Epidemiol. Infect. (1991). **107**, 577–584
Printed in Great Britain

Sudden unexplained death syndrome – a new manifestation in melioidosis?

E. H. YAP[1], Y. C. CHAN[1], K. T. GOH[2], T. C. CHAO[3], B. H. HENG[2], T. W. THONG[1], H. C. TAN[1], K. T. THONG[1], E. JACOB[4] AND M. SINGH[1]

[1] *Department of Microbiology, National University of Singapore, Kent Ridge, Singapore 0511*

[2] *Quarantine and Epidemiology Department, Ministry of the Environment, Singapore*

[3] *Institute of Science and Forensic Medicine, Ministry of Health, Singapore*

[4] *Department of Pathology, Singapore General Hospital*

(*Accepted 18 July 1991*)

SUMMARY

The indirect haemagglutination (IHA) test using sensitized turkey erythrocytes and the indirect immunofluorescence assay (IgM-IFA) was confirmed to be sensitive in the detection of a recent or current *Pseudomonas pseudomallei* infection in 19 culture-confirmed Singapore melioidosis patients. All were found to have antibody titres from 4 to 32768 in the IHA test and 10 to 320 in the IgM-IFA test. When these tests were employed on sera from 16 immigrant Thai construction workers who died of sudden unexplained death syndrome (SUDS) and 73 healthy Thai fellow workers, 93·8% and 68·8% of SUDS cases had IHA titre of $\geqslant 4$ and IgM-IFA titre of $\geqslant 10$ respectively, in contrast to 39·7% and 12·3% found among healthy Thai workers. These data indicate that at the time of death, most of the SUDS patients had an active infection with *P. pseudomallei*, possibly resulting from reactivation of a latent infection. The aetiological role of *P. pseudomallei* as the major cause of SUDS is discussed.

INTRODUCTION

Sudden death in apparently healthy immigrant construction workers from Thailand has been reported in Singapore since 1982 [1]. Death usually occurred during sleep, and some of them were heard by their room-mates to breathe noisily, gasp or groan just before death. There were no prodromal symptoms and post-mortem examination did not reveal the cause of death which was usually attributable to cardio-respiratory failure. These deaths are now known as sudden unexplained death syndrome (SUDS).

We have investigated 16 SUDS cases that occurred in 1990 for evidence of an infectious aetiology as pulmonary haemorrhage was noted in more than half of the cases and myocarditis and pneumonitis in some at necropsy [1]. On the basis of epidemiological investigations which showed that 80–90% of SUDS cases originated from villages in north-eastern Thailand [1, 2] we suspected that

Pseudomonas pseudomallei may have an aetiological role in SUDS because the organism is hyperendemic and infection occurs mainly in males in that region [3]. Our initial study [4] showed that the majority (92·3%) of SUDS cases had indeed been exposed to *P. pseudomallei* as evidenced by the presence of antibodies detected by the indirect haemagglutination (IHA) test. This rate of seropositivity was found to be significantly higher than that in apparently normal, healthy Thais from the same region and living under similar conditions in Singapore.

Although the IHA test is a highly specific and sensitive test for the detection of *P. pseudomallei* infection, it is regarded by most workers to be less useful for detection of recent or current infection [5, 6]. Detection of specific IgM antibodies by the indirect immunofluorescence assay (IgM-IFA) and the enzyme-linked immunosorbent assay (IgM-ELISA) has been shown reliably to indicate active or current infection by *P. pseudomallei* [7]. In this paper, we describe the results of IHA and IgM-IFA tests on sera from bacteriologically confirmed melioidosis patients in Singapore, and from both healthy Thai workers and SUDS cases. On the basis of the serological findings in SUDS cases, we propose that *P. pseudomallei* infection may be aetiologically associated with SUDS.

MATERIALS AND METHODS

Patients and sera

Blood for antibody studies was taken at post-mortem from the heart of 16 SUDS cases between March and August 1990. The ages of these male Thai construction workers ranged from 21 to 45 years (mean 32·3 years). They had come from northeast Thailand and stayed in Singapore from 1 to 37 months (mean 10·4 months) before death. Sera were also collected from 73 healthy, Thai male construction workers (all, except one, from north-east Thailand) who were deployed in the same construction sites as the SUDS cases. The age groups and duration of stay in Singapore were similar to that of the SUDS cases. Nineteen Singapore patients with bacteriologically confirmed melioidosis were also included in the study.

