ORIGINAL ARTICLE

FACTORS AND CHARACTERISTICS ASSOCIATED WITH ASYMPTOMATIC COVID-19 INFECTION IN A DISTRICT SETTING IN MALAYSIA

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ABSTRACT

We aimed to determine the prevalence, characteristics, and risk factors associated with asymptomatic COVID-19 cases in a district setting in Malaysia. We included all confirmed COVID-19 cases within the district from inception to March 2021. Out of 3,051 confirmed COVID-19 cases, 1,862 (61.0%) were asymptomatic. Asymptomatic cases were mostly men, younger, non-Malaysians and had fewer comorbidities. The highest prevalence of asymptomatic cases was from prisons and factories. Male gender (adjusted OR 2.02; 95%CI 1.69 – 2.43), non-Malaysians (adjusted OR 20.00; 95%CI 14.75 – 27.64), current smoking status (adjusted OR 12.06; 95%CI 6.74 – 23.94), prisoners (adjusted OR 19.02; 95%CI 12.30 – 30.57), and higher CT values were significantly associated with asymptomatic COVID-19 infection. To ensure more targeted public health prevention and infection containment strategies, authorities and employers should be more aware of their local population's heterogeneity, risk factors, and unique conditions.

Keywords: COVID-19, Asymptomatic, Associated Factor

INTRODUCTION

The World Health Organization (WHO) declared the novel coronavirus SARS-CoV-2 (COVID-19) pandemic as a global health emergency on 30 January 2020 [1]. As of 20 June 2021, more than 177 million cases were reported, with more than 3 million deaths globally [2]. With a population of 32.66 million [3], Malaysia's infection rate in mid-2021 was at its highest and deadliest since the pandemic started [4]. More than 1.4 million cases were registered in Malaysia until 18 August 2021, with 53,657 cases in the state of Perak itself [5].

The COVID-19 infection is primarily transmitted through respiratory droplets, such as through coughing, and has a mean incubation period of 5 days [6, 7, 8]. Asymptomatic cases were reported to contribute up to 40% of all COVID-19 cases [9, 10]. Although these cases lack symptoms and have better recovery from the infection, there is increasing evidence reporting possible transmission of COVID-19 from asymptomatic or pre-symptomatic persons [6, 11]. A recent meta-analysis demonstrated no significant differences in transmission rates of asymptomatic and symptomatic cases [9]. Although some studies reported younger age groups and the absence of underlying medical diseases as potential determinants of asymptomatic infection [12], there is still no conclusive evidence on the determinants of asymptomatic infections [12].

Asymptomatic cases represent a significant public health problem as they are frequently missed or underdiagnosed, and this complicates public health management and might substantially contribute to the spread of COVID-19 [13,14]. Early detection of an infected person in the community is crucial in controlling COVID-19 transmission. With fewer available resources at the district level than higher populated regions, evaluating risk factors in a district setting will allow for a more targeted response strategy. This will improve and facilitate the use of resources to strengthen transmission control. Notifying the local district health office is compulsory for all positive COVID-19 cases. Thus, we aimed to determine the prevalence, characteristics, and risk factors of asymptomatic COVID-19 cases amongst the positive COVID-19 cases in Kinta District, Perak, Malaysia.

METHODS

We conducted a cross-sectional study in the Kinta District, Perak, Malaysia. We sequentially included all COVID-19 confirmed cases registered from 14 March 2020 until 25 March 2021 at the Kinta District Health Office, Perak, Malaysia. All baseline and outcome data were collected from the Kinta District Health Office database. This study was approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (NMRR-20-2788-57742).

Definitions
Cases were defined as individuals, irrespective of age, nationality, and gender, with a confirmed diagnosis of COVID-19 infection. Confirmed diagnosis of COVID-19 infection was defined by the presence of SARS-CoV-2 viral RNA examined

during a real-time reverse transcription-polymerase chain reaction test (RT-PCR) performed in any of the accredited laboratories by the Ministry of Health Malaysia (MoH) [15].

