intervention, there was significant difference between the group that used white light in comparison to the control group, therefore only the white light had effect towards sleep quality.

Table 5. **Mean difference 2 weeks of intervention**

Intervention group	Compared group	Mean Difference	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
White	Yellow	1.45	0.04	0.01	2.9
Yellow	Control	0.92	0.17	-0.25	2.11
Control	White	-2.38	<0.01	1.03	3.74

Result in table 5, showed PSQI 3 which is a significant difference between the group using white light with control group ($p < 0.01$) after 2 weeks intervention. PSQI measured between the group using white light compared to the group using yellow light ($p = 0.04$). This result showed that only white light had significant effect toward sleep quality while yellow light does not affect sleep quality to a significant level ($p=0.17$) compared with the control group. The results are then analyzed further using General Linear Model – Repeated Measurement (GLM-RM) method. This method was used to determine the effect of colored light toward sleep quality among 3 groups upon 3 measurements.

Table 6. **Accumulation of effect toward sleep quality**

Intervention group	Compared group	Mean Difference	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
White	Yellow	0.48	1	-0.76	1.73
	Control	1.31*	0.02	0.15	2.47
Yellow	Control	-0.48	1	-1.73	0.76
	White	0.83	0.14	-0.19	1.84
Control	White	-1.31*	0.02	-2.47	-0.15
	Yellow	-0.83	0.14	-1.84	0.19

Table 6. Showed that there was a significant difference between white light user with control group ($p=0.02$) while yellow color light does not have significant difference with control group ($p=0.14$). This conclude that after 3 cumulative measurements were analyzed, only white light had significant effect toward sleep quality.

Table 7. **Comparison between Groups with Control**

		Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound
PSQI 1	White light	0.15	-2,11	0,33
	Yellow light	0.64	-0,81	1,32
	Control	0[a]		
PSQI 2	White light	< 0.01	1,16	3,67
	Yellow light	0.02	0,21	2,38
	Control	0[a]		
PSQI 3	White light	< 0.01	1,28	3,48
	Yellow light	0,058	-0,03	1,89
	Control	0[a]		

[a] is used as a reference

Based on the result of parameter estimates GLM-RM in table 7, it showed that before the intervention (baseline), there are no significant differences between the comparison of white and yellow lights with pvalues 0.15 and 0.64 respectively. Therefore, any changes towards the PSQI score would merely have been due to the intervention. It also showed that after one week of intervention, both groups that used white ($p < 0.01$) and yellow light ($p = 0.02$) had significant difference to the control group. But after 2 weeks of intervention, only the group that used white light had a significant difference with the control group ($p<0.01$) while the group of yellow light had no difference ($p=0.058$)

Discussion

This research was conducted in order to examine the effect of using white or yellow light towards sleep quality. In accordance with the results shown above, showed only the white colored light had significant effect towards sleep quality. Before the intervention was conducted, a baseline measurement of PSQI which was analyzed using ANOVA in order to see the homogeneity. Result showed that there was no significant difference between the three groups, therefore any changes on the PSQI score was not caused by the variety of the subjects, but merely due to the interventional methods. As shown in table 3, there were only significant difference between the group that used white light toward control group ($p = 0.022$). This was caused by the blue LED light inside the white LED light that has a wavelength of 450 – 470 nm, whereas that wavelength could stimulate the work of melanopsin which is a part of Photosensitive Retinal Ganglion Cells (pRGCs) located inside the retina[11]. The activation of melanopsin causes a signal to be send through retino-hypothalamic tract and affected suprachiasmatic nucleus (SCN) which would release glutamate and pituitary adenylate cyclase-activating polypeptide (PACAP) that would interact with NMDA and AMPA receptor that would lead to changes in the circadian cycle[11]. Contrarily, yellow LED light with a wavelength of 560 – 590 nm would not stimulate the work of melanopsin, causing minimal effect toward sleep quality. Result of this research corresponds to another research held by Seonjin Lee and Dongwook Kim which shows that sleep latency in subjects using white light was longer than those who used other colored light.[12]

Table 2 result showed a decline over the PSQI score of all group between the 1st week and 2nd week intervention. This could happen due to adaptation toward the use of light during sleep.

Conclusion

The result showed there was a significant effect of using white LED light during sleep compared to control group, whereas there was no significant effect of using yellow LED light during sleep compared to control group.

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Relationship of Flat Foot and Plantar Fascia Thickness in Medical Students of Pelita Harapan University

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Abstract

Background : Plantar fascia plays significant role in supporting the height and structure of Medial Longitudinal Arch (MLA). In flat foot, the MLA is depressed. There is thickening of plantar fascia reported in cases of plantar fasciitis in subjects with flat foot. Therefore, research needs to be done to investigate the relation between flat foot and plantar fascia thickness.

Aim: To understand the relationship between flat foot and plantar fascia thickness among medical students of Pelita Harapan University.

Methods : This study was conducted using cross-sectional method with sampling method of non-random consecutive sampling. Data was collected through examinations performed and adjustments are made using inclusion and exclusion criteria. Flat foot was determined using navicular drop test and plantar fascia thickness was assessed using ultrasonography measurement. Results were analyzed using SPSS 22.0 and statistically tested using Spearman's rho test.

Result : The analysis of the relationship between flat foot and plantar fascia thickness showed positive correlation with correlation coefficient of 0.634 on the right foot and 0.443 on the left.

Conclusion : There is moderate and strong positive relationship between flat foot and plantar fascia thickness among medical students of Pelita Harapan University.

Introduction

Flat foot is a type of foot arch that is present in 10-25% adult population[1] and is associated with the increase of musculoskeletal symptoms felt.[2] Flat foot is characterized by medial longitudinal arch (MLA) depression, eversion of rearfoot and midfoot abduction and dorsiflexion.[3,4] Plantar fascia is one of the most important structure in maintaining the height of MLA. Study done by Huang et al. in 2004 showed that there is an increased plantar fascia thickness in some cases of plantar fasciitis found in subjects with flat foot. This is due to *microtears* and inflammatory response in plantar fascia that results in thickening of the fascia as a compensation and healing process from withstanding heavy load.

Plantar fascia forms a strong fascia band that extends from the rough surface of calcaneus to the toes. Plantar fascia (PF) plays an important role in maintaining foot arch[5], mainly because it provides primary passive support that maintains the structure and height of MLA.[6] In weight bearing position when standing up, the pressure that came from extension of PF acts as a bond on the MLA to minimize the drop of foot arch.[7]

Plantar fasciitis is the most common cause of heel pain.[8] Plantar fasciitis is associated with biomechanical factors like flat foot, foot pronation, heel valgus, sudden weight gain or obesity.[9,10,11] PF thickness > 4mm is associated with plantar fasciitis.[12,13,14] PF thickening is found in cases of plantar fasciitis with flat foot.[10]

Studies have been made to investigate the relation between plantar fasciitis with flat foot, but only a few studies that examines the direct relationship of flat foot and plantar fascia thickness. Hence, this research is done to investigate the relation between flat foot and plantar fascia thickness in university students in Indonesia.

Materials And Method

A total of 34 university students with flat feet from the medical faculty at Pelita Harapan University participated in this study. Data is collected using the navicular drop test method to evaluate flat feet and ultrasonography (US) is used to calculate plantar fascia thickness.
Navicular drop test (NDT) is one of the methods used to evaluate flat feet. Navicular drop is the difference between the distance of *navicular tuberosity* measured to the ground when standing up in a weight bearing position and sitting down (subtalar neutral position).[15] Results showing ≥ 10mm represents flat foot.[16] After the NDT, note the distance of both feet. Next, plantar fascia thickness was measured using ultrasonography.

This is a correlation study with a cross sectional design that is held at Faculty of Medicine on Pelita Harapan University, Karawaci, Tangerang starting from January until April 2019. Samples are obtained using the consecutive sampling technique. Data collected were analyzed using SPSS 22.0 software. Spearman's correlation was used to analyze the data.

Results

Most of the respondents are female (51.8%) with median value of age 20 years old. The mean weight of respondent was 62.41kg with the median of 61kg. The mean height of respondents is 165.4cm with the median value of 165cm. Mean BMI of the respondents are 22.68 kg/m^2 with the median value of 23.25 kg/m^2. Male group have a higher BMI with the mean value of 23.71 kg/m^2 when compared to the mean BMI of female group with the value of 21.95 kg/m^2.

