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ORIGINAL ARTICLE

EASY WAY TO LEARN STANDARDIZATION : DIRECT AND INDIRECT METHODS

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In direct age-adjustment, a common age-structured population is used as standard. This population may actually exist (e.g., United States population, 1999) or may be fictitious (e.g., two populations may be combined to create a standard). In indirect age-adjustment, a common set of age-specific rates is applied to the populations whose rates are to be standardized. The simplest and most useful form of indirect adjustment is the standardized mortality ratio (SMR) (5).

Key words : *easy way, standardization methods, direct, indirect*

Introduction

Comparing mortality and morbidity rates in two or more different geographic areas is important for the evaluation of community health status. As there is a possibility of having different frequency distributions in different populations, a comparison between crude rates would be misleading since crude rates are not very informative about the health status of a population. Standardization for the characteristic(s) responsible for the differences in comparison is necessary. Age and sex are two of the most common variables used for standardization and they are called standardized rates. The difference between crude rates and standardized rates is that crude rates are calculated based on the population under study as a whole whereas standardized rates are based on particular characteristic(s) as standard (Figure 1). If the rates are calculated based on the specific characteristic(s), they are called specific rates (e.g. age specific mortality rate).

This article attempts to help health personnel in the selection and utilization of appropriate standardization methods using illustrated explanations. There are two methods for calculating standardized rates, namely **direct and indirect standardization.** For the example purpose, let us concentrate on the standardization methods based on age-standardized rates.

When age-specific mortality rates for two or more populations are known, direct standardization method can be applied.

Procedure for direct standardization

Calculate the age-specific mortality rates for each age group in each population. Then choose the standard (reference) population from one of the populations (*Note: If the mortality rates of a specific community are compared to the national population, then the national population is considered as a "standard" population). Multiply the age-specific mortality rates of the other population under study to the number of persons in each age group of the standard population. By this way, you will get the expected deaths for each age group of each population. Add the number of expected deaths from all age groups. Finally to get the age-adjusted mortality rates, divide the total number of expected deaths by the standard population (1-4). Now you can conclude by comparing the age-standardized mortality rates of two populations (figure 2).

Figure 1: Concept of direct standardization

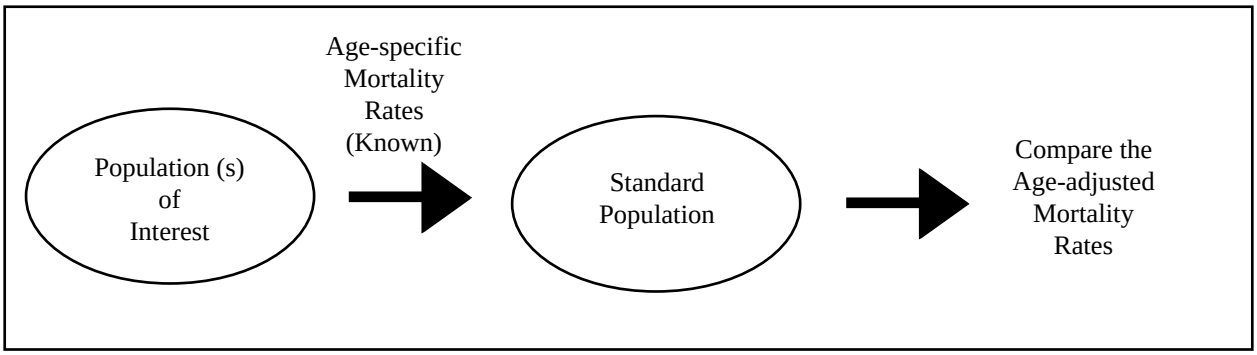

Figure 2: Procedure for application of direct standardization method

Age groups	Population I	No. of deaths in each age group	Age specific death rate		Age groups	Population II	No. of deaths in each age group	Age specific death rate
	B_1	A_1				B_2	A_2	
	$CDR_1=A_1/B_1$					$CDR_2=A_2/B_2$		

*

Age groups	Standard Population	Expected deaths	Age specific death rate		Age groups	Standard Population	Expected deaths	Age specific death rate
	B_1	A_1				B_2	A_2	
	$ADR_1=A_1/B_1$					$ADR_2=A_2/B_2$		

CDR = Crude Death Rate ADR = Adjusted Death Rate

Example1

Table 1: Age-groups, deaths and mid-year populations of two different populations

Population A				Population B		
Age group (years)	Mid-year population	Deaths	Age-specific death rate per 1000	Mid-year population	Deaths	Age-specific death rate per 1000
0-24	18,000	35	1.94	13,000	30	2.31
25-49	11,000	60	5.45	7,000	50	7.14
50-74	9,000	370	41.11	11,000	400	36.36
75 and above	3,000	250	83.33	4,000	380	95.00
Total	41,000	715		35,000	860	
Crude rate per 1000			17.44			24.57

Commenting on crude death rates, population B seems to have higher death rates than population A

As an example, let us say the national population has been chosen as the reference population, the calculation will therefore be as follows in table 2

Table 2: Calculation of expected deaths by applying direct standardization method

Population A				Population B	
Age group (years)	Reference population per 1000	Age-specific death rate	Expected deaths	Age-specific death rate per 1000	Expected deaths
0-24	11,000	1.94	21.34	2.31	25.41
25-49	17,000	5.45	92.65	7.14	121.38
50-74	20,000	41.11	822.20	36.36	727.20
75 and above	3,000	83.33	249.99	95.00	285.00
Total	51,000		1186.18		1158.99

Summation of the total number of expected deaths

Population A = 1186.18

Population B = 1158.99

Age adjusted death rate for population A
= $\frac{1186.18}{51,000}$ x 1000
= 23.3 per 1,000 population

Age adjusted death rate for population B
= $\frac{1158.99}{51,000}$ x 1000
= 22.7 per 1,000 population

Commenting on age-adjusted rates, in fact the risk of death is higher in population A than in population B. It has clearly shown that you may have misleading conclusion if you rely only on crude death rates.

When Age-specific mortality rates of the population (s) of interest are unknown, indirect standardization method is applied

Figure 3: Concept of indirect standardization

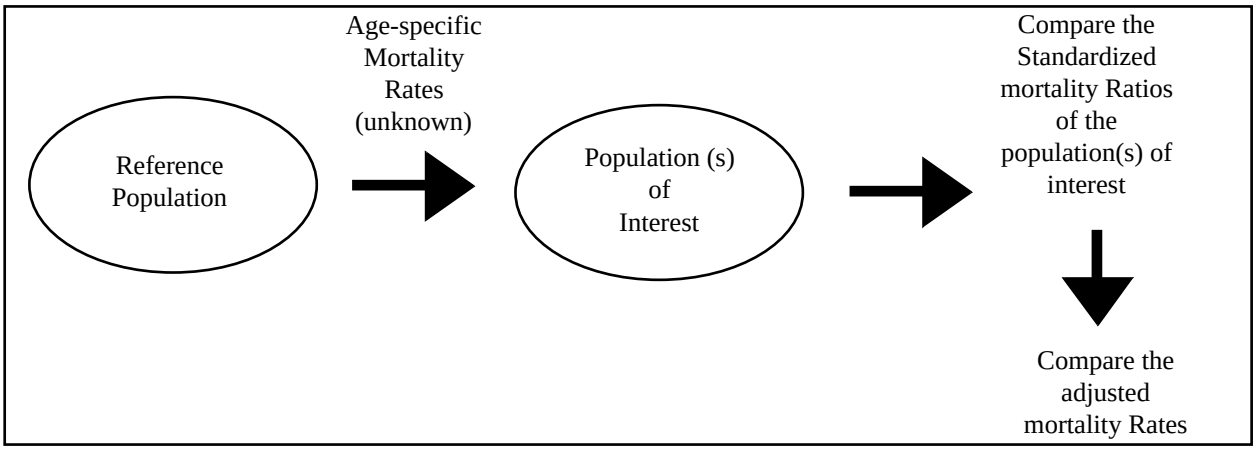

Figure4: Procedure for application of indirect standardization method

Age groups	Population I	No. of deaths in each age group	Age specific death rate		Age groups	Population II	No. of deaths in each age group	Age specific death rate
	B_1	Obs_1				B_2	Obs_2	
	$CDR_1=Obs_1/B_1$					$CDR_2=Obs_2/B_2$		

*

Age groups	Population I	Expected deaths	Standard Age specific death rate		Age groups	Population II	Expected deaths	Age specific death rate
		Exp_1					Exp_2	
	$SMR_1=Obs_1/Exp_1$					$SMR_2=Obs_2/Exp_2$		

Example (2)

Let us say the observed deaths in the populations A and B are as follows:

Observed deaths in population A=120 Observed deaths in population B= 30

Table 3: Calculation of expected deaths by applying indirect standardization method

Population A				Population B		
Age group	Population	Age-specific mortality rate deaths per1000	Expected	Population	Age-specific mortality rate per1000	Expected deaths
0-24	2000	4.0	8.0	1000	4.0	4.0
25-49	2500	7.0	17.5	1500	7.0	10.5
50-74	3500	10.0	35.0	2500	10.0	25.0
75+	4500	30.0	135.0	1000	30.0	30.0
Total			195.5			69.5

Division of the total number of observed deaths by the total number of expected deaths

SMR for population A = $\frac{120}{195.5}$ = 0.61

SMR for population B = $\frac{30}{69.5}$ = 0.43

The risk of death is in fact higher in population A than population B after adjusting for differences by age. Common practice is to compare (SMR) in indirect method.

Let us see the second method which is an indirect standardization.

Procedure for indirect standardization

Choose a reference or standard population. Calculate the observed number of deaths in the population (s) of interest. Apply the age-specific mortality rates from the chosen reference population to the population(s) of interest. Multiply the number of people in each age group of the population(s) of interest by the age-specific mortality rate in the comparable age group of the reference population. Sum the total number of expected deaths for each population of interest. Divide the total number of observed deaths of the population(s) of interest by the expected deaths (figure 4) (1-4).

The ratio of the observed number of deaths to the expected number of deaths is called: "Standardized mortality ratio" or SMR

$$SMR = \frac{\text{Observed number of deaths}}{\text{Expected number of deaths}}$$

Adjusted mortality rates (AMR) can be calculated by the following formula:-

Adjusted mortality rates = Standardized mortality ratio x crude death rate

AMR= SMR x CDR (Standard)

(* Note : if the age-specific mortality rates of the reference population is applied, Crude Death Rate must be calculated from that reference population.)

Conclusion

Standardization methods are not difficult but sometimes the health personnel have some confusion about selecting which method and how to calculate and apply the particular method. It is sincerely hoped that this article may at least contribute to public health medicine by improving the understanding of standardization methods in comparing two or more different populations, which have difference(s) in some characteristic(s).

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ORIGINAL ARTICLE

ABSENCE OF APO B R3500Q MUTATION AMONG KELANTANESE MALAYS WITH HYPERLIPIDAEMIA

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Familial defective apolipoprotein B-100 (FDB) is an autosomal dominant genetic disorder associated with hypercholesterolaemia and premature coronary heart disease. FDB is caused by mutations in and around the codon 3500 of the apolipoprotein B (apo B) gene. Apo B R3500Q mutation is the first apo B mutation known to be associated with FDB and it is the most frequently reported apo B mutation in several different populations. The objective of the present study was to determine the association of apo B R3500Q mutation with elevated plasma cholesterol concentration in Kelantanese population in which both hypercholesterolaemia and coronary heart disease are common. Sixty-two Malay subjects with hyperlipidaemia, attending the lipid clinic at Hospital Universiti Sains Malaysia, Kelantan, were selected for this study. The DNA samples were analysed for the presence of apo B R3500Q mutation by polymerase chain reaction-based restriction fragment analysis method using mutagenic primers. This mutation was not detected in the subjects selected for this study. Apo B R3500Q mutation does not appear to be a common cause of hypercholesterolaemia in Kelantanese Malays.

Key words: *Familial defective apolipoprotein B-100, apo B mutations, hypercholesterolaemia*

Introduction

Low-density lipoprotein (LDL) particles are the major cholesterol transport lipoproteins in the plasma. The plasma cholesterol concentration is mainly regulated by the LDL receptor pathway in which LDL receptors mediate uptake and degradation of the LDL particles. Apolipoprotein B-100 (apo B-100) is the sole protein of the LDL particle and it acts as a ligand for the LDL receptor (1).