The symptomatic case was defined as positive COVID-19 RT-PCR with any of the following self-reported symptoms: fever, cough, general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnoea, anorexia/ nausea/ vomiting, diarrhoea, anosmia or ageusia [15]. The asymptomatic case was defined as positive COVID-19 RT-PCR without the self-reported symptoms listed above.

In this study, we categorised the sources of infection based on the geographical guidelines by the Ministry of Health Malaysia and unique environmental characteristics. Sources of infection were the following: 1) Local community: source likely within the Kinta district; 2) Import A: source likely from outside Malaysia; 3) Import B: source likely from other states within Malaysia; 4) Import C: source likely to be from other districts within the state of Perak; 5) Factory: source likely from factories within the Kinta district; and 6) Prison: source likely from prisons within the Kinta district. Definitions of the local community, Import A, Import B, and Import C were based on the Ministry of Health Malaysia guidelines [5,16]. In addition to these four categories, cases from factories and prisons were categorised separately due to their unique environmental characteristics compared to the general population.

The presence of comorbidity was defined as self-reported history of any but not limited to the following: diabetes mellitus, hypertension, asthma, chronic obstructive pulmonary disease, and chronic kidney disease [17].

Cycle threshold (CT) values were categorised into three groups based on the likelihood of infectiousness. Less or equal to 25 as they are most likely to be infectious [18], 26 to 35. CT values above 35 are considered not contagious [19].

Statistical analysis

Non-normally distributed continuous variables were presented as median (interquartile range), while categorical variables were presented as numbers (percentage). Evaluation of differences between the two groups was performed with unpaired t-test or Mann-Whitney rank test for continuous variables and with chi-squared test or Fisher's exact test for categorical variables. We evaluated the risk factors associated with the absence of symptoms upon confirmation of COVID-19 diagnosis through univariate and multivariate logistic regression. A stepwise backward selection with minimising Akaike information criterion (AIC) was used to select the variables in the multivariate analysis. The stepwise backward selection enabled full consideration of the full model, and all candidate variables were assessed [20]. AIC was preferred as it attempts to select the model by balancing the underfitting and overfitting of model [20]. We also reported the model's discriminative value through C-statistics and pseudo-R2. For all analyses, a two-sided p<0.05 was considered statistically significant. All analyses were performed in R (version 4.0.1) [21].

RESULTS

There were 3,051 confirmed Covid19 cases in the Kinta District up to 25 March 2021. Cases were mostly male, 1,821 (59.7%) with a median age of 31 years (24 – 42 years). The majority of cases were Malaysians (71.6%). There were 51.9% Malays, 6.6% Chinese, 13.4% Indian, 7.8% Bangladeshi, 6.9% Indonesian, 6.0% Myanmar, 5.4% Nepal and 2.1% other races. Only 14.8% of the cases had a history of at least one comorbidity. The reported comorbidities were diabetes (5.2%), hypertension (5.7%), asthma/COPD (1.8%), coronary heart disease (4.2%), and chronic kidney disease (1.4%). There were only 6.0% active smokers. The highest number of cases were reported from the local community (42.1%), followed by factories (35.7%).

Characteristics of the symptomatic and asymptomatic cases

There was a total of 1,862 (61.0%) reported asymptomatic cases and 1,189 (39.0%) symptomatic cases. The asymptomatic cases appear to be younger and of different ethnicities and nationalities. The presence of any comorbidities was also lower in asymptomatic cases (10.8%) than in symptomatic cases (21.0%). The presence of diabetes, hypertension and coronary heart disease was significantly different in asymptomatic cases than in symptomatic cases (p<0.001 for all three comorbidities). The highest proportions of asymptomatic cases by the source of infection were for factories and prisons. The characteristics of asymptomatic and symptomatic cases are presented in Table 1.