Table 1. Subject characteristics

Subject characteristics (n=34)	n	Min	Max
Gender			
Male	14 (41.2%)	-	-
Female	20 (58.8%)		
Age (years)			
Mean ± SD	20.26 ± 0.86	18	22
Median	20		
Weight (kg)			
Mean ± SD	62.4 ± 10.1	47	87
Median	61		
Height (cm)			
Mean ± SD	165.4 ± 8.7	150	188
Median	165		
Body Mass Index (kg/m²)			
Mean ± SD	22.68 ± 2.06	18.6	24.9
Median	23.25		
Plantar Fascia Thickness (cm)			
Right foot			
Mean ± SD	0.33 ± 0.06	0.21	0.45
Median	0.33		
Left foot			
Mean ± SD	0.35 ± 0.06	0.23	0.49
Median	0.34		
NDT (cm)			
Right foot			
Mean ± SD	1.41 ± 0.24	1.1	1.9
Median	1.4		
Left foot			
Mean ± SD	1.48 ± 0.27	1.1	1.9
Median	1.4		

Table 2. Mean BMI in male and female groups

Gender	Mean ± SD (BMI)
Male	23.71 ± 1.78
Female	21.95 ± 1.95

PF thickness and NDT

Correlation between PF thickness and NDT is done using Spearman's rho method. Analysis showed correlation coefficient of 0.634 (p = 0.000) for right foot and 0.443 (p = 0.009) for left foot. Correlation analysis results showed strong correlation between PF thickness and NDT of the right foot and moderate correlation for the left foot.

Table 3. Correlation of PF thickness in left (S) and right foot (D) and NDT

	PF Thickness D – NDT D	PF Thickness S – NDT S
r_s	0.634	0.443

Fig 1. Correlation of PF thickness in right foot (D) and NDT (D)

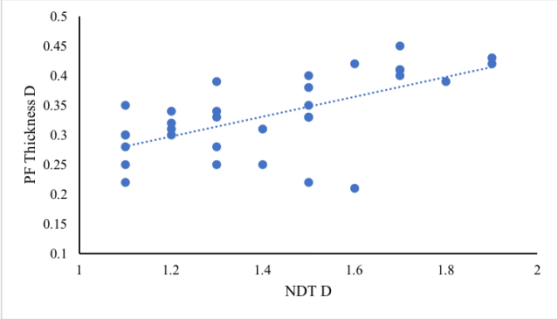
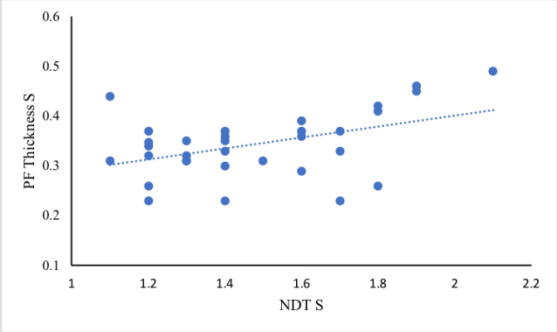


Fig 2. Correlation of PF thickness in left foot (S) and NDT (S)



PF thickness and gender

Analysis using Independent T Test of equal variance is used to assess the mean difference of PF thickness in gender groups. Results shows p value of 0.14 for right foot and 0.04 for left foot. There no significant mean difference found on right foot, but results are significant for left foot.

Table 4. PF thickness in left and right foot and gender

Gender	Mean ± SD	p value
Right foot		
Male	0.35 ± 0.06	0.14
Female	0.32 ± 0.06	
Left foot		
Male	0.37 ± 0.06	0.04
Female	0.32 ± 0.06	

PF thickness and age

Correlation studies between PF thickness and age are done. Results showed no correlation between PF thickness of right and left foot and age with significance of 0.175 for right foot and 0.197 for left foot.

PF thickness and BMI

It is found that there is no relationship between both variables, PF thickness and BMI with significance of 0.111 for right foot and 0.092 for left foot.

Discussion

PF thickness and flat foot

Results showed that there is a strong relationship between PF thickness of the right foot and flat foot because coefficient correlation of 0.634 is in the range of 0.51-0.75 on the other hand, moderate relationship is found on the left foot because coefficient correlation of 0.443 is in the range of 0.26-0.50.[17] This proves that flat foot curvature is associated with an increase in plantar fascia thickness as evidenced by the presence of a strong and moderate positive correlation between the two variables studied. P value also shows that the results obtained are significant.

The results of this study are consistent with research conducted by Wearing SC et al in 2007 comparing PF thickening and the shape of the foot arch using radiography and regional loading when walking in two

groups of individuals with and without heel pain. Results stated that thickening of PF was positively correlated with CMT1 angle in the foot with heel pain (r = 0.89) and the foot without heel pain (r = 0.64).[5]

The findings of this study are accordant with the anatomical structure and pathophysiological processes that occur when the arch of the foot is found flat. The condition that occurs in flat foot is that the subtalar joint will remain pronated after the foot becomes flat and the midtarsal joint is not locked.[18] This abnormal condition continues to occur in a weight bearing position and the talocalcaneus joint is continuously subluxated hence, normal gait cycle is not supported. Tibia turns inward, and the foot will be in excessive pronation to allow flattening of the foot. This abnormal walking mechanism will stretch the PF.[19] Repeated traction on the PF will cause microtears and inflammatory response.[20] Low arch of the feet will increase the pulling force and repetitive stress on the PF which causes microtears so that the inflammatory response arises to repair the damage. This normal repair process is inhibited by microtrauma resulting from repeated heel strikes that will cause a chronic inflammation of the fascia.[14]

PF thickness and gender

Based on the results, there is no significant mean difference found between PF thickness on the right foot in groups of male and female. Contrary to the right foot, a significant mean difference is found on the left foot because the p value was 0.04.

Previous studies conducted by Taş S in 2017 examines the comparison of PF thickness and stiffness and heel fat pad in men and women however, PF thickness measurements were only carried out on the dominant foot. The subjects in this study were 30 women and 30 men with the same age range. The results obtained state that there is a significant difference in PF thickness between groups of women and men with a p value of 0.037. The average PF thickness in the female group is 3.2 ± 0.5 while in men it is 3.5 ± 0.6. This can be interpreted that women have a thinner PF thickness when compared to men.[21] Results are contrary to the study by Abul K in 2015 which assess PF thickness in both foot of 156 subjects without symptoms of heel pain consisting of 88 women (56.4%) and 68 men (43.6%). PF thickness on the left and right foot was found to have a significant difference between groups of women and men with a mean thickness of 2.84 ± 0.42 on the right foot and 2.86 ± 0.44 on the left foot in women while in the male group, the mean thickness was found 3.28 ± 0.56 on the right and 3.3 ± 0.56 on the left. The p value on both female and male foot showed significant results which was <0.001.[22]
The results obtained by the comparative studies above differ from the results in this study, where differences in significance in the right and left foot are found. The difference in significance on the right and left foot can be influenced by differences in the mean and standard deviation of the PF thickness on the left and right foot which may be influenced by differences in NDT values on the right and left foot which are rarely found to have the same value in this study. Different from the comparative studies above which did not examine flat feet but normal feet, so might be no factor that might influence the significant difference of PF thickness found.

The difference in mean PF thickness in the male and female groups can be caused by adaptation as a form of compensation for the increase in load caused by the average male BMI which is greater than women.[21]

PF thickness and age

The relationship between PF thickness and age found to be insignificant in both the right foot (coefficient correlation of 0.175) and the left foot (coefficient correlation of 0.197). The results obtained in this study differ from the results of previous study by Abul K in 2015 which divided 156 samples into two

different age groups. A total of 89 samples (57.1%) are in the age group of 18-39 years while 67 samples (42.9%) are in the age group of 40-65 years. The 18-39 years age group had an average PF thickness of 2.92 ± 0.49 in the right foot and 2.91 ± 0.49 in the left foot, while the 40-65 years age group had a PF thickness in the right foot 3.18 ± 0.54 and the left foot 3.23 ± 0.56. The p value on the right foot 0.003 and <0.001 on the left foot indicates that there are significant differences in PF thickness in the two age groups. In addition to the paired t test, the researchers also conducted a Pearson correlation test between PF thickness and age which obtained r = 0.269 on the right foot r = 0.327 where both values showed a moderate relationship.[22]
The variation of result between this study and previous study mentioned above may be caused by the age range of subjects of this study is narrow, only from 18 to 22 years old.

PF thickness and BMI
As for BMI, relationship with PF thickness were also insignificant in both the right (coefficient correlation of 0.111) and the left foot (coefficient correlation of 0.092). Research conducted by Taş S et al in 2017 in Turkey revealed that the thickness of PF and BMI had a moderate correlation with coefficient correlation of 0.536.[23] It was found that PF thickness increased in overweight and obese individuals with BMI ≥ 25 kg / m^2.
Congruent with Taş S et al, a study conducted by Uzel M et al in 2005 divided 87 subjects into groups with normal BMI and overweight and obese BMI, found a moderate positive correlation in the Pearson correlation test with a value of r = 0.319.[24]
Research results by Abul K in 2015 also support both studies. Similar to the two studies above, Abul K also divided 156 subjects into two groups based on BMI, namely the group with a normal BMI of 75 people (48.1%) while 81 others were categorized as overweight and obese BMI. It was found that the group with normal BMI had a smaller average PF thickness compared to the group with overweight and obese BMI, where the difference between the two was statistically significant with p values of 0.002 for the right foot and 0.001 for the left foot. Correlation relationships between the two variables are also analyzed and it shows a moderate relationship with the value of r = 0.439 for the right foot and 0.457 for the left foot.[22]

The difference in the results obtained can be explained by the exclusion criteria of this study, where samples with a BMI above 25 kg/m^2 were excluded from this study because they were categorized as obese according to WHO Asia Pacific.[25] Therefore, the results obtained are certainly different from the three comparative studies above where subjects with overweight and obesity BMI category were included in the data analysis.