Familial defective apolipoprotein B-100 (FDB) is an autosomal dominant disorder associated with increased plasma cholesterol concentration (2) and may thus increase the risk of premature coronary heart disease. To date, three apo B mutations, apo B R3500Q, apo B R3531C and apo B R3500W that cause defective apo B, have been reported (3).

Apo B R3500Q mutation is the first reported apo B mutation associated with FDB. This mutation is caused by a G to A transition at nucleotide 10708 in exon 26 of the apo B gene resulting in the substitution of arginine by glutamine at codon 3500 of the apo B and reduced affinity of apo B for the LDL receptor (4). The estimated heterozygous frequency of FDB based on apo B R3500Q mutation in general population is 1 in 500 (5).

Identification of underlying specific mutations in primary hyperlipidaemia cases is required for the detection of carriers in the families as early intervention may prevent the development of premature coronary heart disease. In this study, we determined the association of apo B R3500Q mutation with elevated plasma cholesterol

Figure 1.A. Diagram showing the DNA sequence of part of the apo B gene in normal allele and the position of 5' primer: Msp I *recognition sequence is created in codon 3500 of normal allele when a mismatched base is introduced at nucleotide 10706 using mutagenic 5' PCR primer.*

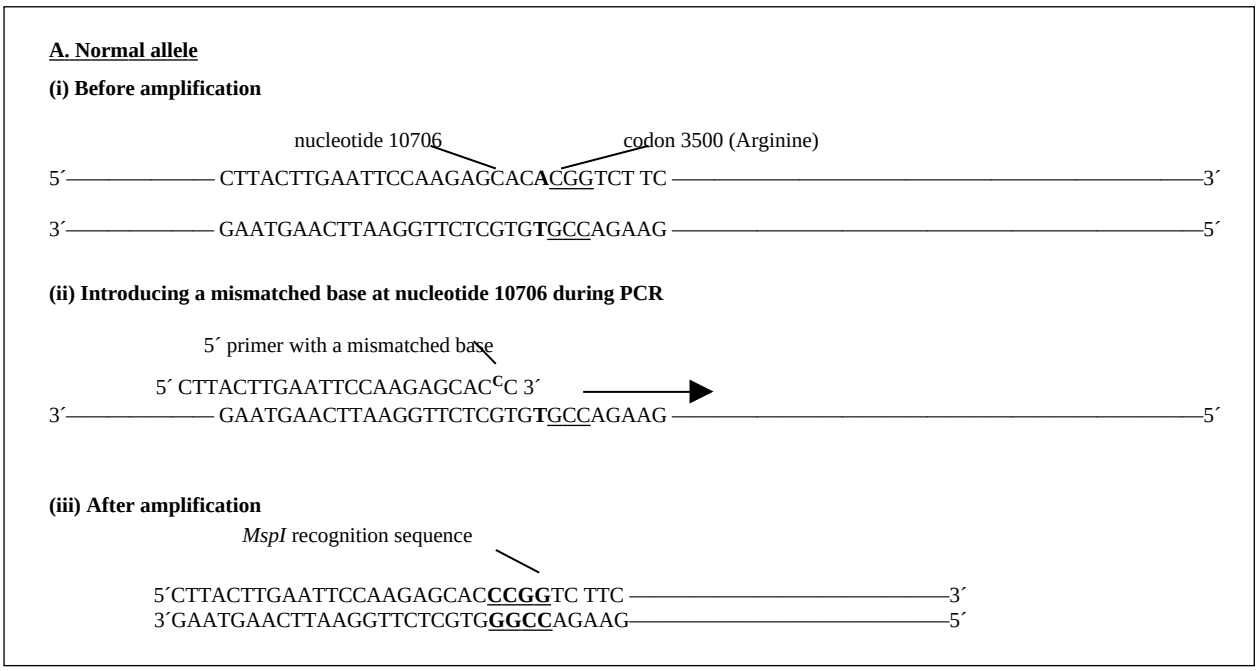
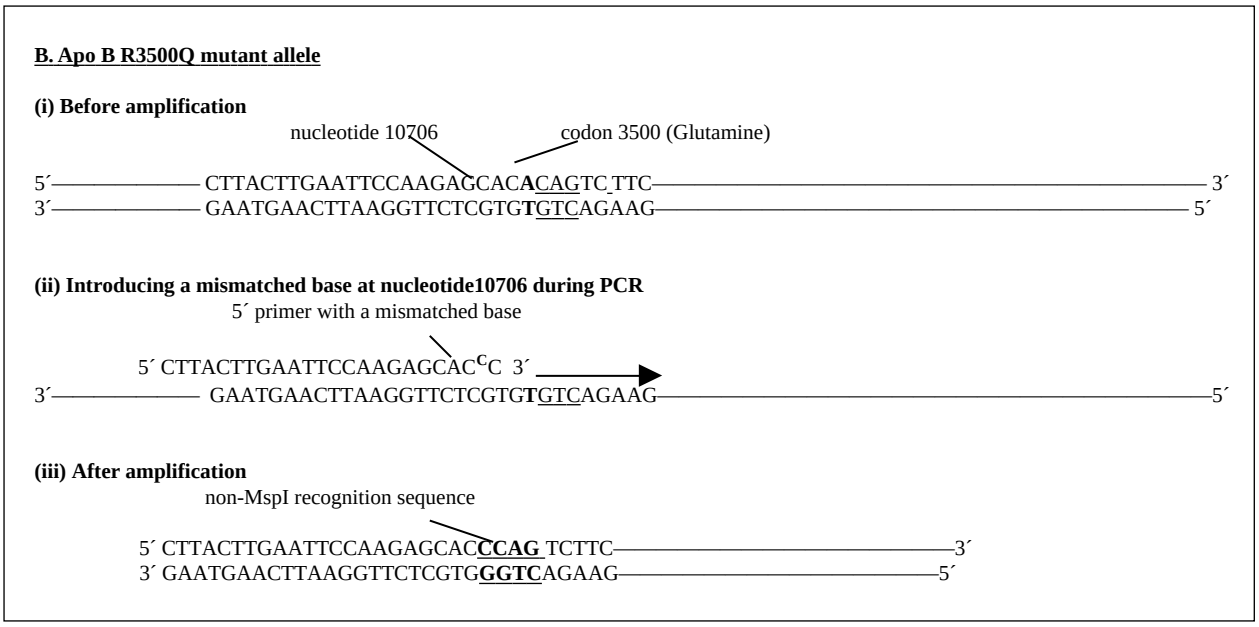


Figure 1.B. Diagram showing the DNA sequence of part of the apo B gene in apo B R3500Q mutant allele and the position of 5' primer: Msp I *recognition sequence is not created in codon 3500 of mutant allele when a mismatched base is introduced at nucleotide 10706 because of G to A transition at nucleotide 10708.*



concentration in Malay subjects attending the lipid clinic at Hospital Universiti Sains Malaysia, Kelantan.

When a point mutation creates or abolishes the restriction enzyme recognition sequence in DNA, it can be detected by polymerase chain reaction (PCR) amplification of the DNA fragments containing target sequence, digestion of the PCR products with restriction enzyme and analysis of the restriction fragments by electrophoresis. If a mutation does not alter any restriction enzyme recognition site, it can be created by PCR, using mutagenic primer with a single-base mismatch, introducing a novel restriction site in the normal allele or in the mutant allele (6). Apo B R3500Q mutation does not create or abolish any restriction enzyme site, however, a recognition sequence for restriction enzyme *Msp I* can be introduced in codon 3500 of the normal allele using mutagenic 5' PCR primer, but not in apo B R3500Q mutant allele (7).

Materials and methods

A total of 62 Malay subjects with hyperlipidaemia, attending the lipid clinic at Hospital Universiti Sains Malaysia, Kelantan, were selected for this study. The study group consisted of 38 males and 24 females, age ranging from 29 to 70 years. Clinical data were obtained from the medical records and subjects were selected on the basis of a plasma total cholesterol pre-treatment concentration of >6.2 mmol/L and individuals with secondary hyperlipidaemia were not included in this study. Their plasma total cholesterol concentrations ranged from 6.31 to 12.35 mmol/L (mean ± SD = 8.02 ± 1.47). Among these subjects, 43 had hypercholesterolaemia alone (plasma triglycerides <2.3 mmol/L) and 19 had mixed hyperlipidaemia (plasma triglycerides ≥2.3 mmol/L). Thirty subjects were classified clinically as familial hypercholesterolaemia (FH) (25 definitive FH and 5 possible FH) based on the criteria of the Simon Broome Register Group (8) and they were not excluded from the study because FDB may present clinically as FH (9).

After an overnight fast (12 hours), 5 ml of venous blood was collected from each subject in a tube containing potassium EDTA as an anticoagulant. Plasma was separated after centrifugation for the determination of lipid profile. For the screening of apo B R3500Q mutation, DNA was extracted from the cellular portion by a simple salting-out procedure (10).

A fragment of 478 bp containing codon 3500 of apo B gene was amplified by PCR using a set of primers (The Bioprocessing Technology Centre, National University of Singapore). These primers correspond to nucleotides 10684-10707 and

Figure 2. Detection of apo B R3500Q mutation according to the length of DNA fragments in the digestion products, bp = base pair.
(A) Schematic representation of the PCR-amplified DNA fragment spanning codon 3500 and 3611 of the apo B gene.
(B) Schematic representation of the expected DNA fragments in Msp I *digestion products according to the presence or absence of* Msp I *cleavage sites at codon 3500 and 3611.*

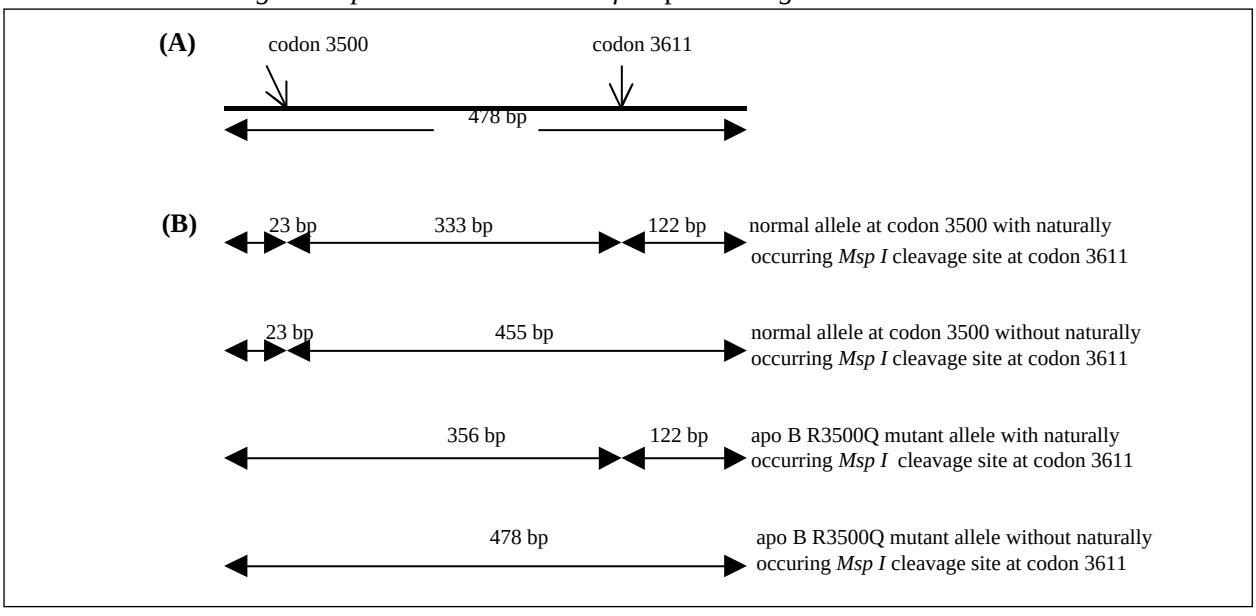
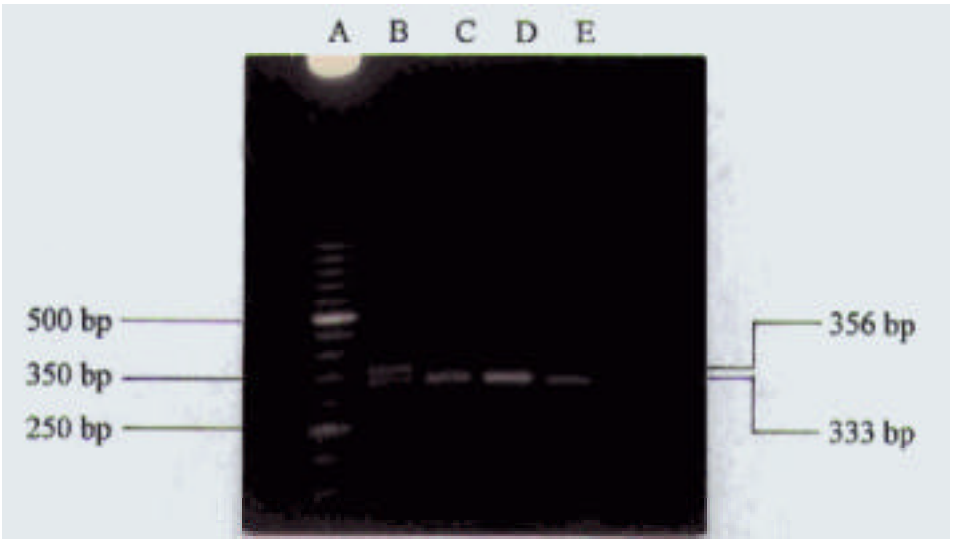


Figure 3. Photograph showing the results of an analysis of Msp I *digestion products by 2% agarose gel electrophoresis, bp = base pair.*
Lane A-50 bp DNA ladder,
Lane B- positive control (heterozygous for apo B R3500Q mutation): 356 bp and 333bp bands obtained,
*Lane **C, D** & **E**-samples from hyperlipidaemic subjects: a single band of 333 bp obtained.*



nucleotides 11138-11161 of the apo B gene respectively. The sequence of the 5' primer was modified, an "A" which is the second last base at its 3' end, was replaced by "C". The terminal 3' end nucleotide of the 5' primer corresponds to the first base of codon 3500.