Antigen

The antigen used in the IHA and IgM-IFA tests was from a local strain of *P. pseudomallei* isolated from a septicaemic patient. The organism was identified by colonial morphology and biochemical reactions using the API 20NE system (API Lab Products, UK).

IHA test

The IHA test was a modification of the procedure described by Alexander and co-workers [8]. *P. pseudomallei* was grown in a protein-free broth at 37 °C for 2 weeks, and heat killed by autoclaving. After centrifugation, the supernatant was used as antigen, which was preserved by addition of 0.5% (v/v) phenol and stored at 4 °C. Sensitized erythrocytes were prepared by coating tannic acid-treated formalinized turkey cells with an optimal dilution of antigen (determined by checkerboard titration) for 30 min at 37 °C. Excess antigen was removed by washing twice with phosphate-buffered saline (PBS), pH 7·2. Before testing, sera were inactivated by heating at 56 °C for 30 min, followed by absorption with non-sensitized turkey cells at 37 °C for 30 min. In the test, serum samples were diluted,

Table 1. *Results of the indirect haemagglutination (IHA) test and the indirect immunofluorescence assay for IgM antibody (IgM-IFA) for the detection of* Pseudomonas pseudomallei *antibodies in 19 Singaporeans with bacteriologically-confirmed melioidosis*

IHA			IgM-IFA		
Titre	Number positive	%	Titre	Number positive	%
< 4	0	0	10	5	26.3
4	0	0	20	6	31.6
8	0	0	40	4	21.1
16	1	5.3	80	2	10.5
32	2	10.5	160	1	5.3
64	4	21.1	320	1	5.3
128	4	21.1			
256	1	5.3			
512	2	10.3			
> 512	5	26.3			

beginning from 1:4, in 1% heat-inactivated, normal rabbit serum in PBS and mixed with an equal volume of sensitized turkey cells in a microtitre plate. Results were read within 1 h at room temperature. Controls included in the test were positive and negative sera, as well as sensitized and non-sensitized cells.

IgM-IFA test

The IgM-IFA test was performed according to the method described by Ashdown [9]. *P. pseudomallei* was grown overnight on trypticase soy agar (BBL, USA). The organisms were washed twice with PBS, pH 7·4, and resuspended to an opacity equivalent to 10^9 cells/ml. Four microlitres of cell suspension were spotted onto each well of a 30-well Teflon-coated glass slide, air-dried and heat-fixed. Serially diluted serum samples were added to each well and incubated at 37 °C for 30 min. After washing, fluorescein-conjugated sheep anti-human IgM (Wellcome Research Laboratories, UK) was added and further incubated for 30 min at 37 °C. The slides were finally washed twice with PBS, air-dried, and examined for specific fluorescent staining of the bacteria using a Polyvar fluorescence microscope (Reichert, Austria).

RESULTS

The IHA test using turkey erythrocytes and the IgM-IFA test as used in this laboratory were evaluated for sensitivity in detection of *P. pseudomallei* infection on sera from culture-positive melioidosis patients in Singapore. The IHA and IgM-IFA antibody titres in 19 patients are shown in Table 1. IHA antibodies were detected in all sera with titres ranging widely from 16 to more than 512. The antibody titre of one serum was as high as 32768 (not shown). All sera also contained IgM-IFA antibodies to *P. pseudomallei* with titres ranging from 10 to 320. About half (57·9%) of the sera had low titres < 40. High IgM-IFA titres (> 40) were generally found in those sera which had IHA titres of > 64, but there was no direct correlation between the antibody titres obtained in the two tests (data not shown).

Table 2 shows the IHA and IgM-IFA antibody titres of the sera from SUDS

https://doi.org/10.1017/S095026880004927X Published online by Cambridge University Press

Table 2. *Comparison of the* Pseudomonas pseudomallei *antibodies in victims of the sudden unexplained death syndrome (SUDS) and that of healthy Thai construction workers as detected by the indirect haemagglutination (IHA) test and the indirect immunofluorescence assay for IgM antibody (IgM-IFA)*

IHA

	SUDS (n = 16)		Healthy Thais (n = 73)	
Titre	Number positive	%	Number positive	%
< 4	1	6.3	44	60.3
4	3	18.3	3	4.1
8	3	18.8	9	12.3
16	1	6.3	7	9.6
32	1	6.3	2	2.7
64	2	12.5	1	1.4
128	2	12.5	3	4.1
256	1	6.3	1	1.4
512	2	12.5	3	4.1

At $\geqslant 4$, $p = 6.1 \times 10^{-5}$ (Fisher's exact test) between SUDS and healthy Thais.