This study has several limitations. The use of NDT as a method for determining flat feet can be influenced by the subjectivity of the examiner. In addition, plantar fascia measurement using USG is only done once. Therefore, further research should utilize a more accurate and precise method to determine flat feet and measure plantar fascia thickness more than once so that the results obtained are more exact.

Conclusion
There is moderate and strong positive relationship between flat foot and plantar fascia thickness among medical students of Pelita Harapan University.

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BRAF V600E Immunoexpression in Papillary Thyroid Carcinoma and Its Association with Prognostic Factors and Histopathologic Variant

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Abstract

Aim: to provide additional information regarding the clinicopathological characteristics of Papillary Thyroid Carcinoma (PTC). Methods: Fifty patient with PTC were reviewed to determine prognostic factors such as age, gender, size of tumor and histologic variant. BRAF V600E mutation was detected by immunohistochemical staining and assessed with H score. Result: BRAF V600E mutations were detected in 17 (34%) cases. There were seven cases with extrathyroidal extension (ETE) p 0,04, 11 cases with lymph node metastasis (LNM) p < 0,001, and 8 cases with tall cell variant p 0,047.The cases with positive BRAF V600E mutation had mean age of 44.71 years, and the size of the tumor between 0.1-4cm. Six cases of them are male and 11 female.

Conclusion: There were significant relationships between BRAF V600E mutation with ETE, LNM, and tall cell variant. There was no significant relationship between BRAF V600E mutation, either with age, gender, or size of the tumor. BRAF V600E immunohistochemical examination can be performed as additional investigation for PTC patients.

Introduction

Thyroid carcinoma is the most common malignancy in endocrine organs. Incidence rate has increased worldwide and including in Indonesia.[1,2,3] Papillary thyroid carcinoma (PTC) is the most common type, which comprises 80-90% of all thyroid malignancies .[4,5] BRAF gene mutation is mutation that often found in the PTC, which is about 20-80% of all thyroid carcinoma. More than 90% of BRAF mutation involves changes of thymine to adenine at nucleotide 1799 (T1799A) in exon 15 resulting in the substitution of valine into glutamine at the point mutation of the amino acid position 600 (BRAF V600E).[2,6,7]
More than 30 studies have been conducted to determine the relation between the clinicopathologic characters of PTC with BRAF V600E mutation. Most of the studies indicated BRAF V600E mutation was associated with advanced disease stage, tumor aggressiveness, high recurrence rate and increased mortality of the patients.[8,9] Some studies have also suggested that the BRAF V600E mutation was associated with age, gender, tumor size, extrathyroidal extension (ETE), and lymph node metastasis (LNM).[2,10-13] Other studies have also shown a significant relationship between BRAF V600E mutation with histopathological variants of PTC such as classical variant, tall cell, and oncocytic.[2,10-13] Our study using immunohistochemical staining with BRAF V600E antibody was expected to provide an additional information regarding the clinicopathological characteristics of PTC in Indonesia

Materials and Methods

Samples: We collected all cases of PTC in the Department of Anatomic Pathology, Faculty of Medicine Universitas Indonesia - Ciptomangunkusumo Hospital (Jakarta, Indonesia) in the period of January 2014 to April 2015. Exclusion criteria were cases with inadequate slides, paraffin blocks were not found and PTC cases with other components, such as Hashimoto's thyroiditis, Anaplastic thyroid carcinoma and Hürtle cell carcinoma. The age and sex were noted based on medical records. The size of the tumors was noted based on medical or macroscopic records and microscopic assessment.

Histology: PTC is defined as a malignant tumor of the thyroid follicular cells marked with pseudo-inclusion, ground-glass appearance, and nuclear grooves.[4,14] Classic variant consist of papillary pattern with fibrovascular stalk, follicular variant consist of follicular growth pattern of> 50% tumor area, follicles with irregular shapes small to medium sized. Tall cell variant is composed of cells with a height of at least 3 times the width of cell constitute cover >50% tumor area and microcarcinoma variant with a diameter of 1 cm or less.[4,14,15] Extrathyroidal extension was assessed by microscopic examination which was an extension to the fatty tissue, muscle, or nerve around the thyroid gland. Lymph node metastatic tumor cells were characterized by the presence of PTC corresponding primary tumor in the lymph node in microscopic examination.

Immunohistochemistry: Sections of 4µm thick paraffin blocks were incubated overnight with primary antibody mouse monoclonal anti-human BRAF V600E (Spring Bioscience®) with a dilution of 1: 200. Positive control is a case of PTC with BRAF V600E mutation detected by Real-Time Polymerase Chain Reaction (PCR). Negative control is from each cases.

Methods of Validation:
Immunohistochemical staining were assessed by two independent observers and then assessed the suitability between the two observers. Semiquantitative scoring were done using the modified H score system.[16] This system includes percentage (%) of positive cells in 1000 tumor cells and also we assessed the staining intensities: 0: negative, 1: weak positive, 2: moderate positive, 3: strong positive. H score for each sample was calculated with the formula of H score = $\boldsymbol{H\ score = \Sigma\ Pi\ (i + 1)}$; Pi is the percentage of tumor cells stained (0-100%) and i is intensity of the staining (0,1,2,3).

Statistical analysis: The data was analized statistically Using IBM SPSS Statistics 20, with Chi-square test, or Fisher's test. The numerical data was analyzed using unpaired t or Mann-Whitney test.

Result

BRAF V600E Immunoexpression

The range H score BRAF V600E is 100-400. We defined the cut-off point to divide the BRAF V600E positive and negative H score with the curve of the receiver operating characteristic (ROC) and obtained the value of area under the curve (AUC) was 0.805 (95% CI 0.625 to 0.984). Cutting point of balance between sensitivity and specificity is 78% in H score of 326.5. H score $\geq$ 326.5 was determined as positive BRAF V600E mutation and < 326.5 as negative BRAF V600E mutation. BRAF V600E mutation was found in 17 (34%) cases by immunohistochemistry. Images of BRAF V600E immuno-expression can be seen in Figure 1.

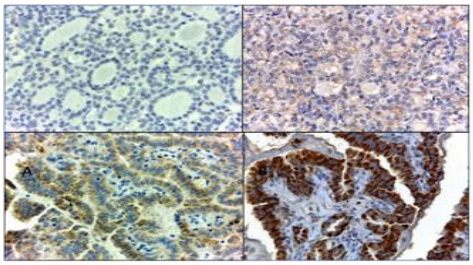

Figure 1 Immunohistochemical staining results BRAF V600E A.Negative (0). B. Weak (+1) C.Moderate (+2) D.Strong (+3).

We found significant relationship between BRAF V600E mutation with ETE and LNM. (Table 1) There were no significant relationships between BRAF V600E mutation either with age, gender, or size of the tumor. Histopathologic variants in this study were follicular, tall cell, classic, and microcarcinoma. These variants were further categorized into 2 groups: tall cell and non tall cell. There were significant relationships between BRAF V600E mutation, both with tall cell and non-tall cell variants.

BRAF V600E Mutation Analysis

Table 1. BRAF V600E Mutation in PTC at Ciptomangunkusumo Hospital, Jakarta, Indonesia

Factors	**BRAF V600E positive**	**BRAF V600E negative**	**p**	**Odds Ratio (OR)**	**95% CI**
Age					
Mean (SD)	44,71 (15.090)	41.58 (15.839)	0,505[a]		
Tumor size (min-max cm)	17 (0,1-4)	33 (0,1-9)	0,134[b]		
Gender					
Male	6	10	0,720[c]		
Female	11	23			
ETE					
Present	7	2	0,04[d]	10,85	1.932 - 60.930
Absent	10	31			
LNM					
Present	11	5	<0,001[c]	10,267	2.592 - 40.669
Absent	6	28			
Variant					
Tall cell	8	6	0,047[c]		
Non Tall cell	9	27			
Total	17	33			

a= Unpair t test; b= Mann-Whitney; c=Chi square; d=Fisher's exact

Discussion

BRAF V600E Mutation on PTC

Many studies have been done to detect BRAF V600E mutation by immunohistochemical staining which is a simple and in-expensive method..[6,11,17,18] Zagzag et al. detected mutations in BRAF V600E, using specific antibody clone VE1 and showed positive results in 89% of cases with a specificity of 100% and sensitivity of 89%.[11]

Previous research stated that the BRAF V600E mutation in the PTC might be heterogeneous, which was proved by specific antibodies.[18,19] Majority of cases in this study demonstrated non homogeneous staining, so we use a scoring system to determine the positivity. Distribution of tumor cells that had mutations in the positive cases also varied. The strongly positive stained cells varies from 34-100% of tumor cells in positive case 9 of which stained > 80%. This finding is in line with research conducted by de Biase et al. and heterogeneous staining was not due to preservation or poor tissue fixation.[19]

Other meta-analysis studies have demonstrated that BRAF V600E mutation in the PTC was an independent prognostic marker associated with poor survival and high recurrence rate.[20] Kim et al. in meta-analysis study conducted in 2012 stated that the PTC with BRAF V600E mutation have a risk of 1, 5 to 2.1-fold to undergo ETE, LNM, and recurrent.[21] Our study involved 50 PTC cases showed positive result in 17 (34%) cases. The analysis showed a significant association between V600E BRAF mutation and prognostic factors, for example, ETE, LNM, and the tall cell variant.