5'primer -5'CTTACTTGAATTCCAAGAGCAC^{C}C3'
(Superscript letter denotes mismatched base)
3' primer - 5'GGTAGGATGATATTTTTGAGGAAC3'

The mutagenic 5' primer introduced its mismatched base at nucleotide 10706 creating a recognition sequence for *Msp I* (CCGG) in codon 3500 of normal allele (Figure 1.A.). In case of apo B R3500Q mutant allele, the mutagenic 5' primer could not create *Msp I* recognition sequence because of G to A transition at nucleotide 10708 (CCAG) (Figure 1.B.)

The amplified PCR products also spanned codon 3611. Since a naturally occurring *Msp I* recognition site is frequently present in this codon, both normal allele and apo B R3500Q mutant allele may also be cleaved at codon 3611.

PCR mixture contained 300-500 ng genomic DNA, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each primer, 2.5 U Taq DNA polymerase and 5 µl PCR buffer (Promega, USA) in a 50 µl reaction volume. PCR was performed in automated thermal cycler (Eppendorf Mastercycler 5330) using the temperature profile of 5 minutes at 96ºC for initial denaturation followed by 35 cycles of denaturation at 96ºC for 2 minutes, annealing at 60ºC for 1 minute and extension at 72ºC for 1 minute. A final extension of 7 minutes was added at the end of the last cycle. In each batch of PCR, a negative control (all reagents except DNA) was included to exclude contamination. The DNA sample from a patient heterozygous for apo B R3500Q mutation was used as a positive control. The PCR products were then digested with *Msp I* (Promega, U.S.A.) at 37ºC for 3 hours. The reaction mixture was prepared by mixing 10 µl PCR product, 10 U restriction enzyme and 2 µl restriction enzyme buffer in a 20 ll volume.

The PCR products and *Msp I* digestion products were analysed by 2 % agarose gel electrophoresis at 90 volts for 1.5 hours. A 50 bp DNA ladder was included in every run of electrophoresis to analyse the size of DNA fragments.

Results

When the DNA samples were analysed, a 478 bp fragment was obtained for all the samples after PCR. The length of DNA fragments in different types of allele after cleavage with *Msp I* depend on the presence of *Msp I* cleavage sites at codon 3500 or

codon 3611 or both codons (Figure 2). The cleavage of a 122 bp fragment from the PCR product after digestion indicates the presence of naturally occurring *Msp I* cleavage site at codon 3611.

When digestion products were analysed by 2% agarose gel, the positive control sample (heterozygous for apo B R3500Q mutation) showed a two-band pattern (356 bp and 333 bp) whereas a single-band of 333 bp was observed in the digestion products of all hyperlipidaemic subjects recruited in this study (Figure 3).

A 356 bp band observed in the positive control was due to the absence of *Msp I* cleavage site

After electrophoresis at 90 volts for 1.5 hour.

The single 333 bp band observed in the digestion products of hyperlipidaemic subjects indicated homozygosity for normal allele at codon 3500 as well as the presence of naturally occurring *Msp I* cleavage site at codon 3611 of both alleles. Apo B R3500Q mutation was not detected in these individuals.

Discussion

Apo B R3500Q mutation is the most commonly reported apo B mutation associated with increased plasma cholesterol concentration (7, 9, 11, 12 and 13). In the previous studies, the frequency of heterozygous FDB with this mutation was estimated to be 1 in 500 and FDB heterozygous were identified in 2-3 % of individuals with diagnosis of FH (9. 11 & 13). In this study, the DNA samples from 62 Malay subjects were analysed to determine the association of apo B R3500Q mutation with increased plasma cholesterol concentration in Kelantanese population. Apo B R3500Q mutation was not detected in the hyperlipidaemic subjects selected for this study. Although this mutation is relatively common in some populations, it does not appear to be a major contributor to elevated plasma cholesterol in the Kelantanese population. Apo B R3500Q mutation was also found to be absent in some populations such as Finnish (14), Japanese (15), South African (16) and French-Canadian (17). Because the number of cases examined in this study is small and hypercholesterolaemic subjects were selected only from the lipid clinic at Hospital Universiti Sains Malaysia, our study group may not represent the whole Kelantanese population. Therefore, further studies involving larger sample sizes are required to confirm the results of this study. On the other hand, screening of mutations other than this apo B R3500Q mutation such as apo B R3531C and apo B R3500W needs to be undertaken since these mutations might be associated with increased plasma cholesterol in the Kelantanese population.

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ORIGINAL ARTICLE

HELICOBACTER PYLORI RELATED FUNCTIONAL DYSPEPSIA IN A DEFINED MALAYSIAN POPULATION

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The objective of the study was to determine the prevalence of *H. pylori* in functional dyspepsia among the three main races in Malaysia. Gastric antral biopsies from 233 (98 males, 135 females; age range: 17-75 years, mean age 39.5 years) patients attending the Universiti Kebangsaan Malaysia (UKM) gastroenterology clinic were assessed for the presence of *H. pylori* by culture and histology. About a third of the cases (79 of 233 (34%); 34 males, 45 females; mean age 42.6 yrs) were positive for *H. pylori.* The presence of *H. pylori* was always associated with antral gastritis. Malay patients were least likely to be positive for *H. pylori* (10 of 88 (11.4%); 5 males, 5 females; mean age 35.7 yrs) compared to the Chinese (43 of 95 (45%); 19 males; 24 females; mean age 40.2 yrs) and Indian patients (23 of 41 (56%); 10 males, 13 females; mean age 48.1 yrs). We found that *H. pylori* were most common among Chinese followed by Indians. However, the relative risk for the Indians was 8.58 and 6.29 for the Chinese compared to Malays. We conclude that the prevalence of *H. pylori* in patients with functional dyspepsia differs considerably with respect to ethnic groups.

Key words : *Helicobacter Pylori, dyspepsia, Malaysia*

Introduction

Functional dyspepsia (FD) is a complex entity of disorders in which their geneses are many and varied but not proven. It is common throughout the world including Malaysia. FD constitutes a significant percentage of upper gastrointestinal disorders and it is at least twice as common as peptic ulcer disease (1.2). In Malaysia, the magnitude of FD is impressive. Kudva et al. (1988) showed that 63% of males and 83% of females referred for endoscopic examination were diagnosed as FD.

The three groups of races, which form major components of the Malaysian population, are the Malays, Chinese and Indians. In Kuala Lumpur, where the present study was conducted; the ethnic proportions are 33% Malay, 53% Chinese and 14% Indians (1). All three races show differences in FD frequency. The Malays are most susceptible and the Indians the least. The reasons for the differences are unclear. Among the numerous attributes implied to cause FD, *Helicobacter pylori* is now recognised as an etiology (2). Our previous findings had shown that a third of FD patients attending the gasteroenterology clinic had *H. pylori* (3). We report here the detailed findings on the relationship between *H.pylori* and age, sex and have made particular reference to the prevalence of *H. pylori* in the main ethnic groups among the patients presenting at the gasteroenterology clinic in UKM.

Methods

Patients

We studied 233 cases that were diagnosed as functional dyspepsia at endoscopy. Patients with gastric or duodenal ulcers, reflux esophagitis, irritable bowel disease, cancer of esophagus or stomach, and previous gastrectomies were excluded. Patients who had a recent history (up to 4 weeks before endoscopy) of antiulcer agents, corticosteroids, non-steroidal antiinflammatory drugs and antibiotic ingestion were also excluded. Patients that were included in the study were patients who did not have any of the exclusion criteria and who were diagnosed as FD which was taken to mean dyspepsia where clinical evaluation and basic laboratory tests failed to reveal an obvious structural cause for the symptoms and in which endoscopy was normal or there was visual evidence of non-erosive gastritis or non-erosive duodenitis. Patients selected were aged between 17 and 75 years. Informed written consent was obtained from these patients. This project was approved by the Ethics Committee of Universiti Kebangsaan Malaysia.

Endoscopies

Endoscopies were done with an Olympus GIF – P2 fibreoptic endoscope. Biopsies were taken from the prepyloric antral area, corpus and duodenal bulb. Biopsies were usually taken from an inflamed area if present, otherwise any part of the mucosa was used. After each patient the biopsy forceps would be sterilised with gluteraldehyde and subsequently rinsed with saline.

Histopathology

Biopsies were immersed in 10% formalin. Sections were stained with hematoxylin and eosin and the presence and severity of gastritis were graded according to the modified Whitehead's criteria (6) and assessed by a histopathologist. The Warthin-Starry stain was used for confirmation when the H & E sections examined were doubtful for the presence of H.pylori.

Statistical Analysis

Dichotomized data was compared using the chi-square test. A logistic regression model was used to determine the association of *H.pylori* with subjects' characteristics after adjusting for age and race. Adjusted odds ratios and 95% confidence intervals were calculated from this logistic model.

Results

H.pylori was demonstrated in 79 of 233 (34.4%) cases studied. The demographic data of the total number of patients studied is summarised in Table 1.

Sex and *H.pylori*

There was no difference in the gender ratio in each age or ethnic groups. The percentage of *H.pylori* positive patients was comparable in both genders (43.7 % males versus 33.3 % females).

Age and *H.pylori*

The age specific prevalence rates of *H.pylori* are 26% in those aged less than 30, 29% in those between the ages of 30 and 49 and 41% for those above 50 years of age. The prevalence of *H.pylori* was noted to be higher in patients more than 50 years of age compared to those below 30 years (P=0.027). The mean age of patients with *H.pylori* were older than those without the organism (43.2 (13.3) years versus 35.9 (12.4) years, P=0.002). The relative risk of patients in the older age (50 years) groups to get the infection is greater compared to those aged less

Table 1. Demographic data of all subjects

H.pylori status	Total no	Age (yrs) (mean (SD))	Sex M:F	Race Malays	Chinese	Indians	Others
Positive	79	43.2 (13.3)	34:45	10	43	23	3
Negative	154	35.9 (12.4)	64:90	78	52	18	6
All	233	35.6 (12.4)	98:135	88	95	41	9

than 30 years (Table 2). After adjustment for race, in comparison to patients less than 30 years, the relative risk of those aged between 30 and 40 years increased slightly but remained unchanged for the group more than 50 years (Table 2).

Race and *H.pylori*

The Indians had the highest race specific prevalence rates (56%) followed by the Chinese (45%). The Malays were the least likely to be positive for *H.pylori* (11.4%). The percentage distribution of infection by race showed that *H.pylori* occurred most commonly in the Chinese followed by the Indians and the Malays (Table 2). Having adjusted for age, the relative risk was increased for the Indians and the Chinese but low in the Malays (Table 2).

H.pylori and gastritis

Endoscopic gastritis was found in 82 of 233 (35%) patients studied. *H.pylori* was detected in 35 of 82 (43%) patients.