Overall, 835 out of 1,189 symptomatic cases reported the presence of fever (27.4%). Other common symptoms include cough (18.3%) and coryza (10.3%). Figure 1 shows an UpSet plot of combinations of self-reported symptoms in descending order of frequency. Fever only (19.9%) was the most common presented, followed by fever and cough (13.6%), then cough only (12.8%).

Factors associated with asymptomatic COVID-19 infection

Male gender (adjusted OR 2.02; 95%CI 1.69-2.43), non-Malaysian nationality (adjusted OR 20.00; 95%CI 14.75-27.64) and history of current smoking (adjusted OR 12.06; 95%CI 6.74-23.94) were associated with asymptomatic presentation. Prisoners were about 19 times more likely to be associated with asymptomatic infection than the local community. The asymptomatic presentation

was also associated with higher cycle threshold (CT) values: CT 26-35 (adjusted OR 1.60; 95%CI 1.32 – 1.93) and CT ≥36 (adjusted OR 2.74; 95%CI 2.05 – 3.70). Cases with a history of interstate travel (import B) and screened at government hospitals had a higher risk of symptomatic presentation. The regression analysis is presented in Table 2.

Table 1: Characteristics of case participants

Variable [a]	Asymptomatic N= 1,862	Symptomatic N= 1,189	p-value*
Age, in years	30 (24.7 – 38.7)	34 (24.0 – 50.0)	<0.001*
≤ 20	192 (10.3%)	188 (15.8%)	<0.001*
21 – 40	1266 (68.2%)	553 (46.5%)	
41 – 60	294 (15.8%)	310 (26.1%)	
≥ 61	105 (5.7%)	138 (11.6%)	
Male gender	1,237 (66.4%)	584 (49.1%)	<0.001*
Ethnicity			<0.001*
Malay	707 (38.0%)	875 (73.6%)	
Chinese	104 (5.6%)	97 (8.2%)	
Indian	249 (13.4%)	161 (13.5%)	
Bangladeshi	232 (12.5%)	5 (0.4%)	
Indonesian	184 (9.9%)	25 (2.1%)	
Myanmar	179 (9.6%)	4 (0.3%)	
Nepal	151 (8.1%)	13 (1.1%)	
Others	56 (3.0%)	9 (0.8%)	
Nationality			<0.001*
Malaysian	1,052 (56.5%)	1,133 (95.3%)	
Non-Malaysian	810 (43.5%)	56 (4.7%)	
Comorbidities			
Any comorbid	201 (10.8%)	250 (21.0%)	<0.001*
Diabetes	71 (3.8%)	89 (7.5%)	<0.001*
Hypertension	69 (3.7%)	105 (8.8%)	<0.001*
Asthma/ COPD	35 (1.9%)	19 (1.6%)	0.664
CHD	53 (2.9%)	75 (6.3%)	<0.001*
CKD	24 (1.3%)	20 (1.7%)	0.464
Other	43 (2.3%)	40 (3.4%)	0.103
Currently smoking	171 (16.3%)	11 (1.0%)	<0.001*
Source of infection			<0.001*
Local community	525 (28.2%)	760 (63.9%)	
Import A	11 (0.6%)	18 (1.5%)	
Import B	44 (2.4%)	127 (10.7%)	
Import C	17 (0.9%)	31 (2.61%)	
Prison	401 (21.5%)	27 (2.3%)	
Factory	864 (46.4%)	226 (19.0%)	
Screening Locality			<0.001*
Government Hospital	50 (2.7%)	171 (14.4%)	
District health facilities	1077 (57.8%)	816 (68.6%)	
Private clinic	705 (37.9%)	163 (13.7%)	
Private hospital	30 (1.6%)	39 (3.3%)	
CT value	29.06 (23.5 – 33.9)	25.80 (21.6 – 30.2)	<0.001*

[a] Information for all variables was available for 1,862 asymptomatic cases and 1,189 symptomatic cases except for age (1,857 asymptomatic cases and 1,189 symptomatic cases) and CT value (1,812 asymptomatic cases and 1,085 symptomatic cases)