BRAF protein is a central regulator in the MAPK pathway, which in turn activates BRAF mutant protein and causes the MEK ERK protein phosphorylation. Active ERK protein moves into the cell nucleus and induce transcription factors and cellular transformation.[2] MAPK pathway dysregulation and / or BRAF mutations can increase transcription of MET gene and will increase the expression of Met as a receptor protein tyrosine kinase which in turn activated by ligand hepatocyte growth factor (HGF) so that the tumor cells are able to migrate and invade the capsule and lymphatic vascular structures.[22,23] Nardone et al 2003 study also expressed high Met protein expression in tall cell variant, and is related to tumor aggressiveness.[24] Some studies have also suggested that the BRAF V600E mutation associated with increased expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs), which increases tumor invasion ability.[25,26] MET gene transcription may also be caused by a mutation or dysregulation of others, namely RAS oncogenes or RET / PTC.[27,28] Similarly to the case study that is not mutated BRAF V600E but can also occur ETE and LNM. So that needs to be further investigated regarding other pathways that play a role in the pathogenesis of PTC such as RAS or RET / PTC.

Adeniran research reported 97 cases of PTC , 42% of them experienced a BRAF mutation, 18% RET / PTC, and 15% RAS mutation.[29] Cases with BRAF mutations generally occur in older patients with classic or tall cell variant, more advanced stage and ETE. Cases of mutated RET / PTC were reported at a younger age and the more numbers of LNM. While the case with exclusively RAS mutations occur in follicular variant of PTC with less LNM.[29]

Some targeted therapies that inhibit BRAF selectively or non-selectively have been approved by the FDA effective and well tolerated by patients with mutations in BRAF V600E.[2,30] It can be administered to PTC patients with advanced stage, have experienced metastasis, and resistance to radiation therapy.[31,32] However, a review by Alonso-Gardoa et al. 2015, said that it is still needed further research as a stable treatment results and benefits of the combination therapy of several therapeutic targets.[30]

Association of BRAF V600E immunoexpression with Age

Meta-analysis study conducted by Lassalle et all[33] in 2010, showed 12 studies that found significant relationship between BRAF V600E mutation with age. Other studies have found no association between age and the V600E BRAF mutation.[11,20,34] Our study did not gain significant relationship between BRAF V600E mutation with age. There is a case study that found mutated BRAF V600E are more found at the age of 45 years or more as many as 12 cases with the oldest 68 years of age. Meanwhile, at the age less than 45 years, only five cases of mutated BRAF V600E. This shows the V600E BRAF mutation is more common in older age. Research Ciampi and Nikiforov in 2007 stated that BRAF V600E mutation is more common in old age, while at a young age the mutations in RET / PTC are more often found.[35]

Association of BRAF V600E immunoexpression with Gender

PTC can occur in women and men, where women are more often in the ratio 2:1 to 4:1.[4,36,37-42] Male gender said to be a poor prognostic factor in the PTC as related to high frequency of tumor recurrences.[43] The study included 16 men, 7 of them with LNM and 6 of them with the ETE. Four cases with both ETE and LNM. Lymph node metastases and ETE also associated with tumor recurrence.43 Several studies stated BRAF V600E mutation linked with male gender.[20,44,45] However, in this study we found no such link. Seventeen cases with BRAF V600E mutation in this study only six (35%) waas male.

Association of BRAF V600E immunoexpression with Tumor Size

Tumor size is an important variable in determining the prognosis of the patient, the larger the size of the tumor the worse the prognosis.[4,36,46-48] Several studies have shown a link between BRAF V600E mutation and tumor with larger size.[34,49-52] Other studies found no such association.[29,44] Our study found no significant association between BRAF V600E mutation with tumor size. However, from seventeen cases the mutated BRAF V600E in this study, we found ten (59%) cases measuring more than 2 cm or more.

Association of BRAF V600E immunoexpression with Histopathological Variant

In this study we found four variants, namely follicular, tall cell, microcarcinoma, and classic. Classic and tall cell variant and said to be related to the BRAF mutation V600E.[10-13] Research by Fernandez et al in 2013 showed BRAF V600E mutation in 72.2% of cases PTC tall cell variant, 77.4% of cases PTC classical variant, and 31.9% of cases PTC follicular variant.[53] Min et al in 2013 have positive results BRAF V600E mutation at 100% tall cell variant, classical variant 79.4%, and 47.6% follicular variant.[54] Study of Ghossein et al 2007 showed tall cell variant have significant association with poor prognostic factors such as older age, extrathyroidal extension, necrosis, and mitosis.[55] Calangiu et al 2014 study also states that PTC patient with tall cell variant and ETE, has a 5-year survival rate is lower than the patients with classic variant.[56] Moreover, tall cell variant generally associate with BRAF V600E mutation and also associated with aggressiveness.[18]

Our study showed a significant relationship between BRAF V600E mutation with histopathological tall cell variant and non tall cell (p 0.047). Fourteen cases with tall cell variant, 8 (57%) were mutated BRAF V600E. Meanwhile, eight cases with the classical variant, 6 (75%) were mutated BRAF V600E, and the follicular variant only 1 (6%) cases of mutated BRAF V600E. Two cases with microcarcinoma variant mutated BRAF V600E, one of them containing tall cell components more than 50%. There are 8 (16%) cases of non-tall cell mutated

BRAF V600E, one with follicular variant, six variants of the classic, and one variant microcarcinoma containing components classical variant. Of the seven cases of classical variant mutated BRAF V600E, five of which with ETE and LNM. This shows that the PTC with a classical variant can also be aggressive.

Association of BRAF V600E immunoexpression with ETE

Extrathyroidal extension is an important prognostic factor in patients with PTC since it is associated with high recurrence rates and mortality.[49,57] Our studies found there is relationship between the V600E BRAF mutation and ETE p 0.04 and OR 10.85 (95% CI 1.932 to 60.93). Nine subjects with ETE, 7 of them mutated BRAF V600E. Other studies also suggested a significant association between BRAF V600E mutation with ETE.[21,29,34,58-60]

Association of BRAF V600E immunoexpression with Lymph Node Metastasis

Lymph node metastasis in PTC associated with the occurrence of recurrency.[61] Our research shows there is a significant relationship between BRAF V600E mutation with LNM p <0.001 and OR 10.267 (95% CI 2.592 to 40.669). Sixteen cases with LN metastatis, 11 were mutated BRAF V600E. Several studies have also suggested an association between BRAF V600E mutation with LNM.[12,34,49,51,56,60] The association between BRAF V600E mutation with the incidence of metastatic lymph nodes is an indicator of recurrence. Several recent studies suggest that in PTC patient with BRAF V600E mutation total thyroidectomy should be performed with prophylactic lymph node dissection.[43,62]

Conclusion

BRAF mutation plays a fundamental role in the pathogenesis of PTC. The positivity of BRAF V600E immuno-expression in this study were 34%. Further research is needed to determine other pathways that play role in the PTC such as RET/PTC, RAS, and so on. There was no significant relationship between BRAF V600E mutation, either with age, gender, or tumor size. There were significant relationship between BRAF V600E mutation with ETE, LNM and histopathological tall cell and non-tall cell variants. Significant correlation between BRAF V600E mutation with LNM and ETE, showed that BRAF V600E immunohistochemical examination can also be performed to predict the prognosis of PTC patients.

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Challenge as Physician to Diagnose Pediatric Patient with Laryngopharyngeal Reflux: A case report and literature review

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Abstract

Background : Laryngopharyngeal reflux (LPR) is the reflux of backflow of gastric acid or refluxate that usually affects the throat and laryngopharynx. Many physicians are unable to differentiate between pediatric LPR with pneumonia. Laryngopharyngeal reflux needs to be widely known and understood by a physician because there are relationships between upper and lower airway disease. In pediatric, LPR may also contribute to many problems in the respiratory tract, the clinical manifestation of pediatric LPR, and remains a challenge for physicians.