Histologic evidence of gastritis was detected in 38% (88 of 233 patients) of the total antral biopsy specimens examined. All 79 of 233 or 100% of patients who were positive for *H.pylori* showed histologic gastritis compared to only 9 of 154 (5.8%) *H.pylori* free patients. *H.pylori* was more commonly found in biopsy specimens that showed acute-on-chronic gastritis than those showing chronic gastritis alone but the reverse was true in *H.pylori* free specimens with gastritis.

The overall findings showed that the association between *H.pylori* and gastritis is highly significant (P<0.001) and confirm the widely observed close relationship between the two. Endoscopic gastritis underestimated the presence of histological gastritis but was a reliable indicator of *H.pylori* associated gastritis when present.

DISCUSSION

Our study shows that about a third of FD patients are infected with *H.pylori*. This finding is in agreement with other reports (7,8). However, many have reported higher frequencies of infection among FD cases, ranging from 50 – 87% (4, 8-10). This is probably due to the difference in the definition of FD used in the various studies and the different population evaluated. The gastric mucosa did not always show gross abnormality even in the presence of *H.pylori.* A previous report (11) had also stated that the findings at gastroscopy cannot be related to the presence of *H.pylori.* Definitive studies on the presence of *H.pylori* should rely on histology. In contrast to endoscopic findings of the gastric mucosa, all *H.pylori* positive cases had histological gastritis. Only a minority of negative cases had histological gastritis. Our data thus suggest that histological gastritis is closely related to the presence of *H.pylori.* The histologic structural changes that occur in the mucosa of infected individuals provide evidence to support the role of this bacterium in a subset of patients with FD. If *H.pylori* were merely an opportunistic pathogen colonising an already abnormal mucosa, the frequency of gastritis would be similar irrespective of *H.pylori* infection. Instead our findings show that the prevalence of gastritis is definitely higher in subjects with *H.pylori*. The clinical improvement and mucosal restoration in

Table 2. Results of Analysis using Logistic Regression.

Factors	% Positive *H.pylori*	% Negative *H.pylori*	Odds Ratio	Odds Ratio (Adjusted for race)	95% Confidence Interval
Age (years)					
<30	20.3*	29.2	1	1	
30-49	22.8	28.6	1.15	1.33	0.95-1.86
>50	56.9*	56.9	1.95	1.94	0.88-4.28
Race					
Malays	12.7	50.3	1	1	
Chinese	54.4**	33.8	9.96	6.29	2.90-13.66
Indians	29.1**	11.7	6.45	8.58	3.42-21.52

*The prevalence of H.pylori is higher in the older age group *P=0.027.*
The Indians and the Chinese have a higher prevalence of H.pylori, ***P=0.001 compared to the Malays.*

subjects freed of the organism after treatment shown in our previous report (12) further support the contention that the organism may have a pathogenic role in a subset of FD patients.

Previous studies have reported that females have a higher frequency of FD (3, 13). In this study, we found that the subset of *H.pylori* related FD indicated no sex preponderance. This confirms the findings of others (14 -15). A study conducted in Singapore (17) showed that *H.pylori* associated FD was more common in the older group. This difference was observed in groups with only a slight age difference (43.8 (16.5) years versus 39.9 (17.2) years, P=0.002). Due to the marginal difference in the age compared, it would be difficult to say with absolute certainty that the infection is more common in the older age group compared to the younger age group. More over, this age related frequency was without the adjustment for race. We found a similar age-related frequency but this relationship was no longer strong on multivariate analysis taking into account race, the range of confidence interval included the value 1. Thus, our study did not show any strong association between age and the frequency of infection. We conclude that at present, there is no overwhelming evidence that *H.pylori* is more common in the older age group. This is in agreement with other studies conducted in the European population (4, 16).

Our cohort study shows that the Malays are the most frequent race afflicted by FD (3). However, we found that the Malays were the least likely to be affected when *H.pylori* related FD was considered. We noted that in Malaysia, the prevalence was notably higher in Indians and the Chinese compared to the Malays. Similar findings were observed among Singaporeans (14). There certainly exist ethnic variation, with rates of 11% in Chinese aged between 30-49 years, and only 2% in Indians of the same age group. To adjust for the potential confounding between age, sex and race, a logistic regression model was fitted in our study with age, sex and race as independent variables. Race was found to be significant but not sex. From this model, the relative risks (Malays as reference) adjusted for age was only slightly reduced for the Indians and the Chinese. It can therefore be concluded that the higher risk of being positive for *H.pylori* in the Indians and Chinese compared to the Malays is real and not likely to be due to age. Others (17-19) have also observed ethnic variation.

Factors that can probably affect *H. pylori* carriage rates are socio-economic status, sociocultural practice and religious dietary taboos practiced by the different races. The Malays appear to be relatively protected against the infection even though they make up a large percentage of FD patients. This further verifies FD as a heterogeneous disorder. Studies to evaluate the above mentioned factors are required for a clearer understanding on the ethnic variation as more reliable comparisons can be made between the different races.

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ORIGINAL ARTICLE

CLINICAL EXPERIENCE OF MEDICAL STUDENTS AT UNIVERSITY SAINS MALAYSIA

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Experience of acute medical, surgical conditions, and clinical procedures of undergraduate students were assessed via a questionnaire survey during the final week of the 1993/1998 programme at the School of Medical Sciences, Univestiti Sains Malaysia. Individual performances were assessed by a scoring system. One hundred and twenty four students responded, (response rate 97%). More than 90% had seen myocardial infarction, cerebrovascular accident, pneumonia, respiratory distress, gastroenteritis, coma, and snake bite. Less than 33% had witnessed acute psychosis, diabetic ketoacidosis, acute hepatic failure, status epilepticus, near drowning, hypertensive encephalopathy, acute haemolysis or child abuse.

Acute surgical/obstetrics cases, seen by >90% students, included fracture of long bones, head injury, acute abdominal pain, malpresentation and foetal distress. Less than 33% had observed epistaxis, sudden loss of vision, peritonitis or burns. Among operations only herniorrhaphy, Caesarian section, internal fixation of fracture and cataract extraction were seen by >80% students. The main deficits in clinical procedures are in rectal and vaginal examinations, urine collection and microscopic examinations. The performance of individual students, assessed by a scoring system, showed 15 students had unacceptably low scores (<149/230, 50%), 37 had good scores (>181.4/230, 70%) and 5 had superior scores (197.6/230, 80%).

Key words : *undergraduate students, clinical experience Universiti Sains Malaysia*

Introduction

The School of Medical Sciences at University of Science Malaysia in Kelantan, Malaysia has a problem based integrated curriculum (1,2). The teaching of basic science and clinical subjects is integrated in a 5-year course. There is a spiral learning process (3), facilitated by exposure to topics on numerous occasions, and early clinical contact which enables students to test their knowledge in a range of clinical scenarios. In the first year, the integration is based on organ systems with emphasis on normal structure and function. Problem based learning and clinical teaching are introduced in the second year of the programme. During the second and third years students learn about the various human organ systems in blocks, during which they have lectures and clinical teaching. In the fourth and fifth years students undertake rotational clinical postings in all clinical disciplines. They clerk patients admitted to the wards under supervision and also do night calls.

The curriculum for undergraduate medical students at the School of Medical Sciences, Universiti Sains Malaysia emphasises the need for medical students to become familiar with the presentation, diagnosis and management of common acute medical and surgical conditions. Each discipline has formulated a list of conditions that should be seen by students, and of practical

procedures that they should observe/carry out during their training and record in department log books. In recent years there has been concern regarding the clinical experience of graduating medical students (4). In a questionnaire survey conducted in selected medical schools in the United Kingdom (UK) the amount of clinical experience gained by medical students, who started their training in 1981, 1986 and 1991, had progressively declined. Some of these changes were attributed to modifications in health care delivery patterns following reorganisation of the National Health Service.

The main aim of this study was to assess the students' actual experience of acute medical and surgical conditions, and of performing defined procedures. A subsidiary aim was to compare our findings with those in the United Kingdom, recognising that patterns of acute disease in the two countries are not identical.

Methods

A questionnaire survey was conducted on the first day of the final week of the 1993/1998 five year undergraduate training programme at the School of Medical Sciences, Universiti Sains Malaysia. Questionnaires were distributed following a routine weekly seminar attended by all fifth year students. Consent was verbal and students who did not wish to participate were asked to leave the questionnaire blank. Students were not given a time limit for answering the questions.

The questionnaire contained questions about the students' exposure to a wide range of acute medical and surgical conditions and surgical operations, and whether they had observed/ performed certain procedures. Enquiry was made about 27 acute medical conditions (internal medicine, paediatrics and psychiatry) and 15 surgical conditions (general surgery, orthopaedics, ophthalmology, obstetrics and otorhinolaryngology). The list of acute medical conditions was adapted from a questionnaire used in a similar study in the United Kingdom (5). However "hypothermia" was removed, as it is an uncommon problem in Malaysia,

Table 1: Number (%) of students who had seen acute medical conditions

Acute medical conditions	Never	Once	More than once	Not answered
Myocardial infarction	4 (3.3)	17 (14.2)	96 (80.0)	3 (2.5)
Cerebrovascular accident (Stroke)	5 (4.2)	6 (5.0)	104 (86.7)	5 (4.2)
Acute poisoning	29 (24.2)	31 (25.8)	55 (45.8)	5 (4.2)
Acute left ventricular failure	13 (10.8)	12 (10.0)	87 (72.5)	8 (6.7)
Pneumonia	2 (1.7)	6 (5.0)	106 (88.3)	6 (5.0)
Acute upper GIT bleeding	26 (21.7)	22 (18.3)	67 (55.8)	5 (4.2)
Meningitis/encephalitis	9 (7.5)	14 (11.7)	91 (75.8)	6 (5.0)
Pneumothorax	23 (19.2)	28 (23.3)	61 (50.8)	8 (6.7)
Acute psychosis	45 (37.5)	24 (20.0)	46 (38.3)	5 (4.2)
Respiratory failure/distress	4 (3.3)	20 (16.7)	90 (75.0)	6 (5.0)
Diabetic ketoacidosis	42 (35.0)	40 (33.3)	34 (28.3)	4 (3.3)
Febrile convulsion	10 (8.3)	11 (9.2)	94 (78.3)	5 (4.2)
Hypoglycaemia	23 (19.2)	30 (25.0)	62 (51.7)	5(4.2)
Status asthmaticus	20 (16.7)	30 (25.0)	64 (53.3)	6 (5.0)
Acute renal failure	16 (13.3)	28 (23.3)	68 (56.7)	8 (6.7)
Acute hepatic failure	48 (40.0)	29 (24.2)	34 (28.3)	9 (7.5)
Subarachnoid haemorrhage	31 (25.8)	36 (30.0)	43 (35.8)	10 (8.3)
Status epilepticus	43 (35.8)	29 (24.2)	40 (33.3)	8 (6.7)
Near drowning	66 (55.0)	27 (22.5)	24 (20.0)	3 (2.5)
Acute gastroenteritis with dehydration	4 (3.3)	15 (12.5)	96 (80.0)	5 (4.2)
Upper airway obstruction (Stridor)	25 (20.8)	31 (25.8)	59 (49.2)	5 (4.2)
Comatous patient	5 (4.2)	16 (13.3)	95 (79.2)	4 (3.3)
Shock	12 (10.0)	27 (22.5)	76 (63.3)	5 (4.2)
Hypertensive encephalopathy	49 (40.8)	38 (31.7)	27 (22.5)	6 (5.0)
Acute haemolysis	60 (50.0)	21 (17.5)	31 (25.8)	8 (6.7)
Snake bite	7 (5.8)	26 (21.7)	84 (70.0)	3 (2.5)
Child abuse	47 (39.2)	30 (25.0)	37 (30.8)	6 (5.0)

and "acute glaucoma" was transferred to the list of acute surgical conditions. Nine additional acute medical conditions selected from those listed in log books were added to the questionnaire. The list of acute surgical conditions was based on those included in the departmental log books. Except for "hemicolectomy" and "craniotomy" the surgical operations recommended were similar to those contained in the UK questionnaire (5). Students were considered to have seen a condition if they had been concerned in the assessment/management of an affected patient immediately after admission to hospital. There were 26 practical questions about procedures that students were expected to have observed or performed, including 20 practical procedures listed in the UK questionnaire (5). Students' exposure to each acute medical and surgical condition was also assessed using a scoring system (5): 1, never seen; 2, seen once; 3, seen more than once; practical procedures were graded as 1, never seen; 2, seen; 3, done with supervision; 4, done alone. Presence at surgical operations was omitted from total score computations. Questions that were not answered were given the minimum score of 1. Thus the minimum scores were 27 for medical conditions, 15 for surgical conditions, 26 for practical procedures, - 68 in total, and the maximum scores were 81 for medical conditions, 45 for surgical conditions, 104 for practical procedures - 230 in total. For computation the minimum mark was 0% and the maximum mark 100%.