Not normally distributed variables are presented as median (IQR), categorical variables are presented as count (percentage). *p-values <0.05 are considered as statistically significant values and were calculated from unadjusted X2 test of independence or Fisher's Exact test (n≤5 in any cell) for categorical variables and from unpaired t-test or Mann-Whitney U (non-normally distributed data) for continuous variables. CHD: Coronary heart disease. CKD: Chronic kidney disease. COPD: Chronic obstructive pulmonary disease.
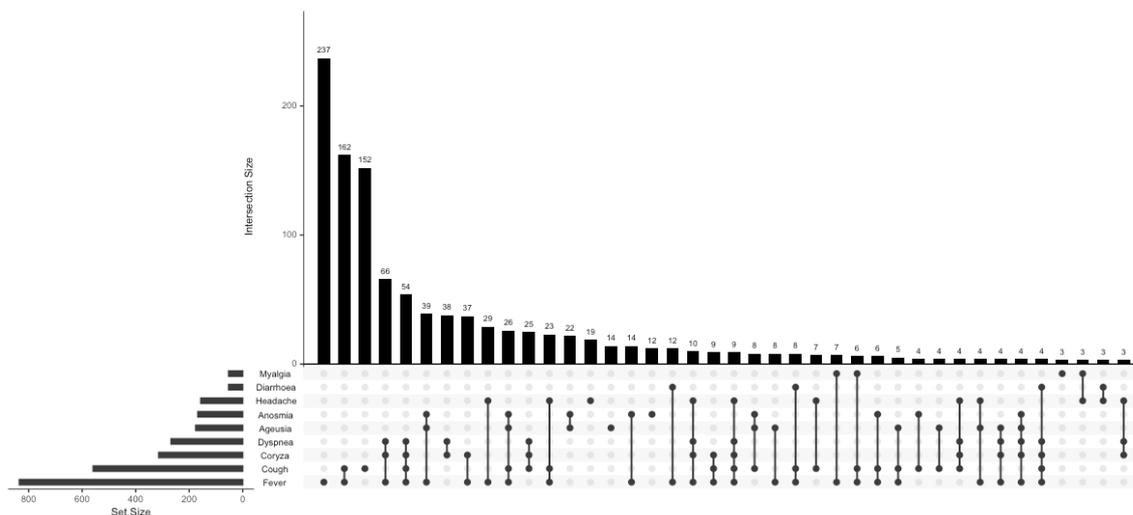
Figure 1: Distribution of self-reported symptoms combinations