Objective : To emphasize the new diagnostic symptoms and signs instrument for pediatric LPR using a fiber-optic laryngoscope, also to remind the correlation between the upper and lower respiratory tract and factors which contribute to pediatric airway.

Case : A case of a 21-month-old girl with sudden onset of hoarseness, stridor, and wheezing was diagnosed with pneumonia, further investigation showed reflux symptoms, vocal cord abnormalities, and subglottic edema that suggest LPR was the final diagnosis.

Conclusion : Pediatric LPR may be difficult to diagnose, there are many differential diagnosis, symptoms, and signs that may occur. The new diagnostic instrument can be used for diagnosing pediatric LPR, it is feasible and applicable in daily practice. Laryngopharyngeal reflux needs to be understood and considered as a differential diagnosis for coughing, hoarseness, in children despite the diagnosis challenge.

Introduction

Laryngopharyngeal Reflux (LPR) is defined by the reflux or backflow of either gastric acid or refluxate that containing pepsin into the aerodigestive tract, usually affects the throat and laryngopharynx[(1)]. In general, this disease can be diagnosed with clinical symptoms and further physical examination. The most common symptom of LPR in children is coughing, choking, or hoarseness. Coughing or hoarseness etiology might be hard to differentiate in children, yet children with dysphonia or hoarseness may have reflux as contributing factors[(2)]. Although the incidence of LPR in children is difficult to obtain, about 20% of infants and children are likely to suffer from reflux disease[(3)].

Extraesophageal reflux disease or commonly called LPR can make many complications in pediatrics. Atypical manifestations including stridor, recurring cough, hoarseness, laryngitis, otitis media, sinusitis, chronic bronchitis, asthma, or recurrent pneumonia may occur[(4)]. LPR also can contribute to developmental failure, laryngomalacia, recurrent respiratory papillomatosis (RRP), chronic cough,

hoarseness, esophagitis, and other disorders.

LPR etiology should be able to distinguish between infection, allergies, neoplasms, or other systemic disorders[5]. It is important as a physician to be able to diagnose this disease, overtreatment or overdiagnosis might occur. Below we present a case with laryngopharyngeal reflux diagnose in children with atypical manifestation.

Case Presentation

A 21-month-old girl came to the ENT clinic with complaints of additional breathing sound when she was playing 12 days before admission. Complaints began suddenly when the patient choked while she was eating. There were hoarseness breathing sounds. The hoarseness worsens when the patient cries or after screaming accompanied by coughing. There were stridor and wheezing also appears during sleep. Stertor can be heard when the patient awakes and cries. The previous history was denied. History of sneezing, runny nose, and fever was denied by parents. There is no history of asthma or allergies in patients.

Before going to the ENT clinic, the patient was brought by his parents to a pediatrician. On examination, intercostal retractions and epigastric retractions were present, wheezing in both lung fields, dominantly heard in the right lung field. The patient was treated for asthma and was given a combination of salbutamol and steroid inhalation therapy for one week. Seven days after the first therapy was given, the patient's condition did not improve, the patient also had a chest X-ray and a full blood laboratory examination with serum immunoglobulins Covid-19. On X-ray examination found infiltrates in the right lung field, current working diagnosis is community-acquired pneumonia, treated with antibiotic therapy, Azithromycin was given for 5 days. The patient still did not show any improvement in clinical symptoms after antibiotic therapy, the patient was then referred to an ENT specialist.

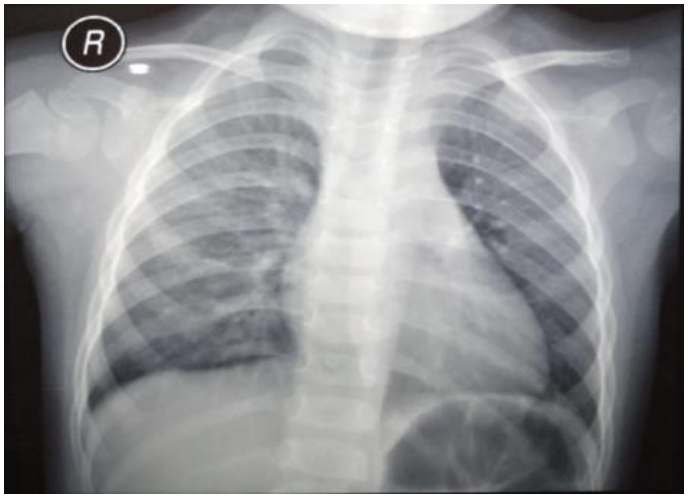

Figure 1. Patient’s X-ray showing infiltrates in the right lung field.

The patient came to the ENT clinic, the patient was compos mentis, and vital signs within normal limits. The patient's nutritional status was normal. On physical examination, there was clear stertor heard when the patient was calm. Patients underwent fiber-optic laryngoscopy examination. The results were found a narrow nasal cavity, livid edema of inferior nasal concha, postnasal drip was present, nasopharyngeal oropharyngeal mucosa and there is no enlarged adenoid, visible standing secretion in the piriformis and vallecular sinuses, edema of arytenoids, vocal plaque and ventricular edema and no visible enlargement of the nasopharynx, also appear standing secretion in the piriformis and vallecular sinuses, arytenoid edema, vocal plaque, and ventricular edema and appear whitish patches as well subglottic edema was found.

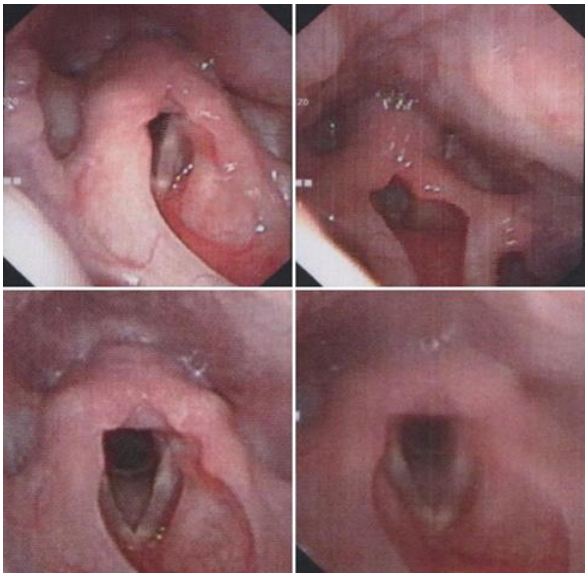

Figure 2. Patient's fiber-optic laryngoscopy showing whitish patch and subglottic edema.

The patient then treated with Omeprazole, administered twice daily, for 2 weeks, mometasone furoate nasal spray therapy 2 times daily, cetirizine, nasal irrigation with normal saline and education to modify lifestyle. Two weeks after oral Omeprazole therapy, the patient's clinical condition was improved, No hoarseness, stridor and stertor, vesicular breath sound in both lung fields. Therapy with Omeprazole is still given for up to 2 months and continuing nasal irrigation. The IgE specific antibody was tested and did not shown to any specific allergen.

Discussion

Complaints of hoarseness, wheezing, stridor, and stertor appeared suddenly after the patient choked during mealtime. Wheezing in children has many differential diagnoses, most common is a tracheoesophageal fistula or malacia, foreign body aspiration, bronchiolitis, and gastroesophageal reflux disease (GERD)(6). The cause of infection in these patients has not been eliminated, the patient was diagnosed by a pediatrician with community-acquired pneumonia, working diagnosis was based on clinical symptoms and X-ray examination shown infiltrates in right lung field, complete blood count within normal limits. After administration of antibiotics and inhalation therapy, the patient's condition did not improve. Complaints were also felt suddenly when the patient was eating, aspiration of foreign bodies involving the upper respiratory tract was yet to be eliminated, stridor was present, that suggest the obstruction of the upper respiratory tract.

The patient has symptoms suggested caused by reflux., wheezing, stridor, cough, hoarseness was present. Refluxate from gastrointestinal or GERD may manifest several problems related to the airway, including laryngomalacia, hoarseness, vocal cord nodules, asthma even life-threatening events(7). A physician needs to know and consider what other symptoms or signs may occur in a patient with GERD.

GERD is often diagnosed by clinical signs and symptoms, where specific testing is not needed and empiric treatment may give an improvement of the symptoms, but when airway manifestation occurs, further workup should be done to determine the underlying factors causing the symptoms, whether it caused by an anomaly of the anatomy or the manifestation of GERD.

Esophagitis, particularly eosinophilic esophagitis (EE) is described as a chronic idiopathic inflammatory disorder of esophagus that can mimic airway symptoms of GERD. The airway manifestation in EE includes wheezing, stridor, dyspnea on exertion, hoarseness, and croup. EE is a clinicopathologic diagnosis, in which symptoms and dense eosinophilia on esophageal biopsy findings lead to the diagnosis. Another testing that can be done to diagnose EE is IgE levels, blood eosinophil level, or genetic testing(7). The gold standard for diagnosing EE is made by tissue biopsy of esophagus. Clinical symptoms of esophageal dysfunction, more than 15 eosinophils in one high power field, lack of responsiveness to high dose PPI, and normal pH monitoring of distal esophagus were also included in diagnostic guidelines of EE(8). Patient clinical symptoms have improved after PPI treatment, although the patient did not undergo esophagus biopsy and esophageal pH probe monitoring, other laboratory examination within normal limit, no increased level of blood IgE and eosinophil also within normal limit. Thus, the diagnosis of EE can be eliminated in this patient.