Statistical analysis of frequencies was performed using the EPI6.0 (Centers for Disease Control, Atlanta) software for personal computers.

Results

Questions were completed by 124 students (response rate 97%) in less than 30 minutes. Acute medical conditions that were each seen by >90% of students included myocardial infarction, cerebrovascular accident, pneumonia, respiratory distress/failure, acute gastroenteritis with dehydration, coma, and snake bite (table 1).

Some conditions (eg, myocardial infarction, cerebrovascular accident, pneumonia, acute gastroenteritis with dehydration) had been seen on two or more occasions by most students, whereas others (eg, acute psychosis, diabetic ketoacidosis, acute hepatic failure, status epilepticus, near drowning, hypertensive encephalopathy, acute haemolysis and child abuse) had not been seen by one third or more of the students.

Acute surgical cases, seen by >90% of students, included fracture of long bones, head injury, acute abdominal pain, malpresentation and foetal distress (table 2).

Some conditions (eg, fracture of long bones, head injury, acute abdominal pain malpresentation and foetal distress) were seen on two or more occasions by most students, whereas others (eg, epistaxis, sudden loss of vision, peritonitis, burns) had not been seen by one third or more of the students.

Among operations (table 3) only

Table 2: Number of students (%) who had seen acute surgical conditions

Acute surgical conditions	Never	Once	More than once	Not answered
Myocardial infarction	4 (3.3)	17 (14.2)	96 (80.0)	3 (2.5)
Epistaxis	37 (30.8)	38 (31.7)	35 (29.2)	10 (8.3)
Sudden loss of vision	51 (42.5)	28 (23.3)	31 (25.8)	10 (8.3)
Acute glaucoma	29 (24.2)	26 (21.7)	60 (50.0)	5 (4.2)
Fracture of long bones	2 (1.7)	11 (9.2)	103 (85.8)	4 (3.3)
Head injury	1 (0.8)	8 (6.7)	110 (91.7)	1 (0.8)
Peritonitis	41 (34.2)	28 (23.3)	42 (35.0)	9 (7.5)
Acute abdominal pain	5 (4.2)	7 (5.8)	103 (85.8)	5 (4.2)
Intestinal obstruction	15 (12.5)	21 (17.5)	79 (65.8)	5 (4.2)
Burns	65 (54.2)	35 (29.2)	14 (11.7)	6 (5.0)
Antepartum haemorrhage	20 (16.7)	14 (11.7)	78 (65.0)	8 (6.7)
Postpartum haemorrhage	19 (15.8)	26 (21.7)	70 (58.3)	5 (4.2)
Pre-eclampsia/Eclampsia	9 (7.5)	15 (12.5)	92 (76.7)	4 (3.3)
Abnormal presentation of labour	5 (4.2)	7 (5.8)	103 (85.8)	5 (4.2)
Foetal distress	3 (2.5)	8 (6.7)	106 (88.3)	3 (2.5)
Obstructed labour	22 (18.3)	26 (21.7)	70 (58.3)	2 (1.7)

herniorrhaphy, Caesarian section, internal fixation of fracture and cataract extraction were seen by 80% or more of the students. By contrast, one third or more students has never seen prostatectomy, mastectomy, gastrectomy, hemicolectomy, amputation, skin grafting, mastoidectomy or craniotomy.

Table 4 shows the experience gained by students in practical procedures. While more than 90% of students had done venepuncture, urine testing with dipstick, setting up an intravenous drip, electrocardiogram, suturing and intramuscular injections, one third or more had not carried out a rectal examination, vaginal examination with speculum, bladder catheterisation (male patient), subcutaneous injection, Mantoux test, microscopic examination of urine, urine collection or peripheral blood smear.

The performance of individual students, assessed by a scoring system, showed a mean (SD) score of 170.7 (19.1). Fifteen students had unacceptably low scores (<149/230, 50%), 37 had good scores (>181.4/230, 70%) and 5 had superior scores (197.6/230, 80%).

The figure shows the 'performances' of the students overall, and in each component of the assessment.

Discussion

The results of this questionnaire survey show that most students gained broad clinical experience during their five years undergraduate medical training. However a third or more had not seen certain common medical conditions including acute psychosis, diabetic ketoacidosis, status epilepticus, near drowning, hypertensive encephalopathy and child abuse. Reduced clinical experience in these areas may reflect a paucity of clinical cases or perhaps missed opportunities to learning from such patients. With the possible exceptions of diabetic ketoacidosis and child abuse necessitating hospital admissions, the other conditions are common in Northern Malaysia and ought to have been seen. While most students had seen acute surgical conditions a third or more students had not encountered such conditions as epistaxis, sudden loss of vision, acute glaucoma, peritonitis or burns. Again hospital admissions for these conditions is not rare in this society. Generally most students had not seen the surgical operations listed, except for herniorrhaphy, Caesarian section, appendicectomy, internal fixation and cataract extraction. As witnessing surgical operations is regarded as optional experience in undergraduate training, it was not mandatory for students to have seen these operations. Moreover certain operations, like gastrectomy are less common nowadays because of the extensive use of H_2-blocking agents for the treatment of peptic and duodenal ulcers. In contrast changes in medical practice and improved availability of treatment have increased the number of internal fixation and cataract operations, with enhanced opportunities to witness such procedures.

A large proportion of students had not gained experience in common clinical or technical

Table 3: Number (%) of students who had seen surgical operations

Surgical operations	Never seen	Seen once	Seen 2-4 times	Seen >4 times	Not answered
Herniarrhaphy	13 (10.8)	46 (38.3)	53 (44.2)	5 (4.2)	3 (2.5)
Caesarian section	1 (0.8)	14 (11.7)	78 (65.0)	27 (22.5)	0 (0)
Cholecystectomy	28 (23.3)	43 (35.8)	43 (35.8)	4 (3.3)	2 (1.7)
Appendicectomy	27 (22.5)	45 (37.5)	46 (38.3)	2 (1.7)	0 (0)
Prostatectomy	94 (78.3)	18 (15.0)	5 (4.2)	0 (0)	3 (2.5)
Mastectomy	66 (55.0)	37 (30.8)	15 (12.5)	1 (0.8)	1 (0.8)
Thyroidectomy	33 (27.5)	41 (34.2)	42 (35.0)	3 (2.5)	1 (0.8)
Gastrectomy	98 (81.7)	19 (15.8)	2 (1.7)	0 (0)	1(0.8)
Hemicolectomy	85 (70.8)	22 (18.3)	9 (7.5)	1 (0.8)	3 (2.5)
Internal fixation of fracture	16 (13.3)	30 (25.0)	62 (51.7)	12 (10.0)	0 (0)
Amputation	83 (69.2)	27 (22.5)	7 (5.8)	1 (0.8)	2 (1.7)
Cataract extraction	3 (2.5)	32 (26.7)	71 (59.2)	14 (11.7)	0 (0)
Skin grafting	43 (35.8)	34 (28.3)	37 (30.8)	6 (5.0)	0 (0)
Mastoidectomy	88 (73.3)	25 (20.8)	6 (5.0)	1 (0.8)	0 (0)
Craniotomy	52 (43.3)	48 (40.0)	19 (15.8)	0 (0)	1 (0.8)

procedures including rectal examination, vaginal examination with speculum, subcutaneous injection, Mantoux test, microscopic examination of urine, urine collection and peripheral blood smear. It is disturbing that teaching and practice in rectal and vaginal examinations was missed as these aspects of physical examination should have been performed by every student. In this survey some students had done more complex procedures such as repair of episiotomy, pleural fluid aspiration, bag and mask ventilation and exchange transfusion while failing to gain experience in more simple and common procedures.

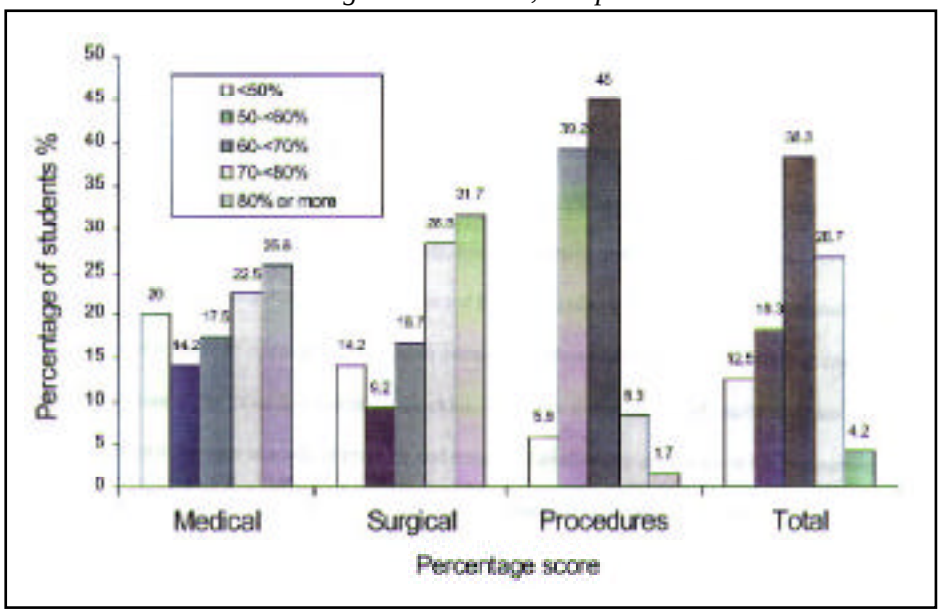
When compared with the clinical experiences of medical students in the United Kingdom study (5) a smaller proportion of our students had seen upper gastro-intestinal bleeding, acute psychosis and diabetic ketoacidosis. However more had had the opportunity to see acute glaucoma, respiratory failure, febrile convulsions and acute renal failure. Our medical students witnessed fewer surgical operations than those in the UK, except for internal fixation of fractures and cataract extraction. A larger proportion of our students also had not done a rectal or vaginal examination, which are unsiderred essential components for the physical examination of certain patients. However our students had more experience in ECG recording, the giving of intramuscular injections and repair of episiotomy.

The distribution of scores for the various components of the survey, and the overall 'performance' of students is of interest. Even though most students scored above 50% in acute medical conditions 20% did poorly (scores <50%) for reasons that require further investigation. There was perhaps more opportunity to gain experience in acute surgical conditions. Only 14.2% of students scored below 50% and 60% of students scored 70% or above. Although most students obtained reasonable scores for acute medical and surgical conditions a significant proportion (20% medical and 14.2%

Table 4: Students'(%) experience of practical procedures

Practical procedures	Never seen	Seen	Done with Supervision	Done alone	Blank
Rectal examination	1 (0.8)	45 (37.5)	44 (36.7)	29 (24.2)	1 (0.8)
Vaginal examination with speculum	2 (1.7)	77 (64.2)	36 (30.0)	4 (3.3)	1 (0.8)
Urine collection	1 (0.8)	42 (35.0)	32 (26.7)	44 (36.7)	1 (0.8)
Urine testing with dipstick	0 (0)	1 (0.8)	5 (4.2)	113 (94.2)	1 (0.8)
Microscopic exam of urine	7 (5.8)	55 (45.8)	42 (35.0)	14 (11.7)	2 (1.7)
Bladder catheterisation (male patient)	2 (1.7)	39 (32.5)	44 (36.7)	34 (28.3)	1 (0.8)
Bladder catheterisation (female patient)	1 (0.8)	11 (9.2)	49 (40.8)	58 (48.3)	1(0.8)
Venepuncture	1 (0.8)	2 (1.7)	7 (5.8)	110 (91.7)	0 (0)
Setting up intravenous drip	0 (0)	0 (0)	5 (4.2)	115 (95.8)	0 (0)
Arterial puncture	3 (2.5)	27 (22.5)	27 (22.5)	62 (51.7)	1 (0.8)
Intramuscular injection	0 (0)	2 (1.7)	33 (27.5)	85 (70.8)	0 (0)
Subcutaneous injection	4 (3.3)	65(54.2)	17 (14.2)	33 (27.5)	1 (0.8)
Mantoux test	10 (8.3)	82 (68.3)	16 (13.3)	12 (10.0)	0 (0)
Ring block (Local anaesthetic)	14 (11.7)	57 (47.5)	36 (30.0)	12 (10.0)	1 (0.8)
Suturing in casualty	1 (0.8)	8 (6.7)	65 (54.2)	46 (38.3)	0 (0)
Repair of episiotomy	1 (0.8)	56 (46.7)	50 (41.7)	13 (10.8)	0 (0)
External cardiac massage	18 (15.0)	56 (46.7)	39 (32.5)	6 (5.0)	1 (0.8)
Bag and mask ventilation	3 (2.5)	36 (30.0)	70 (58.3)	10 (8.3)	1 (0.8)
Endotracheal intubation	0 (0)	13 (10.8)	102 (85.0)	5 (4.2)	0 (0)
Lumbar puncture	0 (0)	118 (98.3)	1 (0.8)	0 (0)	1 (0.8)
Pleural fluid aspiration	7 (5.8)	105 (87.5)	6 (5.0)	0 (0)	2 (1.7)
Peripheral blood smear	3 (2.5)	49 (40.8)	44 (36.7)	23 (19.2)	1 (0.8)
Bone marrow aspiration	31 (25.8)	87 (72.5)	1 (0.8)	0 (0)	1 (0.8)
Peritoneal dialysis	4 (3.3)	110 (91.7)	4 (3.3)	0 (0)	2 (1.7)
Exchange transfusion	27 (22.5)	72 (60.0)	17 (14.2)	2 (1.7)	2 (1.7)
Electrocardiography (ECG)	0 (0)	0 (0)	2 (1.7)	118 (98.3)	0 (0)

Figure 1: Proportion (%) of students in relation to scores obtained for acute medical and surgical conditions, and procedures.



