Seroprevalence of Scrub typhus in East Khasi hills and South West Khasi hills districts of Meghalaya, India

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Abstract
This study investigates the seroprevalence of Scrub typhus in Meghalaya, India's east and south west Khasi hills districts. The Pasteur Institute in Shillong, Meghalaya, conducted a retrospective observational study from November 2022 to May 2023. The study involved 146 individuals with clinical suspicion of Scrub typhus, using the Scrub typhus Detect IgM Elisa. The IgM Elisa diagnosed Scrub typhus IgM levels >11 as positive, 9-11 as equivocal and <9 as negative. The R statistical program was used for statistical analysis. The Chi-square test was used to determine correlations between gender and age groups. Significant data was defined as a p-value of 0.05 or lower.

Acute pyrexia patients suspected of Scrub typhus tested positive for IgM Scrub typhus Elisa. Gender distribution was not significant, with males (72.7%) being the majority of positives. Age groups 0-10 years have the highest IgM 7/17 seropositive rates (41.2%), with majority patients aged 21-30. Males, particularly in rural areas, are engaged in outdoor activities more frequently than females, with 27.3% of males being positive and 72.7% being negative. Equivocal males are 42.9% more likely to engage in outdoor activities than females, who are more likely to spend time in the kitchen. Scrub typhus is endemic in many Indian regions, requiring ongoing surveillance. Patients with fever, rash, myalgia and liver failure should be evaluated, especially in high-altitude regions during the monsoon season. Undifferentiated fevers lasting over a week and not responding to traditional antibiotics warrant a clinical and diagnostic workup for Scrub typhus.

Keywords: Scrub typhus, *Orientia tsutsugamushi*, IgM, Elisa, Infection.

Introduction

Scrub typhus is a zoonotic bacterial infection that is re-emerging in the "tsutsugamushi triangle" of South and Southeast Asia, the Asian Pacific rim and Northern Australia[7]. This is a noteworthy development. According to the World Health Organisation, Scrub typhus is probably one of the most underdiagnosed and underreported febrile diseases requiring hospitalisation[24]. In endemic places, Scrub typhus accounts for up to 23% of all fever episodes, with an estimated 1 million cases every year. In India, Scrub typhus is a significant contributor to acute febrile sickness[12]. The prevalence is found to be higher in the winter and wet seasons. The seasonality of Scrub typhus is influenced by the local climate, temperature and amount of rainfall[9].

Increased humidity in India following the rainy season encourages the development of mite eggs into chiggers, which spreads Scrub typhus[23]. Understanding seasonality may be useful when adopting preventative steps. It is a significant contributor to acute undifferentiated febrile illness (AUFI) and is brought on by the arthropod-borne Gram-negative obligate intracellular bacillus *Orientia tsutsugamushi*. Intimate relationships and coexistence among *O.tsutsugamushi* are two of the four conditions necessary for the development of a microfocus of infection. Zoonotic tetrads include rats, chiggers and secondary or transitional vegetation[6]. Humans and other vertebrates can contract the disease from the trombiculid mite's larvae (chiggers)[22].

The mite goes through four phases of development: egg, larva, nymph and adult[22]. In rats and humans, horizontal transmission happens unintentionally after being bitten by chiggers and transmission occurs in rodents[8]. Although there has been one case of transplacental spread in a pregnant woman who delivered a preterm baby with hepatosplenomegaly, meningitis, sepsis and scrub IgM positive[20]. Vertical transovarial transmission happens in mites[8]. The mites for the summer type (March to November) and winter type (September to December) of Scrub typhus are Leptotrombidium deliense and L. scutellare respectively. Rattus losea, R. flavipectus and Apodemus agrarius are the reservoir hosts (rodents) for the former and A. agrarius, Cricetulus triton and R. Norvegicus are for the latter[10].

Aside from the six significant serotypes of *O. tsutsugamushi* Gilliam, Karp, Kato, Shimokoshi, Kawasaki and Kuroki, there are more than 30 strains that are antigenically distinct[16]. Hematogenous and lymphatic pathways are both used by infection to disseminate[10]. The endothelial cells of the various systems are the target place for multiplication[13]. Immunity mediated by cells as well as humoral mechanisms are crucial for preventing Scrub typhus[13].