Table 1. Symptoms and Signs that May be Associated with GERD in Infants and Children 0 to 18 years old[9]

Symptoms	Signs
General	
Discomfort / Irritability Failure to Thrive	Dental erosion
Feeding refusal Dystonic neck posturing	Anemia
Gastrointestinal	
Recurrent regurgitation with/ without vomiting in the older child Heartburn / Chest pain	Esophagitis
Epigastric pain Hematemesis	Esophageal stricture
Dysphagia/ odynophagia	Barret Esophagus
Airway	
Wheezing	Apnea spells
Stridor	Asthma
Cough	Recurrent pneumonia Associated with aspiration
Hoarseness	Recurrent otitis media

In an adult, reflux screening using the scoring system can be used, namely by using a scoring system published by Belafsky et.al, Reflux Symptoms Index (RSI) and Reflux Findings Score (RFS), classification symptoms of laryngopharyngeal reflux[10]. The use of RSI and RFS scoring can be easily applied to the ORL-HNS practice as an objective parameter at a low cost and easy to use[11]. Children are not a small adult and their growth are dynamic. For pediatrics, the study by Zulka proposes a new diagnostic instrument in pediatrics[12]. The new instrument was compromised by reflux symptoms and laryngeal signs. The condition of vocal cords abnormality or subglottic edema is obligatory. One of the reflux symptoms and 2 laryngeal signs are needed to establish the diagnosis of LPR in a pediatric patient. LPR is diagnosed if the total score is more than 5 with any positive laryngeal sign.

The instrument consists of symptoms of frequent throat clearing, choking, annoying cough, and signs of vocal cord abnormalities and subglottic edema. Symptoms of reflux are interpreted by number, from zero to five, which increased in number also increased in severity of the complaint. The patient has frequent throat clearing symptoms in number 4, -3 for symptoms of choking and annoying cough in number 4, for laryngeal signs patient has a whitish patch (1) and presence ofsubglottic edema (2), based on this instrument, the patient was diagnosed with LPR.

Table 2. Reflux Symptoms and Signs Instrument for Pediatric

Symptoms						
Frequent throat clearing	0	1	2	3	4	5
Choking	0	-1	-2	-3	-4	-5
Annoying cough	0	1	2	3	4	5
Signs						
Vocal cord abnormalities	0 = Normal 1 = Whitish patch 2 = Reinke edema / nodule / granuloma					
Subglottic Edema	0 = Normal 2 = Present					

Chronic LPR may be associated with certain complications, especially in children. Pediatric LPR is thought to cause many respiratory and airway-related problems such as rhinosinusitis, otitis media effusion, or asthma[4]. There is still no gold standard for diagnosing LPR in children[11]. The investigation that can be performed on LPR patients includes fiber-optic laryngoscopy and 24H pH probe monitoring, impedance working, and upper endoscopy[3]. A 24-hour double probe is placing a probe in the nose with separated two measuring endpoints, one in the proximal and second in the distal esophagus, it measures the number of reflux events (pH<4) and duration (greater than 5 minutes) also total and percentage of duration pH below 4[13]. This examination is difficult to assess and hard to be implemented, in this case, the patient did not undergo 24-hour pH probe monitoring. Distal pH probe monitoring examination results have a high rate of false negatives in pediatric patients with extraesophageal symptoms of GERD, affected by non-acidic reflux commonly present in proximal esophagus and pharynx[3]. A study showed pediatric patients with GERD that underwent distal probe pH monitoring and anti-reflux therapy found distal pH studies were not predictive of positive response to anti-reflux therapy and should be avoided in this patient population. Hereby, single distal pH probe monitoring is not considered as an adequate study for a diagnostic instrument for LPR.

Examination with fiber-optic laryngoscopy or endoscopy visualization of the aerodigestive tract is useful for assessing whether LPR or GERD causing

the problem. Examination of vocal cord abnormalities and subglottic edema are useful for diagnosing LPR. Fiber-optic laryngoscopy is chosen because painless and required a short time, less than 2 minutes, and a new diagnostic instrument can also be used for diagnosing pediatrics with LPR. Zulka's reflux symptoms and signs instrument can be used for diagnosing pediatric with LPR, based on clinical symptoms and fiber-optic laryngoscopy examination results. This instrument is more feasible and more applicable in daily practice.

LPR is closely related to Otitis Media or Rhinosinusitis. The prevalence ratio of the incidence of effusion otitis media in pediatric patients with LPR was 4.5 times higher than those without LPR[(4)]. Patients with Chronic Rhinosinusitis and LPR have a correlation, both improve each other symptoms and worsen the quality of life. LPR is one of the factors that contribute to nasal mucociliary clearance damage(14). If the nasal function was impaired, it will cause pulmonary aspiration of nasal contents, triggering nasal-bronchial reflex, and increased absorption of inflammatory mediators that may be responsible for lower airway dysfunction[(15)]. Neural communications are linking upper dan lower airways, the trigeminal nerve that responsible for an afferent sensory impulse from the nose, from nasopharynx via glossopharyngeal nerve and efferent impulses to the bronchi via vagal nerve mediate bronchoconstriction and contribute to nasobronchial reflex that responsible for interactions between upper and lower airways

Nasal mucociliary clearance (NMC) is one of the innate defense mechanisms of the nasal and paranasal sinuses. The mucus, which is secreted by the upper respiratory tract, traps particulates, allergens, and pathogens which will be transferred to the pharynx by the cilia and will be swallowed[(15)]. Nasal mucociliary clearance (NMC) damage can predispose to infections of the nose, paranasal sinuses, and respiratory tract. Disorders of the mucociliary clearance time can occur in patients with LPR, refluxate in the form of hydrochloric acid and pepsin directly affect mucociliary function, the vagus nerve-mediated autoimmune response results in nasal mucosal edema which affects cilia motility and Helicobacter pylori which are contained in refluxate damage mucosal lining directly[(16)]. Decreased laryngopharyngeal receptor sensitivity may result from refluxate, which could potentially result in an increased risk of aspiration that may lead to aspiration pneumonia[(17)].

Treatment with nasal irrigation as an adjunct for common cold/rhinosinusitis, chronic sinusitis, allergic rhinitis, and after nasal surgery. Nasal irrigation moisturizes nasal mucous membranes, reduces crusting, and improves mucus retention from the nose and sinuses[(17)]. Administration of low-salts and isotonic solution associated with a significant reduction in microbial antigen concentration. In general, this therapy is cheap and easy to do even for children. Intranasal steroid administration, in this case, mometasone furoate. The use of mometasone furoate as therapy and/or prophylaxis of nasal symptoms of allergic rhinitis and perennial allergic rhinitis has become the standard treatment with a level of evidence Ia[(18)]. Treatment with mometasone furoate in the pediatric population has also shown excellent efficacy for the management of adenoid hypertrophy and effusion otitis media, despite concomitant atopy. In pediatric patients suffering from allergic rhinitis, it shows better clinical improvement, better efficacy, safer and lower total cost of care. Based on the history and physical examination, that the risk of allergies cannot be ruled out, so the cause and management of allergies that triggered allergies, this treatment was given.

Successful anti-reflux drug treatment can also be used as a diagnostic tool with improved clinical symptoms after therapy. In the pediatric population, this still requires further study. Empirically, therapy using proton pump inhibitor (PPI) can be given to patients with LPR. Omeprazole and Lansoprazole can be used. Administration of Omeprazole at a dose of 0.5 -2 mg / Kg Body Weight per day is divided into two doses and in twice the administration, morning, and evening 30 minutes before meals. The therapy can be given up to 2-3 months. If after treatment symptoms did not improve, further re-examination is necessary. Prokinetic agents are not recommended, especially with

metoclopramide and cisapride, due to the dangerous side effect of the extrapyramidal syndrome.

Ideally, for comprehensive management patients with LPR, a team of speech /swallowing therapists, radiologists, gastroenterologists, and otolaryngologists should work together to evaluate and treat patients with LPR[3]. The most important thing to note is lifestyle modification. Taking a good diet is as effective as PPI therapy. In general, alkaline, protein, low-fat and low-acid foods are quite effective because they are easily digested and can reduce transient relaxation of the esophageal sphincter associated with LPR episode. Caffeine, mint, and citrus diet should be avoided. The patient also needs to avoid large meals before activity and eating shortly before bedtime[2]. In infants or children, especially those who are still breastfeeding, changes in position can reduce reflux up to 80% and reduce regurgitation rates by about 65%(3). Other conservative management that can be applied are elevation of the head of the bed, avoidance of caffeine, mint, and citrus, avoidance of large meals before activity, and avoidance of meals shortly before sleeping.