Twenty percent, 14.2%, 5.8% and 12.5% of students obtained unacceptably low scores (<50%) for acute medical and surgical , procedures and total scores respectively. Twenty five percent, 31.7%, 1.7% and 4.2% of students obtained very good scores (≥80%) for acute medical and surgical, procedures and total scores respectively.

surgical) obtained low scores (<50%) for these conditions. Most students gained experience in practical procedures required for their training during their clinical rotations with only 5.8% students scoring less than 50%. Overall the total scores indicated that most students gained a broad experience in acute medical and surgical conditions and practical procedures with only 12.5% students scoring less than 50%. More reassuring was the high scores (>70%) scored by some 20% of the students. The range of scores emphasises the wide spectrum of attainment in these areas.

In conclusion most students gained a broad experience in acute medical and surgical conditions, though a large proportion had seen relatively few surgical operations. While most students had witnessed/undertaken most of the recommended practical procedures there were unacceptable gaps in clinical examination attainments. These results will provide a baseline for future comparisons. The limited 'exposure' gained by the 12% of students with unacceptability low scores leaves little scope for complacency on the part of students as well as faculty.

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ORIGINAL ARTICLE

COMPARISON OF THE PATTERN OF NOSOCOMIAL INFECTION BETWEEN THE NEONATAL INTENSIVE CARE UNITS OF HOSPITALS KUALA TERENGGANU AND UNIVERSITI SAINS MALAYSIA, KELANTAN

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Nosocomial infection is a common problem in the Neonatal Intensive Care Unit (NICU) and a knowledge of the pattern of nosocomial infection will contribute greatly to the intensification of infection control measures and the development of antibiotic policies in the NICU. This study aims to compare the incidence and clinical characteristics of neonates with nosocomial infection in NICU of both Kuala Terengganu Hospital (HKT) and Universiti Sains Malaysia Hospital (HUSM). Neonates who had both clinical signs of sepsis and positive blood cultures, 48 hours after admission to NICU, from 1st January to 31st December 1998, in both hospitals were retrospectively studied. Among neonates admitted to NICU, 30 (5.4%) in HKT and 65 (3.6%) in HUSM had nosocomial infection (p = 0.07). The mean duration of hospitalisation was shorter (HUSM 37 days, HKT 49 days; p = 0.02), and the number of neonates with predisposing factors for infection is higher (HUSM 100%, HKT 73.3%; p < 0.001) in HUSM compared with HKT. There were no differences in gestation, mean age of onset of infection and mortality between both hospitals. The most common organism isolated from the blood in HKT was *Klebsiella pneumoniae* (33.3%), and in HUSM *Klebsiella aerogenes* (24.6%). Half of *Klebsiella pneumoniae* isolates were resistant to cephalosporins and aminoglycosides in HKT and a similar number of *Klebsiella aerogenes* isolates were resistant to piperacillin and aminoglycosides in HUSM. In conclusion nosocomial infection is a common problem in both hospitals. Except for more frequent predisposing factors for infection in HUSM, and a longer duration of hospital stay among neonates in HKT, the clinical characteristics of neonates with nosocomial infection in both hospitals were similar.

Key Words: *nosocomial infection, neonates, Neonatal Intensive Care Unit*

Introduction

Nosocomial infections are defined as infections that manifest 48 hours after admission to the neonatal intensive care unit (1). However, the incubation period of neonatal infections differs and some perinatally acquired infections are known to manifest after 48 hours of life, especially those with maternal predisposing factors for sepsis such as chorioamnionitis, premature rupture of membranes or maternal infections (1). In this situation, it may be difficult to determine whether the infection was

hospital or perinatally acquired.

The incidence of nosocomial infection in the Neonatal Intensive Care Units (NICU) varies among countries and medical centres. It was reported to be 15.3% in infants hospitalised for more than 48 hours in NICU at the University of Utah Medical Centre, Salt Lake City (1) and 5.2% in Kuala Lumpur Hospital (2). The incidence of nosocomial infection was also noted to be higher (32.6%) in low birth weight (1000-1499 gm) babies (3). Low birth weight and prematurity are predominant risk factors for nosocomial infection because of the immaturity of the immune system in these babies (4). Other risk factors include overcrowding, understaffing, usage of invasive devices and procedures, administration of parenteral nutrition and endotracheal intubation. Common organisms causing nosocomial infection in NICU were gram negative bacilli, coagulase negative staphylococci and fungi. Nosocomial infection has been shown to cause significant mortality especially in premature and low birth weight babies.

The antibiotic policies of the two NICU's in Kuala Terengganu Hospital and Universiti Sains Malaysia Hospital could influence the antibiotic sensitivity patterns of the bacteria isolated. For HKT, intravenous penicillin and netilmicin were used for newborn infections before 48 hours of life. For nosocomial infections, intravenous cefotaxime and amikacin were used while awaiting culture results. Intravenous vancomycin would replace cefotaxime in the presence of a Methicillin Resistant *Staphylococcus Aureus* outbreak in the NICU, especially for skin, joint and bone infections. As for HUSM, penicillin and gentamicin were used for infection in the first 4 days of life and amikacin and piperacillin for infections after 4 days of life. Vancomycin would replace piperacillin if the baby also had umbilical infection.

The objective of this study was to compare the incidence and clinical characteristics of nosocomial infection in the Neonatal Intensive Care Unit (NICU) Kuala Terengganu Hospital (HKT) and Universiti Sains Malaysia Hospital (HUSM) in Kelantan.

Materials and methods

A retrospective study was done in the NICU of two tertiary hospitals, Kuala Terengganu Hospital Terengganu and Universiti Sains Malaysia Hospital, Kelantan. All neonates who developed nosocomial infection from 1st January 1998 to 31st. December 1998 in both hospitals were included in the study. Cases of nosocomial infections were identified from a register in the NICU which recorded all neonates who had positive blood cultures. Clinical information on these babies were then obtained from hospital records.

Nosocomial infection was defined as any infection that manifested clinically 48 hours after admission to the NICU and had a positive blood

Table 1: Clinical characteristic of neonates with nosocomial infection

Clinical characteristics	HKT		HUSM		p value
	n	%	n	%	
Sex					0.3
Male	18	60	37	56.9	
Female	11	36.7	28	43.1	
Undetermined	1	3.3	0	0	
Birth weight (kg)					0.25
< 1.00	1	3.3	7	10.7	
1.00 – 1.49	9	30	25	38.5	
1.50 – 1.99	8	26.7	12	18.5	
2.00 – 2.49	3	10	1	1.5	
≥ 2.50	9	30	20	30.8	
Gestational age (weeks)					0.31
< 28	4	13.3	5	7.7	
28 – 32	6	20	25	38.5	
33 – 36	8	26.7	16	24.6	
≥ 37	12	40	19	29.2	

culture. Only the first episode of infection was studied so that the incidence of nosocomial infection for the year could be calculated. Babies were excluded from the study if maternal predisposing factors for sepsis such as premature rupture of membranes, maternal fever, and maternal infections (urinary tract infection, and chorioamnionitis) were present. Mothers who had positive VDRL during antenatal visits were also not included in the study.

The incidence of nosocomial infection was calculated as the number of patients who developed nosocomial infection divided by the total number of admissions to the NICU during the year 1998. The age of onset of infection was defined as the age when clinical features suggestive of infection were first detected. Death was attributed to that particular episode of infection if it occurred within seven days after the diagnosis of infection.

Data collected were analysed using the SPSS programme, for personal computers. The Chi square test was used to compare categorical variables and the Mann-Whitney test used to compare continuous variables. A p value of less than 0.05 was considered significant.

Results

The total neonatal admissions to NICU during 1998 was 561 in HKT and 1,806 in HUSM. This difference was probably due to different admission policies of the two hospitals. The total number of neonates with nosocomial infection was 30 in HKT and 65 in HUSM giving an incidence of 5.4% and 3.6% respectively (p = 0.06). The clinical characteristics of neonates with nosocomial infection were as shown in Table 1.

The proportion of male neonates in HKT (60%) was similar to that in HUSM (56.9%). One neonate in HKT had undetermined sex at the time of infection. In HKT, the percentage of neonates with birth weight of 1.00-1.49 kg and ≥ 2.50 kg were similar, whereas, in HUSM most neonates had birth weight of 1.00-1.49 kg (38.5%). The proportion of term babies with nosocomial infection was 40% in HKT and 29.2% in HUSM (p = 0.3).

Neonates with nosocomial infection in HUSM had significantly more risk factors than those in HKT (Table 2).

A large number of neonates were ventilated before developing nosocomial infection in both HKT (63.3%) and HUSM (70.8%). All neonates in HUSM received parenteral nutrition prior to the onset of infection compared to only 40% in HKT. Forty seven (72.3%) neonates in HUSM had a central line compared to only 10 (33.3%) in HKT. Overall all neonates in HUSM had one or more of these risk factors compared to 73.3% of neonates with nosocomial infection in HKT (p = <0.001)

The mean age at onset of infection was 11.1 days in HKT and 9.9 days in HUSM (p=0.92) (Table 3).

The mean duration of hospitalisation of neonates in HKT (48.7 days) was significantly longer (p=0.015) than that in HUSM (36.5 days) (Table 4).

The mortality rate in neonates with nosocomial infection in HKT (n = 5, 16.7%) was similar to that in HUSM (n = 14, 21.5%) (p = 0.58).

In HKT, more gram positive (62%) than gram negative (39.9%) micro organisms were isolated (Table 5). In HUSM however, the proportion of gram positive (44.6%) and gram negative (47.7%)

Table 2: Risk factors in neonates with nosocomial infection

Risk Factor	HKT		HUSM		p value
	n	%	n	%	
Ventilation	19	63.3	46	70.8	0.47
Parenteral nutrition	12	40	65	100	< 0.001
Central line[1]	10	33.3	47	72.3	< 0.001
Invasive procedure[2]	2	6.7	18	27.7	0.02

[1] Central line: neonates who have umbilical arterial or venous, femoral or internal jugular lines

[2] Invasive procedures: neonates who have undergone any form of surgical procedures or exchange transfusion

organisms isolated was similar. *Klebsiella pneumoniae* (33.3%) and *Klebsiella aerogenes* (24.6%) were the most common organisms isolated in neonates with nosocomial infection in HKT and HUSM, respectively. Candida sepsis however was detected only in HUSM (7.7%).

In HKT the organisms isolated were tested for sensitivity to cefotaxime, ceftazidime, amikacin, gentamicin, netilmicin and imipenem. Out of 10 *Klebsiella pneumoniae* isolates, 90% were resistant to cefotaxime, 90% to ceftazidime, 60% to amikacin, 50% to gentamicin, and 90% to netilmicin. All *Klebsiella pneumoniae* isolates were sensitive to imipenem. The only isolate of *Pseudomonas aeruginosa* was sensitive to all antibiotics tested and the only isolate of *Escherichia coli* was resistant to cefotaxime, ceftazidime and netilmicin.