IgM-IFA

	SUDS (n = 16)		Healthy Thais (n = 73)	
Titre	Number positive	%	Number positive	%
< 10	5	31.3	64	87.7
10	2	12.5	3	4.1
20	2	12.5	2	2.7
40	3	18.8	3	4.1
80	2	12.5	1	1.4
160	2	12.5	0	0

At $\geqslant 10$, $p = 1.1 \times 10^{-5}$ between SUDS and healthy Thais.

cases and the control group of healthy Thai workers. Fifteen out of 16 (93·8%) SUDS sera had IHA antibodies, with titres spreading over a wide range from 4 to 512. In contrast, of 73 healthy Thai workers only 29 (39·7%) had IHA antibodies at a titre of 4 and greater and the difference in seropositivity rates in the two groups with IHA antibodies at titres $\geqslant 4$ is statistically significant ($P = 6{\cdot}1 \times 10^{-5}$). Of 16 SUDS cases, 11 (68·8%) had IgM-IFA antibodies with titres ranging from 10 to 160. Only 9 of 73 (12·3%) healthy Thai workers, on the other hand, had IgM-IFA antibodies with titres from 10 to 80. The difference in seropositivity rates in the two groups is also statistically significant ($P = 6{\cdot}1 \times 10^{-5}$).

Table 3 summarizes the major necropsy findings in the heart, lungs and kidneys of the SUDS cases. The lungs in all cases showed haemorrhage, congestion or oedema. Dilation, pericarditis or haemorrhage were observed in the heart of five cases (nos. 7, 10, 11, 12 and 13), and renal calculi in the kidneys of two cases (nos.

https://doi.org/10.1017/S095026880004927X Published online by Cambridge University Press

Table 3. *Relation between IHA and IgM-IFA test titres and significant post-mortem findings in SUDS cases*

No.	IHA titre	IgM-IFA titre	Significant post-mortem observations
1	512	160	Lungs – lymphocytic interstitial infiltrates, some polymorphs and histiocytes; patchy acute intra-alveolar haemorrhage
2	256	40	Heart – interstitial mononuclear infiltrates with foci of hypereosinophilia Lungs – congestion with focal intra-alveolar haemorrhage and hemosiderin laden macrophages
3	negative	negative	Lungs – as in 1, and intra-alveolar oedema
4	8	20	Heart – focal interstitial lymphohistiocytic infiltrate Lungs – as in 1, and lymphoid interstitial pneumonitis
5	512	160	Lungs – as in 1.
6	8	negative	Lungs – congestion, oedematous with haemorrhage
7	64	40	Heart – pericarditis over right ventricle
8	16	10	Lungs – congestion, oedema and haemorrhage
9	4	negative	Lungs – congestion, oedema, alveolar haemorrhage Kidneys – congested, with a staghorn calculus of the right kidney
10	64	80	Heart – right side dilated, congested myocardium Lungs – congestion, oedema
11	8	negative	Heart – right side dilated Lungs – congestion oedema, with thick yellowish mucus expressed from cut surfaces of both lungs
12	4	40	Heart – right side dilated Lungs – congested, oedema Kidneys – congested, oedema, worse in left kidney; fibrous adhesions of capsules; large, hard greenish-black staghorn calculus in left kidney, loculations of pus in upper two major calyces Spleen – severe congestion, friable
13	128	80	Lungs – moderate oedema Heart – pericardial surface show petechial haemorrhages
14	4	negative	Lungs – as in 6
15	32	10	Lungs – oedema, emphysema, chronic bronchitis
16	128	20	Heart – epicardium showed diffuse fibrous adhesions to the pericardium; congested myocardium Lungs – congested, oedema; fibrous adhesions inferiorly with diaphragm and posteriorly with the chest wall and between the lobes

9 and 12). In the two cases with kidney impairment, one had an IgM-IFA antibody titre of < 10 while the other had a titre of 40.