Table 2: Association of risk factors and asymptomatic Covid-19 infection

Variable	Univariable regression OR (95%CI)	P-value	Adjusted OR (95%CI)[a] OR (95%CI)	P-value
Age, in years				
≤20	0.44 (0.35 – 0.55)	<0.001	0.95 (0.73 – 1.24)	0.731
21 – 40	Reference	NA	Reference	NA
41 – 60	0.42 (0.34 – 0.51)	<0.001	1.02 (0.81 – 1.28))	0.858
≥61	0.35 (0.26 – 0.47)	<0.001	1.08 (0.77 – 1.50)	0.661
Gender				
Female	Reference	NA	Reference	NA
Male	2.17 (1.86 – 2.54)	<0.001	2.02 (1.69 – 2.43)	<0.001
Nationality				
Malaysian	Reference	NA	Reference	NA
Non-Malaysian	16.14 (12.13 – 21.95)	<0.001	20.00 (14.75 – 27.64)	<0.001
Comorbidities				
Absent	Reference	NA	Reference	NA
Present	0.46 (0.37 – 0.56)	<0.001	0.80 (0.59 – 1.07)	0.133
Asthma/COAD				
No	Reference	NA	Reference	NA
Yes	0.81 (0.44 – 1.42)	0.469	1.73 (0.94 – 3.29)	0.085
Diabetes				
No	Reference	NA	Reference	NA
Yes	0.49 (0.35 – 0.68)	<0.001	0.94 (0.64 – 1.36)	0.736
Smoking				
No	Reference	NA	Reference	NA
Yes	10 (21 (5.79 –20.04)	<0.001	12.06 (6.74 – 23.94)	<0.001
Infection source				
Local community	Reference		Reference	NA
Import A	>100 (<0.01 – NA)	0.958	>100 (<0.01 – NA)	0.956
Import B	0.20 (0.13 – 0.28)	<0.001	0.50 (0.33 – 0.74)	<0.001
Import C	0.35 (0.17 – 0.66)	0.002	0.94 (0.46 – 1.83)	0.852
Prison	11.18 (7.66 – 17.02)	<0.001	19.02 (12.30 30.57)	<0.001
Factory	3.47 (2.92 – 4.13)	<0.001	1.03 (0.79 – 1.35)	0.816
Screening Locality				
District health facilities	Reference	NA	Reference	NA
Government Hospital	0.24 (0.16-0.34)	<0.001	0.35 (0.23-0.51)	<0.001
Private clinic	3.20 (2.64-3.90)	<0.001	0.99 (0.77-1.28)	0.95
Private hospital	0.52 (0.30-0.87)	0.010	0.86 (0.49-1.50)	0.60
CT Value				
≤25	Reference	NA	Reference	NA
26 - 35	1.52 (1.29 – 1.78)	<0.001	1.60 (1.32 – 1.93)	<0.001
≥36	2.89 (2.24 – 3.75)	<0.001	2.74 (2.05 – 3.70)	<0.001

Complete case analysis for 2,892 cases
[a] *AUC 0.85, Pseudo-R2 0.32 in the logistic regression*

DISCUSSION

Overall, two-thirds of the cases reported in the Kinta district were asymptomatic. However, this study's prevalence of asymptomatic cases was higher than in other studies [9, 10]. A meta-analysis of 79 studies reported that only 20% of cases remained asymptomatic during follow-up [22]. The study's cross-sectional nature enabled asymptomatic status only to be collected at diagnosis. Therefore, the prevalence of asymptomatic cases in this study may be overestimated due to the inclusion of both those who remained asymptomatic throughout the infection and pre-symptomatic cases (i.e. those who develop symptoms later) [22].

Gender, nationality, smoking status, infection source, screening locality and CT Value were associated with the risk of asymptomatic COVID-19 infection. Male cases were two times more likely to be asymptomatic than female cases. This finding did not concur with existing evidence that the male gender is a risk factor for incidence, severity, and mortality [23,24,25]. Sensitivity analysis on different sources of infection still resulted in the male gender having a higher risk of asymptomatic infection than females.

Non-Malaysian citizens had 20 times higher odds of having no symptoms than Malaysians. The non-Malaysian citizens were mainly migrant workers who worked in factories and low-skilled labour industries. In Malaysia, migrant workers undergo a medical examination before working in the country and before renewing work permits [26]. Given the compulsory routine medical examinations and the nature of their work, migrant workers are less likely to have concurrent comorbidities. Additionally, the educational background of these migrant workers may limit their knowledge and awareness of the COVID-19 infection and symptoms, thus potentially underreporting their symptoms.

Smoking is a known predisposing factor to respiratory diseases [27]. The overall smoking prevalence in our study was 6% which was significantly lower than the national prevalence of 21% [28]. The underreporting of smoking status may be due to reporting bias from the inclusion of only the current smoking status of case participants. This may not reflect their smoking history, especially for institutionalised case participants. However, the relationship between smoking and COVID-19 is complex. One study highlighted the smoker's paradox –a lower prevalence of smokers was reported with higher odds of developing asymptomatic COVID-19 infection [29]. Goyal et al. [30] found that smokers made up about 5% of the hospitalised population. A large study of the general population of the United Kingdom reported that smoking was associated with reduced risk for COVID-19 mortality (OR: 0.88) [31]. In a study conducted in Malaysia, despite a low prevalence of active smokers in the study population, the authors reported a higher risk of COVID-19 complications with smoking status [32]. It has been proposed that nicotine, an agonist of the cholinergic anti-inflammatory pathway and nitric oxide produced during smoking, could maintain airway dilation, thereby possibly protecting against the severity of COVID-19 [33,34]. Although the lower prevalence of smokers was seen in COVID-19 cases, the numerous proven adverse health effects of smoking are unlikely to outweigh its benefits.