In HUSM the bacterial isolates were tested for sensitivity to piperacillin, amikacin, gentamicin and imipenem. Out of 16 *Klebsiella aerogenes* isolates 56% was resistant to piperacillin, 19% to amikacin, 25% to gentamicin and 6.3% to imipenem. There were 2 *Pseudomonas aeruginosa* and 2 *Escherichia coli* isolates. Except for one *Escherichia coli* isolate all were sensitive to the antibiotics tested. Except for one isolate of *Klebsiella aerogenes* all gram negative bacteria were sensitive to imipenem.

Discussion

In this study, the incidence of nosocomial infection in NICU HKT (5.4%) was not significantly higher than in HUSM (3.6%). Reports in the literature have used different denominators for calculating infection rates, including the number of live births, length of stay in nurseries or number of infants discharged. Other hospitals in Malaysia have recorded higher nosocomial infection rates, 23.8% (5) and 47.6% (6). Omission of neonates with repeated infections could have accounted for the lower incidence of nosocomial infection in this study. Comparisons of incidence rates will be more accurate if all calculations were made using the total number of live births as the denominator.

In this group of neonates with nosocomial infection, the proportion of males was more than females in both hospitals, concurring with other studies (7-10). Very low birth weight infants (1.00 – 1.49 kg) were also noted to have more nosocomial infections in this and other studies (1,9-11).

Most neonates in HKT (63.3%) and HUSM (70.8%) with nosocomial infection were ventilated before the onset of infection. This is not surprising as the endotracheal tube provides a portal of entry for micro-organisms into the respiratory tract subsequently causing systemic infection. Neonates who were >12 hours of age at the time of intubation, had a duration of intubation >72 hours or were re-intubated ≥2 times, were shown to have a high risk of respiratory tract colonisation(12). Neonates on positive pressure ventilation were also exposed to regular endotracheal suctioning which may cause mechanical trauma to the tracheal mucosa. This leads to defects in anatomical barriers leading to infection. Storm demonstrated that transient bacteraemia, without clinical signs of septicaemia, occurred five minutes after endotracheal suctioning (13). Therefore, endotracheal suctioning must be considered a potential risk for the development of systemic infection. Gentle endotracheal suctioning must be performed whenever necessary and at 4 to 6 hours intervals if necessary. Other studies in Malaysia also showed a high percentage of

Table 3: Age at onset of nosocomial infection

Age at onset of infection (days)	HKT		HUSM	
	n	%	n	%
3 - 7	13	43.3	26	40
8 - 14	11	36.7	27	41.5
15 - 21	2	6.7	7	10.8
22 - 28	3	10	5	7.7
> 28	1	3.3	0	0
Total	30	100	65	100

nosocomial infection in ventilated neonates; 72% in Queen Elizabeth Hospital in Sabah (9) and 57.9% in Hospital USM in Kelantan (3).

All neonates with nosocomial infection in HUSM and 40% in HKT had received parenteral nutrition before the onset of infection. The higher proportion of neonates receiving parenteral nutrition in HUSM was probably due to different unit policies. As a routine, parenteral nutrition was administered to all patients admitted to NICU in HUSM before starting enteral feeding. In HKT however, parenteral nutrition was not a routine and the total number of patients who received parenteral nutrition was limited to five neonates at any time. The relationship between parenteral nutrition and infection is well established, especially if a central line was used because it is in direct contact with the central circulation. Sepsis associated with parenteral nutrition has been reported to be as high as 45% in neonates and children (14). In a recent study, an incidence of 3.6 infections per hundred days of total parenteral nutrition use was reported (15).

The percentage of neonates with nosocomial sepsis who also had a central line was higher in HUSM (72.3%) compared to HKT (33.3%). In previous studies the prevalence of umbilical catheter related sepsis varied from 3% to 16% (16-19). All the central lines used in HKT were umbilical vein or umbilical artery catheters. Besides the umbilical route, central venous lines were also inserted via the femoral vein or internal jugular vein in HUSM. The association between infection and the number of lines per patient could not be determined as the number of lines per patient was not counted in this study. Umbilical arterial or venous catheters provide a convenient intravenous access in sick neonates. However, these catheters carry a high risk of bacteraemia in vulnerable neonates. The risk for catheter related sepsis from umbilical arterial catheter has been shown to be related to both very low birth weight and longer duration of antibiotic therapy (16-19). Prolonged antibiotic therapy has been shown to be associated with catheter related sepsis, possibly because neonates on prolonged antibiotics have a higher prevalence of resistant organisms, or it may be just a marker for overall severity of illness and susceptibility to infection in these neonates. Low birth weight and infusion of hyperalimentation fluid were two factors which influence the risk for catheter related sepsis in infants with umbilical vein catheters. Blood stream infection rate has been shown to be significantly more in those who had central catheters in situ (15%) compared to those without catheters (2%) (20). Wong et al demonstrated that all patients with nosocomial infection had an intravascular catheter but only 42% had an umbilical catheter (9). In very low birth weight babies however, there was no significant difference in the rate of late onset sepsis in those who had or did not have an umbilical arterial catheter [3].

Almost one third of patients with nosocomial infection in HUSM had undergone invasive procedures whereas, in HKT, only two neonates had surgery. This difference was probably because HUSM has a Paediatric Surgical Unit and all surgical cases from the state were referred to HUSM.

The majority of first positive blood culture occurred during the first two weeks of life in both hospitals and the mean age at onset of infection was similar in both hospitals. The onset of nosocomial infection has been shown to occur at a later age in other studies, 16.5 days (11) and 13.6 days (6). The widespread use of antibiotic might have delayed or masked the features of infection. The duration of hospitalisation in neonates with nosocomial infection may be affected by various factors. Besides multiple episodes of infection, premature neonates are also prone to develop complications such as persistent ductus arteriosus, intraventricular haemorrhage and chronic lung disease. Infants with nosocomial infection have a longer duration of

Table 4: Duration of hospital stay of neonates with nosocomial infection

Duration of hospital stay (days)	HKT		HUSM	
	n	%	n	%
≤ 14	1	3.3	10	15.4
15 - 30	9	30	21	32.3
> 30	20	66.7	34	52.3
Total	30	100	65	100

hospitalisation (50.5 days) than non-infected babies (14.5 days) (1). The mean length of hospital stay was even longer in very low birth weight babies with nosocomial infection (86 days) compared to that of infants without infection (61 days) (21). The mean duration of hospitalisation among very low birth weight babies with nosocomial infection in a previous study 3 was shorter (31.1 days) than that in the present study. Due to limited data the reasons for the difference in the duration of hospitalisation among neonates in HKT and HUSM could not be explained.

The types of gram negative organisms isolated were similar to those in other studies. *Klebsiella peumoniae* (7), *Escherichia coli*, *Pseudomonas aeruginosa* and Acinetobacter species (3,5) were noted to be common gram negative organisms in previous studies. The pharynx and gastrointestinal tract of neonates in NICU were frequently colonised by these organisms and the number of neonates colonised is proportional to the duration of hospital stay. These organisms are usually transmitted from medical personnel or contaminated patient care items to neonates.

Compared to other studies gram positive organisms were still important causative organisms as they were the causative organisms seen in about half of the neonates in this study. Improved survival of premature neonates and the increasing use of invasive procedures nowadays, has resulted in the increasing prevalence of commensal organisms such as *Staphylococcus epidermidis* and Candida. Wider use of parenteral nutrition in HUSM could have accounted for the high number of neonates with candida sepsis. Among the gram positive organisms, coagulase negative staphylococci has been reported to be the commonest organism isolated (20-23). Neonates with coagulase negative staphylococci

Table 5: Types of micro organisms isolated from blood cultures in neonates with nosocomial

Types of micro organisms	HKT		HUSM		p value
	n	%	n	%	
Gram Negative Organisms					
Klebsiella pneumoniae	10	33.3	0	0	< 0.001
Klebsiella aerogenes	0	0	16	24.6	0.003
Acinetobacter species	0	0	6	9.2	0.09
Enterobacter species	0	0	5	7.7	0.12
Escherichia coli	1	3.3	2	3.1	0.95
Pseudomonas aeruginosa	1	3.3	2	3.1	0.95
Gram Positive Organisms					
Staphylococcus aureus	3	10	4	6.2	0.51
Staphylococcus epidermidis & other coagulase-negative staphycocci	12	40	3	4.6	<0.001
Methicillin resistant *Staphylococcus aureus*	2	6.8	6	9.2	0.68
Methicillin resistant *Staphylococcus epidermidis*	1	3.3	15	23.1	0.02
Streptococcus viridans	0	0	1	1.5	0.50
Fungus					
Candida species	0	0	5	7.7	0.12
Total	30	100	65	100	

infection were more likely to be premature and had central lines (24). Coagulase negative staphylococci usually adhere to catheter surfaces and produce extracellular substances, including slime, thus making them inaccessible to phagocytes and antibiotics. Exposure to intravenous lipid emulsions, and the duration in which the non-umbilical central venous catheters were left in-situ had a clear linear relationship with coagulase negative staphylococci infection (25). Fleer et al showed that contaminated total parenteral nutrition fluid was the causative factor of many *Staphylococcus epidermidis* infections in their NICU (26). Even though more babies had invasive procedures, central lines and parenteral nutrition in HUSM, coagulase negative staphylococci was more commonly isolated in HKT. Contamination of blood culture specimens could have affected the results as the skin of neonates is commonly colonised with coagulase negative staphylococci.

In conclusion nosocomial infection is a common problem in both hospitals and the incidence and mortality rate of nosocomial infection in HKT and HUSM were similar. Except for more frequent predisposing factors for infection in HUSM, and a longer duration of hospital stay among neonates in HKT, the clinical characteristics of neonates with nosocomial infection in both hospitals were similar. Both gram positive and gram negative organisms continued to be the major causative organisms in nosocomial sepsis. The common causative organisms *Klebsiella pneumoniae* and *Klebsiella aerogenes* were resistant to most commonly used antibiotics but still sensitive to imipenem. A surveillance programme should be set up in each hospital to allow early identification of infection in at risk neonates. This will hopefully lead to better infection control and to earlier treatment of nosocomial infection in order to reduce the morbidity and mortality.

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Malaysian Journal of Medical Sciences, Vol. 7, No. 1, January 2000 (41-46)

ORIGINAL ARTICLE

RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS TO DIFFERENTIATE STRAINS OF *VIBRIO VULNIFICUS* ISOLATED FROM COCKLES AND SHRIMPS.

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A random amplified polymorphic DNA (RAPD) fingerprinting method has been developed to differentiate *Vibrio vulnificus* strains isolated. Twenty-nine strains isolated from cockles and twenty-one strains isolated from shrimps were analyzed. A total of 10 primers were screened with *Vibrio vulnificus* strains to identify those capable of generating DNA polymorphisms and two primers were selected. Primer GEN 1-50-01 and GEN 1-50-08 produced polymorphisms in most strains tested, with the band sizes ranging from 10.0 to 0.25 kb pair. Dendrogram analysis showed that primer GEN 1-50-01 produced 10 clusters and 24 single strains at a 40% similarity, whereas primer GEN 1-50-08 produced 11 clusters and 20 single strains at a 40% similarity. This study revealed the potential use of PCR fingerprinting in epidemiological studies.

Key Words: *Vibrio vulnificus, RAPD-PCR, cockle, shrimp*

Introduction

Vibrio vulnificus was first described by Riechelt *et al*. in 1976 (1) as *Beneckea vulnifica*. This organism is a member of the genus *Vibrio* which are defined as gram-negative, non-sporing rods that are straight or have a single, rigid curve. They are motile and most have a single polar flagellum (2-4). It is a marine bacterium that can cause three types of human infections such as primary septicaemia, gastroenteritis and wound infection (5).

The presence of *Vibrio vulnificus* is not associated with pollution. These bacteria are naturally marine organism that thrives in shallow, coastal waters in temperate climates throughout the world (6). Raw seafood such as oysters, eels, shrimps and fish are example of sources of these bacteria (7). A variety of DNA-based typing methods have been applied to identify *Vibrio vulnificus* species, including plasmid profiles and ribotyping analysis. Each of these approaches has provided useful insights into evolutionary and epidemiological relationships of several *Vibrio* species (8). However, while a variety of molecular subtyping approaches are available, the most general procedure for the comparison of genomes is RAPD analysis. RAPD

technique, developed by William *et al.* (9) produces reproducible and often distinctive sets of DNA fragments by subjecting genomic DNA to PCR primed by short (10 mer) oligonucleotide primers of arbitrary sequences.