India's Himachal Pradesh saw the first occurrences that were recorded in 1934. We do not have precise figures for India due to lack of knowledge, unavailability, the high expense of diagnostic testing and the fact that it is not a disease that

can be reported. Frequently, Scrub typhus can result in severe consequences, some of which might be fatal. These complications include pneumonia, myocarditis, meningoencephalitis, acute renal failure and gastrointestinal bleeding. Early identification is crucial since treatment normally works quite well and prompt antimicrobial medication may reduce the risk of sequelae. It is advisable to advise empiric therapy for patients with undifferentiated febrile illness who have evidence of multiple system involvement in poor nations with scarce diagnostic resources[3].

The Weil-Felix test was the first to identify cases of Scrub typhus. It is simple to use, inexpensive and effective, but because it uses Ag derived from Proteus strains, its specificity is limited. The specificity of Elisa is good (87%) but it is expensive, time-consuming and cannot be used on a single serum. On the other hand, ICT is simple to use, has about equal sensitivity and specificity and can test just one serum.

To determine the cause of fever, investigations were conducted on every patient with fever who visited the medical OPD or casualty. These tests included a peripheral blood smear to check for parasites that cause malaria, a complete blood count and serology for dengue, leptospirosis, Scrub typhus and retroviral infections. Patients who required indoor admission and were positive for IgM antibodies to Scrub typhus, were included in the study. In our hospital's microbiology department, a test for serum IgM for Scrub typhus was conducted. As and when necessary, an electrocardiogram, arterial blood gas analysis and a chest X-ray were ordered. Analyses of the cerebrospinal fluid (CSF) and a brain CT were completed, if clinically necessary.

Renal and liver function tests were performed as part of biochemical studies and the results were recorded. Patients with multiple organ dysfunction were treated according to conventional practise, when necessary, using renal replacement therapy or ventillatory support. Doxycycline was administered orally or intravenously to all patients along with other supporting services. Azithromycin was given to ANC patients. Final results were recorded and the cause of death was assessed.

A number of people present with an acute febrile illness, test negative for typhoid, dengue, malaria and other prevalent causes of fever. For Scrub typhus, several of them test positively. Therefore, the goal of this retrospective study is to investigate the seroprevalence of Scrub typhus in two Meghalayan districts: East Khasi Hills and South West Khasi Hills.

Material and Methods

Pasteur Institute, a State Government run research institution in Shillong, Meghalaya, North-east India, conducted this retrospective observational study between November 2022 to May 2023. The investigation used serum samples from 146 individuals with a clinical suspicion of Scrub typhus. To separate the serum, the blood samples were centrifuged at 3000 rpm for 5 minutes. Before being examined, serum samples were kept at a temperature of 80 °C.

Scrub typhus IgM detection by ELISA (enzyme-linked immunoassay): InBios International's Scrub Typhus Detect™ Kit, which contains recombinant p56kDa type specific antigens of *Orientia tsutsugamushi* Karp, Kato, Gilliam and TA 716 strains, was used in accordance with the manufacturer's instructions to detect IgM antibodies to *Orientia tsutsugamushi*. Optical densities (ODs) > 0.50 were deemed positive when evaluated by the Elisa reader HumaReader HS. The cut-off was established in accordance with the kit protocol's instructions for calculating the endemic cut-off titre. From healthy volunteers, the cut-off was computed as follows: mean OD (0.23) + 3 standard deviations (0.09) = 0.50. In the IgM Elisa kit, a Scrub typhus IgM level >11 was regarded as positive, 9-11 was considered equivocal and level <9 was ruled out to be negative.

Quality assurance: Each slide had a positive and a negative control and tests were repeated if either one failed in a screening or titration slide. Both the antibody and antigen controls were re-titrated in cases of continued assay failure to determine if the antibody endpoint had changed or the antigen had lost its reactivity. Before repeating the experiment with the material, fresh controls and antigens were optimised as needed.

Statistical analysis: R statistical programme was used to conduct the statistical analysis. To determine whether there is any correlation between the various groups based on gender and age, the Chi-square test was used. Significant data was defined as a p-value of 0.05 or lower.

Ethical Clearance: Only demographic information about patients was kept in the Institute database to allow manual file retrieval based on patient codes. Due to the retrospective nature of the study, all data were anonymously evaluated without the consent of any specific patient. Hence, Institute Ethics Committee approval was not required.