Compared to local cases, prisoners had 19 times higher odds of having an asymptomatic infection at diagnosis. It has been reported that more than half of the prisons in the world exceeded their actual capacity [35]. Overcrowding in prisons has been previously linked to adverse health outcomes and transmission of infectious diseases such as tuberculosis and skin infections [36]. Various countries have reported rapid progression of COVID-19 infection in their prisons contributed by overcrowding and poor ventilation inside them [37,38]. Several studies reported a high prevalence of asymptomatic cases amongst prison inmates [39,40]. This might be elucidated because inmates tend to be younger and have fewer comorbidities.

Given the restrictions in place at the prisons, the interviewers could not assess the inmates' symptoms and past medical history in person. Thereby potentially also contributing to reporting bias. Import B cases were more likely to be symptomatic by about 50% compared to local cases. Most of these cases had interstate travel history from the COVID-19 hotspot region seeking treatment due to being symptomatic 41. Hence, more likely to be symptomatic as compared to local cases.

Interestingly, sampling location was significantly associated with asymptomatic COVID-19 infection. Compared to the sample taken by district health offices, the samples obtained at government hospitals were 65% less likely to be asymptomatic. Government hospitals mainly carried out screening for symptomatic cases who presented with respiratory-like illness, especially those that required hospitalisation. Whereas district health offices primarily conducted screening of close contacts of positive cases which may or may not have symptoms during screening. This concurs with another study that reports the higher chances of being symptomatic from hospital screening [42].

RT-PCR tests are considered the gold standard for diagnosing COVID-19 [43]. However, RT-PCR does not measure the viral load within a clinical specimen [44]. CT values of RT-PCR represent the number of amplification cycles required for the viral RNA to exceed a threshold level [45]. These values are inversely related to viral load and assess viral RNA concentration within a clinical

specimen. Lower CT values correspond to higher viral RNA concentration and vice versa. We found that higher odds of asymptomatic COVID-19 infection was associated with increasing CT value categories. This concurs with other studies that associate symptomatic infection with lower CT values [46,47].

This study has the advantage of including all COVID-19 cases reported in the district. In Malaysia, all suspected, probable and confirmed cases of COVID-19 are required by law to be reported to the local district health office under the 'Prevention and Control of Infectious Disease Act 1988 [48]. Although the findings of this study are comprehensive to the Kinta district setting, these results may not be generalisable to other districts or countries. The inclusion of pre-symptomatic cases as asymptomatic cases also limits the findings of this study.

The findings of this study suggest that the screening threshold of persons with higher risks of asymptomatic infection to be lowered to prevent the spread of the disease better. Male gender, non-Malaysian, prisoners, and anyone who is currently smoking should be tested even if asymptomatic if they had close contact with a known case of COVID-19.

It is also important to note that these trends occurred before the Government of Malaysia's extensive and comprehensive vaccination efforts. Despite the evidence in unvaccinated individuals, similar risk factors should be sought out even in fully vaccinated individuals. As a large majority of the adult population in the Kinta district have achieved full vaccination status, further evaluation of the risk factors and outcomes of COVID-19 infection in this population is needed.

CONCLUSION

Male gender, non-Malaysian, current smoking status, prisoners and higher CT value were more likely to present with asymptomatic COVID-19 infection. To ensure more targeted public health prevention and infection containment strategies, authorities and employers should be more aware of their local population's heterogeneity, risk factors, and unique conditions.

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