Conclusion

To diagnose pediatric LPR is often a challenge for physicians. Children are not a small adult and their growth are dynamic. The physician needs to know and understand the correlation and manifestation that may occur caused by LPR that affects the upper and lower airway. Pediatric LPR may manifest many atypical symptoms that result in difficulties in diagnosing and treating patients with LPR. The new instrument can be used to diagnose pediatric LPR, it is feasible and applicable in daily practice. Laryngopharyngeal reflux needs to be listed as a differential diagnosis in pediatric with cough, stridor, or hoarseness. By knowing and understand the manifestation that may occur, physicians may be able to diagnose pediatric LPR properly and provide comprehensive management.

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Chest CT as a complement to RT-PCR to confirm and follow-up COVID-19 patients

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Abstract

Background : The first case of COVID-19 in Indonesia was recorded in March 2020. Limitation of reverse-transcription polymerase chain reaction (RT-PCR) has put chest CT as an essential complementary tool in the diagnosis and follow up treatment for COVID-19. Literatures strongly suggested that High-Resolution Computed Tomography (HRCT) is essential in diagnosing typical symptoms of COVID-19 at the early phase of disease due to its superior sensitivity (97%) compared to chest x-ray (CXR).
The two cases presented in this case study showed the crucial role of chest CT with HRCT to establish the working diagnosis and follow up COVID-19 patients as a complement to RT-PCR, currently deemed a gold standard.

Introduction

In late December 2019, a newly emerging infectious disease of unknown origin caused an outbreak in Wuhan, China. The International Committee on Taxonomy of Viruses (ICTV) named the virus SARS-CoV-2 on 11 February 2020 and the disease was announced as COVID-19 by World Health Organization (WHO)[1]. Diagnosis of COVID-19 has been a real challenge, especially in some countries with limited resources and insufficient health system. Indonesia is one of many countries impacted by the virus. The first case was identified in Depok, West Java, on 2 March 2020. It spread to nearby cities, most notably to Jakarta, which has become the epicenter of COVID-19 in Indonesia.
RT-PCR, as a standard reference, has been reported to have some degree of false-negative results caused by some factors including sampling operations, specimens source (upper or lower respiratory tract), sampling timing (different period of the disease development) and Chest CT may be considered as a primary tool for the current COVID-19 detection in epidemic areas[3].

some experts have suggested that chest CT can be regarded as a diagnostic standard of COVID-19[3].The essential aspects of controlling COVID-19 are early diagnosis, early isolation, and early treatment[23]. Recently, some studies reported that chest HRCT has higher sensitivity compared to RT-PCR and CXR[2,3]. Chest HRCT, as a routine imaging tool for pneumonia diagnosis, is relatively easy to do and a fast modality for diagnosis. Chest HRCT provides typical radiologic features in almost allpatients with COVID-19, such as ground-glass opacities (GGO), multifocal patchy consolidation, and/or interstitial changes with a peripheral distribution. The sensitivity of chest CT was great in Wuhan (the most affected city by the epidemic) and the sensitivity values were very close to each

other (97%, 96%, and 99%, respectively). In the regions other than Wuhan, the sensitivity varied from 61 to 98%.[2] Study from Ai et al. showed the sensitivity, specificity, and accuracy of chest CT in indicating COVID-19 infection were 97%, 25%, and 68%[3]. The varieties of the sensitivity results were caused by inter-observer difference interpretation, severity, and disease progression at the time of examination[2].

We looked into the possibility of using chest HRCT as a complement of RT-PCR to diagnose COVID-19, especially during a limited supply chain and unavailability of fast results of RT-PCR and chest CT.

Case Report

Case 1. A 43-year-old female with a history of asthma and pulmonary tuberculosis (TB) presented to the emergency department with moderate fever for three days accompanied by dry cough, myalgia, vomiting, diarrhea, anosmia, and fatigue. Otherwise, the patient looked healthy. Vital signs showed increased body temperature (38.7ºC), slight tachycardia, and normal blood pressure, with the rest physical examination was unremarkable. Initial laboratory results showed normal white blood cell count without lymphopenia. Inflammation marker revealed the increase of C-reactive Protein (CRP) at 40 mg/L, other markers such as Erythrocyte Sedimentation Rate (ESR) and Lactate Dehydrogenase (LDH) within the normal limit (Table 1). Other blood samples taken for NS1 antigen dengue and serology (widal) for typhoid fever were negative.

The CXR (Figure 1) showed fibrosis in the right upper lung zone, consistent with scarred TB. Subsequent HRCT revealed bilateral, multifocal sub-pleural GGO and crazy paving consistent with minimal typical COVID 19 pneumonia (Fig 1 b-d). The nasopharyngeal swab was positive seven days later. Meanwhile, the patient has already been on oseltamivir 2 x 75 mg, chloroquine 2 x 300 mg, and azithromycin 500 mg daily for ten days. The second RT-PCR test 14 days apart showed conversion to negative, but three consecutive nasal and oropharyngeal swabs showed positive results again. The patient's husband was confirmed to have COVID-19 as well and both of them practiced self-isolation in their home, but unfortunately, they were still shared the same room. Contrary to the positive RT-PCR results, the symptoms resolved and the laboratory and inflammation marker turned back to normal. The second HRCT conducted 35 days from the first one revealed complete resolution of the lesion even though RT-PCR was still positive (Fig. 2 a-d).

CXR and HRCT case 1

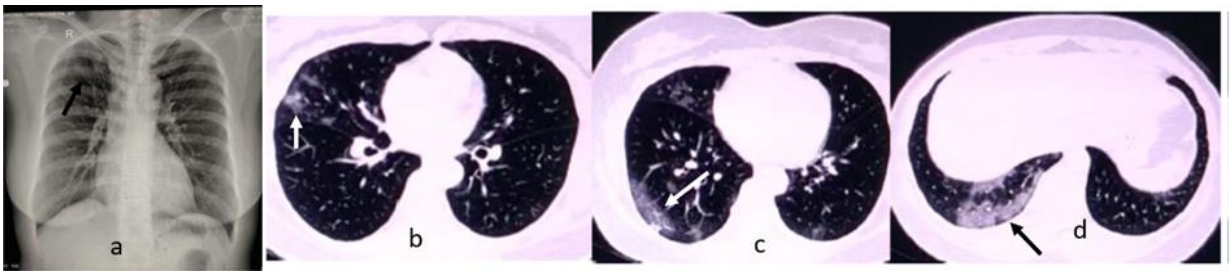

Figure 1. (case 1) CXR shows the right posterior upper lung fibrosis (a). HRCT shows multifocal sub pleural GGO in the middle and posterior right lung lobe (b.c). Subpleural nodule in the left lower lung lobe (head arrow c) and crazy paving found in the right subpleural posterior lung lobe (arrow d) suitable with minimal typical COVID 19

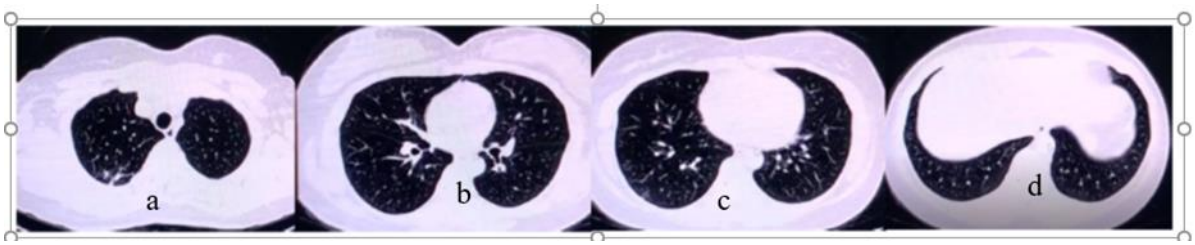

Figure 2. (Case 1) follow up HRCT was done 34 days after first HRCT and all of the lesions were disappear (b.c.d) except right upper posterior lung fibrosis same with old HRCT suggestive sequel TB (a)

Case 2. A 51 years old male health care worker developed fever symptoms for four days, followed by myalgia, malaise, and dry cough. He was diagnosed with typhoid fever based on a Tubex test result, which increased to four (reference < 2). He was treated for typhoid fever with Ceftriaxone 2g/day intravenously for three days without any improvement. Because the serum antibody for SARS-CoV-2 was positive on days seven, he was recommended to get other examinations, which were nasopharyngeal swab, CXR, and HRCT. CXR (Fig.3a) showed GGO in the right lower lung zone. Four days later, a descriptive finding of the HRCT (Fig 3b-d) showed multifocal sub-pleural GGO, consolidation, and fibrosis both in the right superior and lower lung lobe, suitable to moderate typical pneumonia COVID-19 at the progressive stage. He was treated by standard regimen, including oseltamivir 75 mg twice daily, chloroquine 500 mg twice daily, and azithromycin 500 mg orally for ten days. He continued to do self-isolation at his home. The first two initial and days 14 of RT-PCR swabs were negative. On the 10^{th} day, he reported that he had no fever. However, he felt shortness of breath after a mild exercise, which never happened to him. Another chest CT obtained approximately 20 days later showed some resolutions of GGO and consolidation suitable for the convalescence stage (Fig. 4 d-f)

CXR and HRCT case 2.