Results

146 patients experiencing acute pyrexia had a clinical suspicion of Scrub typhus and 15.1% (22/146) of them tested positive for the IgM Scrub typhus Elisa. 80.1 % (117/146) of the IgM Scrub typhus Elisa results were negative while 4.8% (7/146) were equivocal.

The gender distribution of scrub typhus cases did not differ significantly in which 0-10 years were 76.5% males and 23.5% were females, 11-20 years comprised of 55.6% males and 44.4% females, 21-30 years were 72.1% males vs. 27.9% females, 31-40 years were 77.3% males vs. 22.7% females, 41-50 years were 77.3% males vs. 22.7% females, 51-60 years were 46.2% males vs. 53.9 females, 61-70 years

were 60% males vs 40% females and 71-80 years were 16.7% males vs. 83.3% females (Figure 1).

Age groups 0-10 years (Figure 2 and table 1) had the highest seropositive IgM 7/17 (41.2%), indicating they are the most vulnerable. The relationship between age group and positive rates had no statistically significance. The majority of patients are in the 21–30 demographic (Table 1).

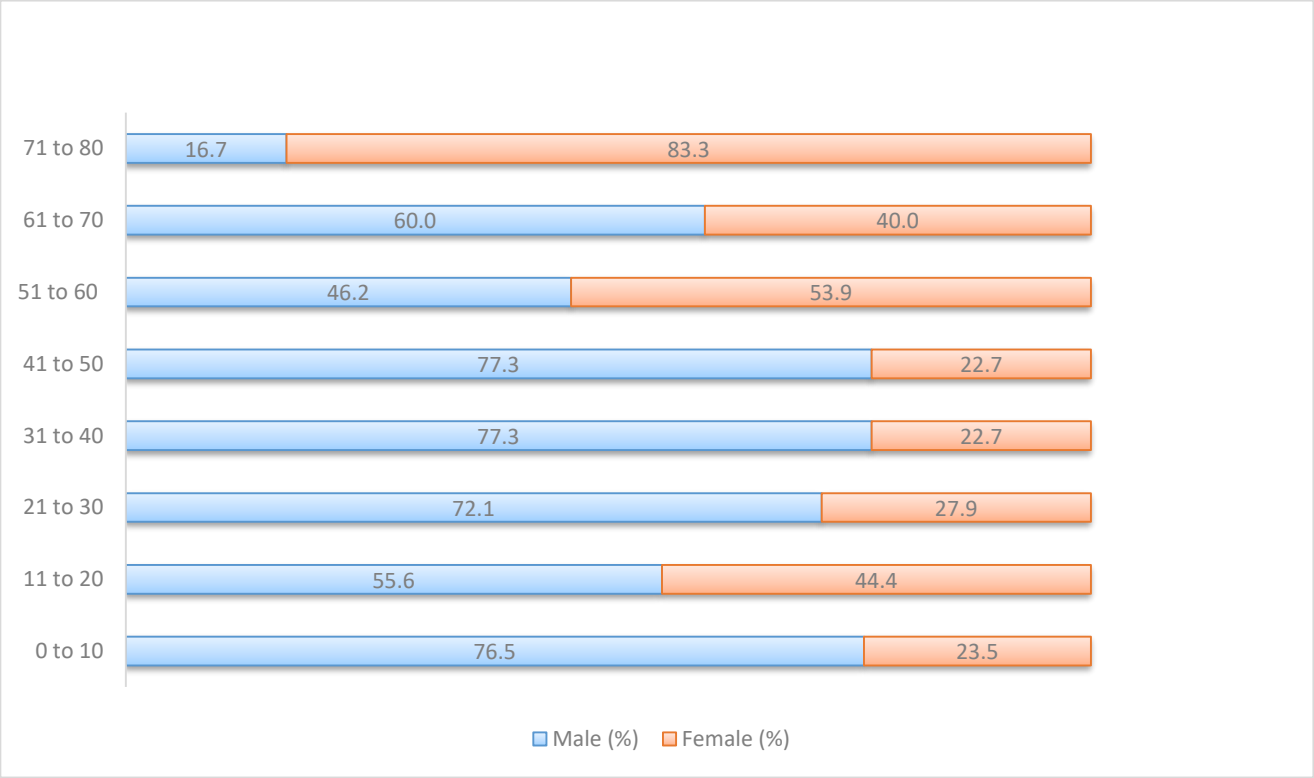

Figure 1: Gender distribution for each age group

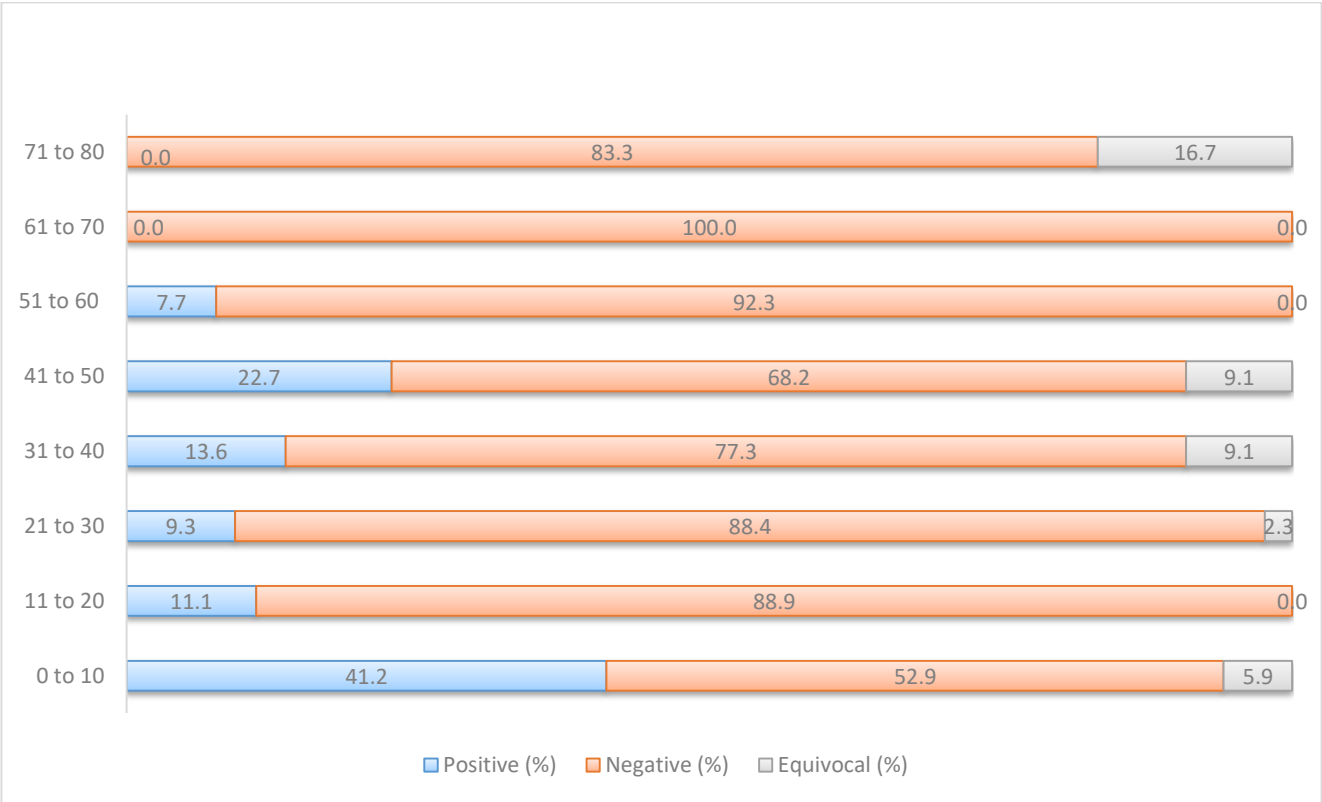

Figure 2: Percentage of positive, negative and equivocal patients for each age group

The percentage of males versus females who were positive, was 27.3% vs. 72.7%; the percentage of males versus females who were negative, was 33.3% vs. 66.7% and the percentage of males versus females who were equivocal, was 42.9% vs. 57.1%. This shows that males, especially in rural areas, engage in outdoor activities more frequently than females, who are assumed to devote more time in the kitchen. Positive rates for age 0-10 years stand at 41.2% being the most prevalent, 41-50 years at 22.7%, 31-40 years at 13.6%, 11-20 years at 11.1% while 21-30 years at 9.3% and 51-60 years at 7.7% whereas no positive cases were detected in age groups of 61-70 years and 71-80 years. Equivocal stands at 16.2% for 71-80 years, 9.1% for both 31-40 years and 41-50 years, 5.9% for 0-10 years and 2.3% for 21-30 years respectively.