DISCUSSION

The IHA test using turkey cells has been shown by us to be highly sensitive and specific in the detection of a recent or current *P. pseudomallei* infection in patients with clinical melioidosis [10]. All culture-positive patients in Singapore had IHA antibodies with a wide distribution of titres, from 16 to 32768. In contrast, of 783 sera from normal healthy adults (683 national servicemen, 50 blood donors and 50

https://doi.org/10.1017/S095026880004927X Published online by Cambridge University Press

workers from the Sewage Department) only 5 servicemen had IHA antibodies with titres greater than 16. An IHA antibody titre of > 40 has been suggested by some workers on melioidosis as the cut-off value for a recent or current infection with *P. pseudomallei* [5, 11]. At the cut-off IHA titre of ⩾ 32, 94·7% of melioidosis patients (Table 1) and 0·4% (5/783) of normal, healthy adults were positive for *P. pseudomallei* antibodies. In addition, all melioidosis patients had IgM-IFA antibodies (⩾ 10), the presence of which are considered by most workers to indicate a recent or current *P. pseudomallei* infection [5, 6].

The IHA and IgM-IFA test results on sera from SUDS cases showed that these individuals have had a recent or current infection with *P. pseudomallei* at the time of death. SUDS cases had significantly higher seropositivity rates for IHA antibody than in healthy Thai workers at cut-off titres of either ⩾ 4 or ⩾ 32, suggesting that recent or current *P. pseudomallei* infections are more common among SUDS cases than in healthy Thai workers. At the cut-off IHA titre of ⩾ 32, 50% of the SUDS cases were negative, compared with the 95% positivity rate found in melioidosis patients. A possible explanation for this difference could be that the SUDS cases had not had time to produce antibodies.

The recent or current infections with *P. pseudomallei* demonstrated in SUDS cases are likely to represent reactivation of latent infections acquired in their home country. Latent infections are common in north-eastern Thailand [12]. Although there have been only 17 episodes of reactivations of *P. pseudomallei* reported in the literature [13], it is probably a more common occurrence among Thais in north-eastern Thailand than hitherto realized. This is shown by our finding of IgM-IFA antibodies in 12% of normal, healthy Thai workers in this study.

It is also possible that the SUDS patients might have acquired their recent or current *P. pseudomallei* infection in Singapore as the organism is endemic in the country. We have also tested sera from other immigrant construction workers (from India, Sri Lanka, China and Malaysia) and found a seropositivity rate of 11·6% which is significantly lower than that found in SUDS cases and the normal healthy Thai construction workers (unpublished data). It is more likely that the Thai workers had already been infected in their home country before their arrival in Singapore.

Necropsy studies of two SUDS cases lend support to the hypothesis of reactivation of a latent *P. pseudomallei* infection. In the pathologist's report of one case (no. 12), the worker died of septicaema with left pyonephrosis. This worker was apparently well on the day of his death when he woke up at 4 a.m., washed his clothes, had a bath, cooked and ate breakfast. He complained of headache at 6 a.m., and at 7 a.m., he fell and became unconscious. He experienced breathing difficulties which finally led to his death at 8 a.m. Post-mortem examination showed the presence of a large, greenish-black calculus of the pelvi-calyceal system with loculations of pus in the upper two major calyces in the left kidney. He had an IHA titre of 4 and an IgM-IFA titre of 40. In the other case (No. 9), a staghorn calculus was found within the pelvis of the right kidney, but no IgM-IFA (< 10) was detected and the IHA titre was 4. It is unfortunate that no microbiological investigations were conducted on these two cases as *P. pseudomallei* was not suspected to be aetiologically associated with SUDS. Septicaemia with renal

impairment, often associated with renal calculi, is common in north-eastern Thailand and is significantly associated with melioidosis [3].

Reactivation of melioidosis has been reported in patients in association with trauma, burns, steroid administration, cancer, diabetes and infectious diseases due to *Streptococcus pneumoniae*, plasmodium, and influenza A virus (12). In SUDS, reactivation of *P. pseudomallei* infections may be caused by stress, resulting in lowering of host resistance. Immigrant Thai workers from the farming areas of north-eastern Thailand are under immense stress which may arise from maladjustment to an urban environment, separation from the family, socio-economic problems, long hours of arduous work with little sleep, lack of recreation and heavy alcohol consumption. Stress of both physical and psychological nature are known to modify immune responses in both man and animals [14]. It is pertinent to note that about three-quarters of SUDS cases occurred within their first year of arrival in Singapore. Stress as a risk factor would have been minimized after a period of adjustment.