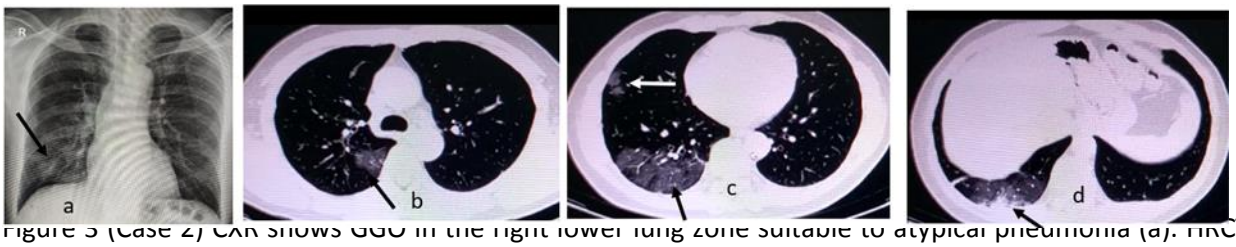

Figure 3 (Case 2) CXR shows GGO in the right lower lung zone suitable to atypical pneumonia (a). HRCT in the same day shows multifocal subpleural GGO (b c), subpleural consolidation and fibrosis in the right superior and lower lung lobe (d) suitable to moderate typical COVID-19 progressive stage

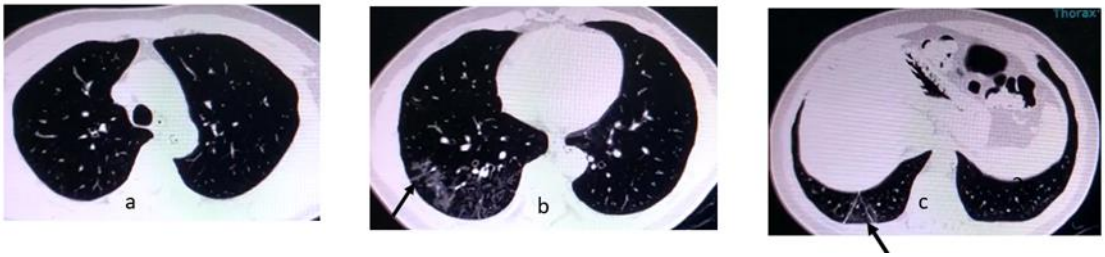

Figure 4. (Case 2) Follow up HRCT in 20 days from the first HRCT shows improvement. Decrease right lower GGO (b). Other lesions disappeared (a.c) suitable for convalescence stage.

Discussion

Viral nucleic acid test by RT-PCR assay, the current diagnostic criteria, plays a vital role in diagnosing COVID-19, despite its moderate sensitivity and long processing time. A nasopharyngeal swab is the most popular site for obtaining a viral sample, with sensitivity reported from previous reports ranging from 30-60%[4,5]. Several factors that can influence the RT-PCR test are the sampling procedure, the disease phase, and the detection kit's performance[6]. Supplementing a non-invasive imaging modality such as chest CT will enhance the diagnostic interpretation in suspected cases[3].

In case no 1, the first HRCT carried out on the third day of symptoms showed bilateral, multifocal sub-pleural GGO and crazy paving consistent with minimal typical COVID 19 pneumonia. The therapy was started based on the clinical, inflammatory marker, and HRCT report because the RT PCR result was only positive in the next seven days[7,8]. The subsequent HRCT was done 34 days after the first HRCT and all of the lesions have disappeared. According to several studies, COVID 19 patients have a GGO image on chest CT at the early stages of the disease, which is from day one to four of infection, then at the period of consolidation during the disease progression from day five to 13, and at the peak stage of the disease. Additionally, several patterns such as GGO, consolidation, crazy paving patterns, linear curves, and parenchymal bands can be found at the peak stage of the disease. During recovering phase of COVID-19, the lungs' initial finding on chest CT scan is a small subpleural GGO that grows larger with a crazy paving pattern and initial consolidation. Lung consolidations increase until two weeks of the first symptoms and the lesions were gradually absorbed,

leaving extensive GGO and sub-pleural parenchymal bands over the time[9,10].

A recent report regarding SARS-CoV-2 stated that about 21,4% of patients experienced prolong nucleic acid detection by RT-PCR test for SARS CoV-2 after a negative result.[3] It was reported that RT-PCR conversion's median time was 19,5 days (range 17-24 days). Our female patient's (case1) RT-PCR positivity timeline from the first to the fourth swab nasopharyngeal was 35 days. Previous studies in SARS-CoV-1 and MERS-CoV indicated that viral RNA could be detected in clinical specimens of patients for more than 30 days after the onset of symptoms[2]. Another study reported a certain number of COVID-19 patients might experience a prolonged nucleic acid conversion regardless of symptoms or radiology. Trace of viral detected by RT-PCR was not necessarily correlated with the ability of transmission[3], because upper respiratory tract was thought to be the main target of SARS-CoV-2 which is often located higher in the upper respiratory tract specimen. This should potentially be caused by prolonged viral shedding in the upper and lower respiratory tract[2,5]. Two negative SARS-CoV-2 RNA PCR tests, at least 24 hours apart, was recommended by the WHO as one of several criterias to release COVID 19 patient from isolation. Prolonged periods of detectable SARS-CoV-2 RNA has suggested a sustained viral replication in some kinds of host cells in patients with COVID-19.

RT-PCR conversion was compatible with the improvement of radiological abnormalities in majority cases. However, extensive studies are urgently needed to explore the duration of infectivity[2]. There is a discrepancy in CT and RT-PCR findings, as there are patients who have positive RT-PCR results without lesion on initial chest CT[1]. At the same time, some case evaluations showed the existence of about 3.5% of disease progression after RT-PCR results turning negative. Even though, after recovery, radiologic abnormalities showed mark improvement, but fibrotic changes remain the same.[1,5] Typical chest CT findings had a high sensitivity for initial false-negative RT-PCR, asymptomatic, and mild symptoms patients. Our second case, the male patient was treated by COVID 19 standard regimen based on the clinical symptoms, typical HRCT findings and positive serum antibody for SARS-CoV-2 result, despite negative RT-PCR result.

From another review, 5% of patients had initial false-negative RT-PCR results and then turned positive after the test are repeated. Many cases with initial false-negative RT-PCR have been reported to have typical COVID 19 chest CT[9]. Although the RT-PCR offers a valuable method in the diagnostic process, we have to be careful in interpreting the duration of viral shedding and infectivity status because it does not distinguish between infectious and non-infectious virus.

Recommendation

These two cases proved the pivotal role of thoracic HRCT scan in diagnosing and following up the case of confirmed COVID-19. As RT-PCR has some limitations, we suggest implementing multiple modalities besides RT-PCR, including clinical features, serial chest HRCT, an inflammation marker, antibody testing, and perhaps lung function test. Chest HRCT can be used in the management of diagnosing COVID-19 as well as follow up treatment.

Table 1. Clinical and laboratory findings

	Patient 1	Patient 2	Reference
Sex	Female	Male	
Age	43	51	
Comorbidities	Asthma		
Symptoms			
Fever	yes	yes	
Cough	yes	yes	
Nausea	yes	no	
Malaise	yes	yes	
Anosmia	yes	no	
Laboratory results			
Haemoglobin (g/dL)	13.3	13.6	13.2 – 17.3
Thrombocyte (10^3/uL)	185	149	150 - 440
Leukocyte (10^6/uL)	4.9	3.2	3.8 – 10.6
Lymphocyte (%)	32	33	25 - 40
Eosinophil (%)	0	1	2 - 4
Monocyte (%)	21	14	2 - 8
CRP (mg/L)	40	80	< 5
ESR (mm/hr)	26	62	0 – 30
LDH (U/L)	349	507	< 480
Therapy			
Oseltamivir	yes	yes	
Chloroquine	yes	yes	
Azitromycine	yes	yes	
RT-PCR	+.-,+,+,+	-,-,-	
Antibody test	Non-reactive (14 days)	Reactive twice (7 and 14 days)	

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Conflict of interests: The authors declare that they have no competing interest

Contributions: All the authors contributed equally for the conception or design of the work; the acquisition, analysis, or interpretation of data for the work; drafting the work or revising it critically for important intellectual content; final approval of the version to be published. All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Consent for publication: The patients gave their written consent to use their personal data for the publication of this case report and any accompanying images. The patient understands that their name and initials will not be published.