Discussion

In many Nations, the prevalence of Scrub typhus ranges from 0-8% to 60%[21]. Compared to earlier Indian studies where the prevalence of Scrub typhus ranges from 13 to 63%[1,15,17], the current study's prevalence of the bacterial infection was 15.06%. In general, acute undifferentiated fevers are particularly for all intents and purposes widespread in really tropical countries like India because of the sort of optimal hatching conditions for mosquitoes and mites during the monsoon and post-monsoon seasons. The prevalence of infectious vectors increased human-vector contact, moderately high temperatures, high humidity, particularly prolonged showers and lush vegetation growth, all contributing to the spread of disease.

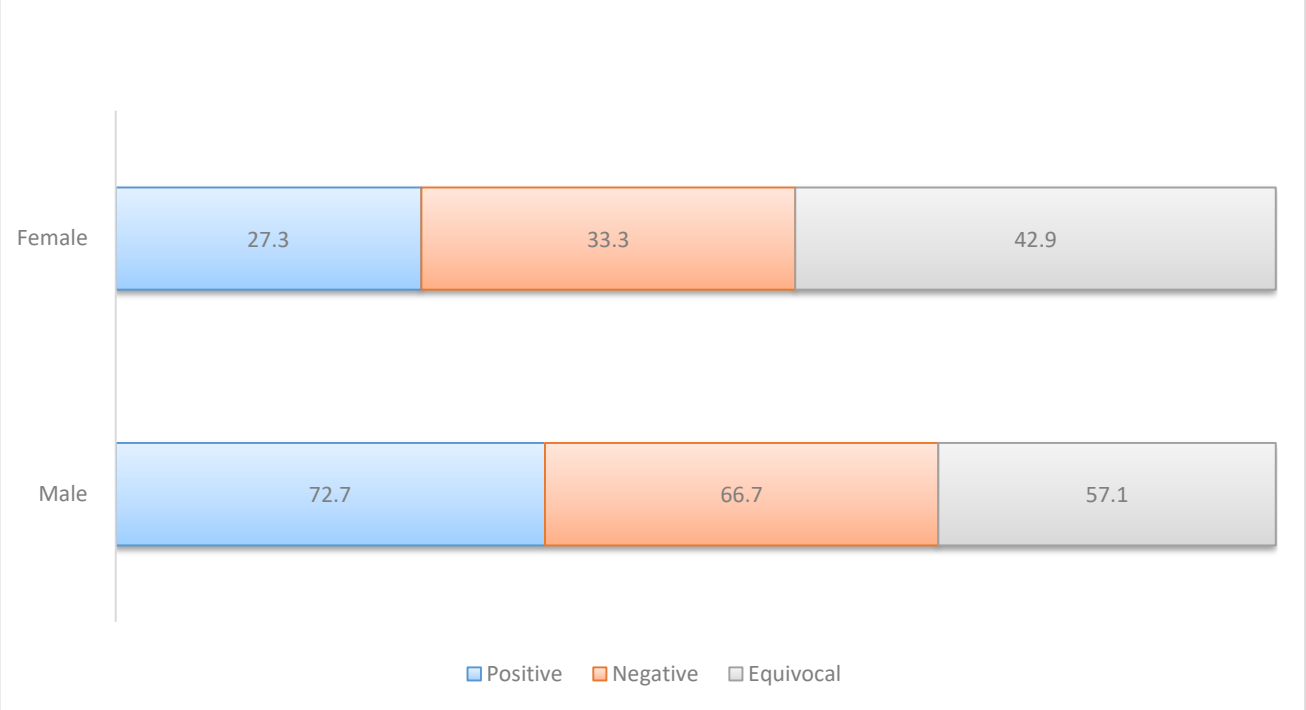

Figure 3: Percentage difference of positive, negative and equivocal patients for females vs. males