Other risk factors in SUDS have also been proposed, such as thiamine and potassium deficiencies [5]. However, preliminary results of blood thiamine in healthy Thai workers indicated that the levels were within normal limits, though in the lower range [2]. In the present study, 3 of 16 SUDS cases (nos. 9, 13 and 15 in Table 3) had received vitamin B complex supplementation in their diets for periods of 1 week, 3 weeks and 2 months, respectively, before they died. Similarly, the serum potassium levels of healthy Thai workers examined were in the lower normal range [2].

The aetiological significance of *P. pseudomallei* in SUDS remains to be determined. The immediate cause of death in SUDS seems to be ventricular fibrillation and its precipitation can be very sudden. A number of SUDS cases were reported to have died while chatting with friends after experiencing breathing difficulties. Death may be due to an anaphylactic-type reaction involving constituents of *P. pseudomallei*, such as the mortality enhancing polypeptide [15]. It is also possible that a lethal toxic factor may be involved. In experimental studies, Niggs and colleagues [16] had observed that mice and hamsters inoculated with *P. pseudomallei* died within 1 or 2 days but showed no gross lesions, a situation not unlike SUDS in humans.

ACKNOWLEDGEMENT

We are grateful to Ms Geetha Baskaran for typing this manuscript.

REFERENCES

1. Goh KT, Chao TC, Chew CH. Sudden nocturnal death among Thai construction workers in Singapore. Lancet 1990; **335**: 1154.
2. Committee on Epidemic Diseases. Sudden unexplained death syndrome among Thai workers in Singapore. Epidem News Bull 1990; **16**: 45–51.
3. Chaowagul W, White NJ, Dance DAB, et al. Melioidosis: A major cause of community-acquired septicemia in northeast Thailand. J Infect Dis 1989; **159**. 890–9.
4. Yap EH, Chan YC, Goh KT, et al. *Pseudomonas pseudomallei* and sudden unexplained death in Thai construction workers. Lancet 1990; **336**: 376–7.
5. Ashdown LR. Relationship and significance of specific immunoglobulin M antibody response in clinical and subclinical melioidosis. J Clin Microbiol 1981; **14**: 361–4.

6. Khupulsup K, Petchlai B. Application of indirect hemagglutination test and indirect fluorescent antibody test for IgM antibody for diagnosis of melioidosis in Thailand. Am J Trop Med Hyg 1986; **35**: 366–9.
7. Ashdown LR, Johnson RW, Koehler JM, Cooney CA. Enzyme-linked immunosorbent assay for the diagnosis of clinical and subclinical melioidosis. J Infect Dis 1989; **160**: 253–60.
8. Alexander AD, Huxsoll DL, Warner AR, Shepler V, Dorsey A. Serological diagnosis of melioidosis with indirect hemagglutination and complement fixation tests. Appl Microbiol 1970; **20**: 825–33.
9. Ashdown LR. Demonstration of human antibodies to *Pseudomonas pseudomallei* by indirect fluorescent antibody staining. Pathology 1981; **13**: 597–601.
10. Yap EH, Chan YC, Ti TY, et al. Serodiagnosis of melioidosis in Singapore by the indirect haemagglutination test. Singapore Med J 1991. In press.
11. Leelarasamee A, Bovornkitti S. Melioidosis: review and update. J Infect Dis 1989; **11**: 413–25.
12. Kanai K, Dejsirilert S. *Pseudomonas pseudomallei* and melioidosis, with special reference to the status in Thailand. Japan J Med Sci Biol 1988; **41**: 123–57.
13. Tanphaichitra D. Tropical disease in the immuno-compromised host: melioidosis and pythiosis. Rev Infect Dis 1989; **11**: S1629.
14. Kiecolt-Glaser JK, Glaser R. Psychological influences on immunity. Psychosomatics 1986: **27**: 621–4.
15. Lusby M, Levine HB. An intracellular mortality-enhancing material from *Malleomyces pseudomallei*. J Immunol 1958; **80**: 446–553.
16. Nigg C, Heckly RJ, Colling M. Toxin produced by *Malleomyces pseudomallei*. Proc Soc Exper Biol Med 1955; **89**: 17–20.