Table 1
Positivity rate of baseline characteristics of study population

Characteristics		Positive n (%)	Negative n(%)	Equivocal n(%)	p-value
Sex	Male	16(72.7)	78(66.7)	4(57.1)	0.73
	Female	6(27.3)	39(33.3)	3(42.9)	
Age group	0 to 10	7(41.2)	9(52.9)	1(5.9)	0.12
	11 to 20	2(11.1)	16(88.9)	0(0)	
	21 to 30	4(9.3)	38(88.4)	1(2.3)	
	31 to 40	3(13.6)	17(77.3)	2(9.1)	
	41 to 50	5(22.7)	15(68.2)	2(9.1)	
	51 to 60	1(7.7)	12(92.3)	0(0)	
	61 to 70	0(0)	5(100.0)	0(0)	
	71 to 80	0(0)	5(83.3)	1(16.2)	

The various etiological agents and similar clinical manifestations of AUFI make diagnosis and treatment even more difficult. In this study, males were infected by far the highest percentage, which is consistent with research from South India[15,18] which demonstrates that, contrary to popular belief, men spend more time outdoors than women do, especially in rural areas where women are generally thought to spend more time indoor doing house chores. Due of the need for a habitat where the adults have an abundant prey population and where mammals constantly go through, mites can have a very patchy distribution. Additionally, the local climate must not require the mites to burrow too deeply for protection, as this stops them from laying eggs.

Even though the climate of the north-eastern Indian State of Meghalaya is one that is conducive to the breeding of the vector, Scrub typhus is prevalent in a significant portion of that country. The hilly Khasi hill region is ongoing industrial and infrastructure development that favours the breeding of vectors. The severity of this disease's clinical symptoms ranges from mild illness to deadly illness with multi-organ failure. Fever, coughing and shortness of breath were the three most typical presenting symptoms. In these situations, fever is virtually always present and was observed in every case. Fever can last for a short period (less than 7 days) or a long period (more than 7 days). Rama et al[14] reported 28% and 72% of patients with fever of varying lengths.

Similar to prior investigations, thirteen patients exhibited altered sensorium[4,5]. Although its prevalence varies from 7-97%, the appearance of an eschar is a crucial clinical clue in the diagnosis of Scrub typhus[11]. Eschar, a pathognomonic sign of Scrub typhus, was present in 75% of the patients in Chanta et al[2]'s study and was typically painless. In Sudhakar et al[19]'s study, all patients with Scrub typhus were diagnosed and had eschar. In our study, however, only thirty three patients (23.6%) had eschar. As a result, the eschar mark alone could not be used to raise a suspicion of the disease.

If patients are admitted later, mortality will be reduced as a result of the administration of oral doxycycline for patients with fever. As a result, the illness is not fatal. But the prognosis is poor when many organs are involved.

Conclusion

In conclusion, since Scrub typhus is endemic in many regions of India, surveillance should continue there. Patients who present with fever, rash, myalgia and liver failure, should also be evaluated for Scrub typhus, especially if they are from high-altitude regions where the disease is prevalent, especially during the monsoon season. All undifferentiated fevers lasting longer than a week and not responding to traditional empirical antibiotics justify a clinical and diagnostic workup for Scrub typhus. Important life-threatening consequences that increase the case fatality rate, include multi-organ failure, renal, hepatic and respiratory dysfunctions. Therefore, in northeast India, greater knowledge of the disease's presentation, clinical characteristics, laboratory findings and patient management will aid in lowering both morbidity and death from this infectious disease. One of the possible diagnoses for acute febrile sickness should be Scrub typhus. To spread awareness among the general public, the Government must organise programmes and health camps to increase clinicians understanding of Scrub typhus.

In order to inform people about disease transmission and preventive measures, scientists like microbiologists require more emphasis on the ground level. Additionally, to provide information for health policy, further study on this curable infection in India is necessary including epidemiological and basic research. Undeniably, rate of Scrub typhus should be at the bare minimum if India wants to eradicate this bacterial infection for good.

Acknowledgement

The staff member of the Ganesh Das Govt. M&CH hospital's infertility clinic, specifically Smt. Banroilang Ksanlah and Smt. Restitula Kharbuli, are gratefully acknowledged by the authors for entering and compiling the information from the medical professionals.

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(Received 28th April 2024, accepted 06th July 2024)