In this study we determined the DNA diversity of *Vibrio vulnificus* strains by RAPD-PCR which allows rapid and sensitive differentiation between the strains.

Materials and methods

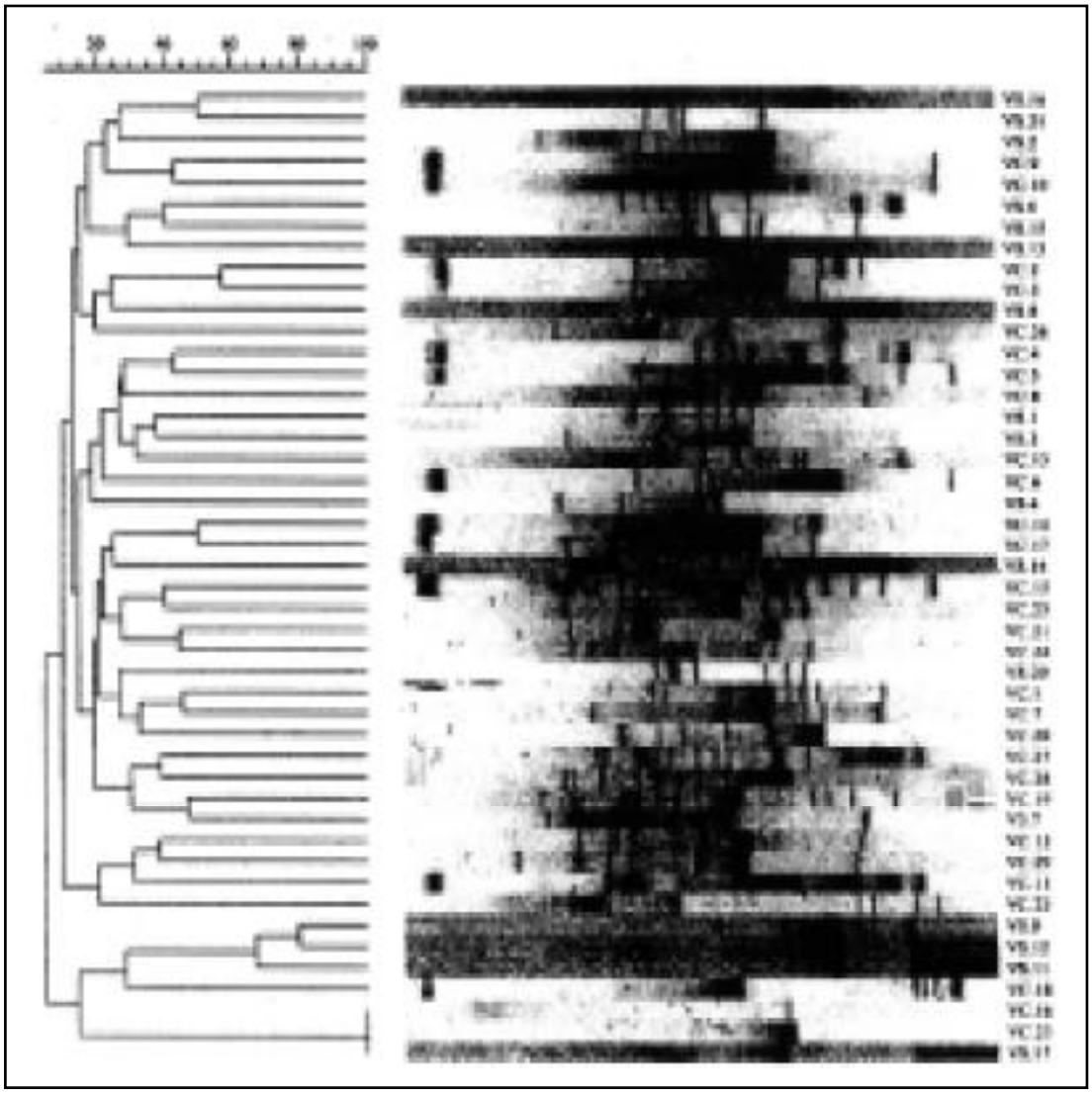
Bacterial isolates

Twenty-nine and twenty-two isolates of *Vibrio vulnificus* from cockles and shrimps purchased from wet markets and supermarkets in Seri Serdang, Seri Kembangan, Subang Jaya, Seremban, Penang and Kuching were examined. All isolates were grown on Thiosulfate Citrate Bile Salts Agar plates (TCBS) and were characterized by standard biochemical tests (4).

Genomic DNA isolation

Genomic DNA extraction was done according to the method by Ausubel *et al.* (10). About 1.5 ml of the bacterial culture was decanted into a sterile eppendorf tube and was centrifuged for 1 minute, at 10,000 rpm. The supernatant was decanted completely and the pellet was resuspended in 700 ml Glucose-EDTA-Tris-HCl buffer. Subsequently, 50 ml of 10% sodium dodecyl sulphate and 5 ml of 20 mg ml^{-1} proteinase K were added to the

Figure 1: Dendrogram produced from cluster analysis of Vibrio vulnificus DNA fingerprints with primer GEN 1-50-01. The similarity index is indicated on top of the plot. Strains VS.5, VS.10, VS.18 and VS.19 were untypeable.
VC = Vibrio vulnificus from cockle, VS = Vibrio vulnificus from shrimp.



suspension and the cells were lysed for 20 minutes at 60ºC in a water bath shaker. After the incubation, 500 ml of Phenol-Cheoroform-Isoamyl Alcohol mixture were added and mixed gently for 5 minutes. The mixture was centrifuged for 1 minute at 12,000 rpm. About 200 ml of the aqueous upper layer was collected in an eppendorf tube and then 200 ml of 3 M sodium acetate and 800 ml of isopropanol were added. The precipitated DNA was recovered by centrifugation for 10 minutes at 12,000 rpm. The pellet was washed with 600 ml of 70% ethanol. After centrifuging for 10 minutes at 12,000 rpm the pellet was dried at room temperature for 30 minutes. The dried pellet was resuspended in 50 ml of sterile water and used immediately for PCR analysis.

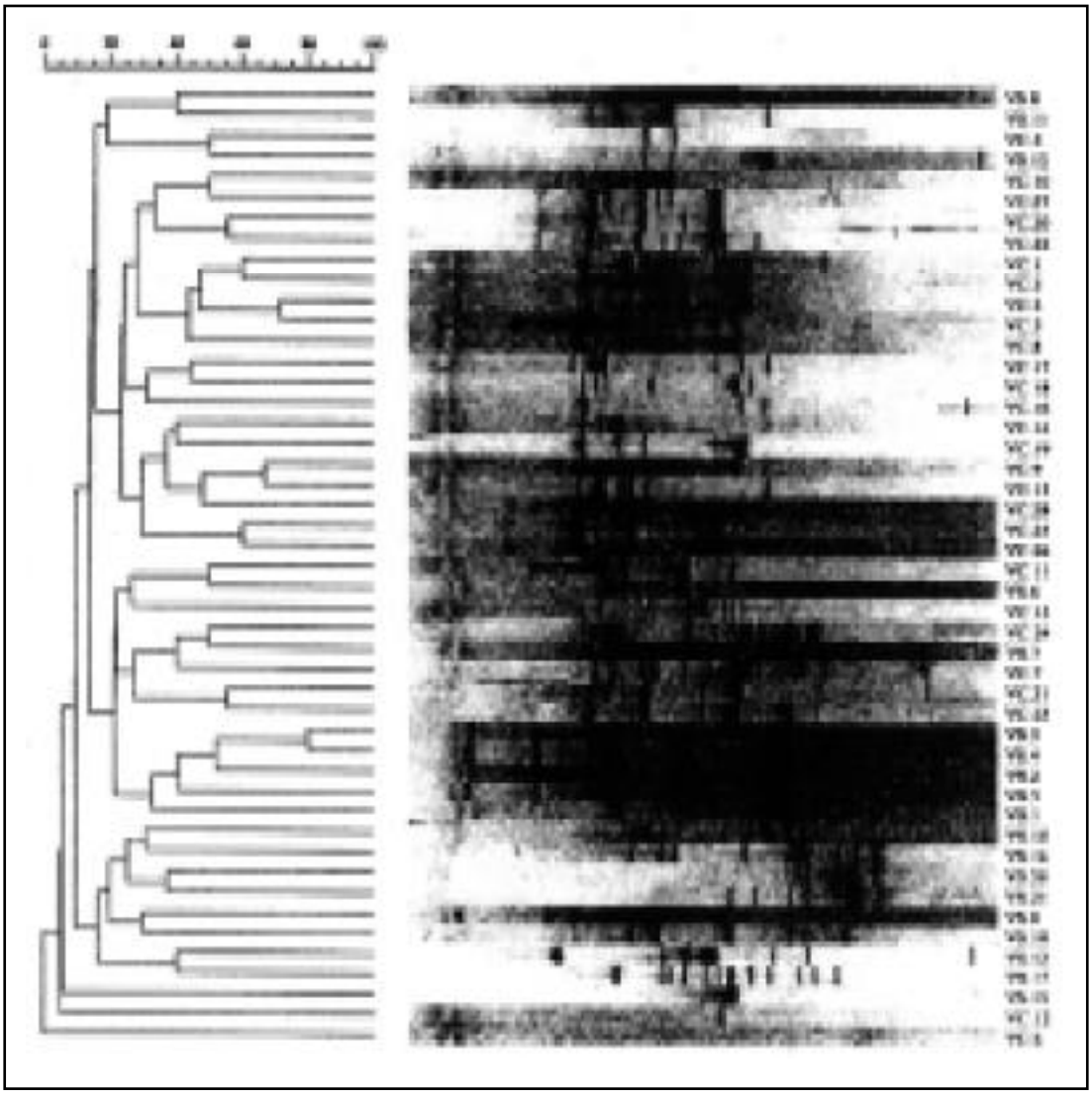
DNA primer

One set of randomly designed 10-mer oligonucleotides (with G+C content of 50%) was obtained from Genosys Biotechnologies., Inc., USA. All primers were screened to determine the ones that can generate clear polymorphic bands.

RAPD-PCR fingerprinting

Reaction mixtures consisted of 2.5 ml 10x reaction buffer, 0.5 ml of 10 mM dNTP mix, 2 mM of primer (Genosys Biotechnologies Inc., USA), 2 ml of 25 mM MgCl2, 0.5 ml of *Taq* polymerase (Promega, USA), 1 ml (20-40 ng)genomic DNA and made up to 25 ml with sterile distilled water. A thermal cycler (Perkin Elmer Model 2400) was used

Figure 2: Dendrogram produced from cluster analysis of Vibrio vulnificus DNA fingerprints with primer GEN 1-50-08. The similarity index is indicated on top of the plot. Strains VC.29, VS.14 and VS.19 were untypeable.
VC = Vibrio vulnificus from cockle, VS = Vibrio vulnificus from shrimp.



for amplification. The reaction was subjected to 45 cycles at 94 ^{0}C for 2 min, 36 ^{0}C for 1 min and 72˚C for 5 min. A final elongation step of 72 ^{0}C for 5 min was included. The PCR amplification products were visualized by running 10 ml of the reaction mixture on a 1.2% agarose gel, which was then stained with 5 mg ml^{-1} ethidium bromide and examined over UV light. A 1 kb DNA ladder (Promega, USA) was used as DNA size markers.

Cluster analysis of RAPD-PCR

The gel picture was first scanned into a digital format as a tagged information file format image using the program Gelcompar ver. 4.1 (Applied Maths, Ghent, Belgium). Dendrogram based on the similarity coefficients were then generated by the unweighted pair-group method of average linkage (UPGMA).

Results

The initial experiments were performed with a subset of *Vibrio vulnificus* strains to identify primers that provide polymorphic band patterns. RAPD-PCR using primers GEN 1-50-01 and GEN 1-50-08 resulted in amplification of genomic DNA of *Vibrio vulnificus* generating fragments of DNA ranging in sizes between 0.25 to 10.0 kilobase pairs (kb). Out of 50 strains examined, 46 and 47 different RAPD patterns were generated using primer GEN 1-50-01 and GEN 1-50-08 respectively. Four and three strains showed no band with primer GEN 1-50-01 and GEN 1-50-08 respectively. Though these strains were untypeable using the respective primer, the combination of the amplification patterns of the two primers allowed all the *Vibrio vulnificus* strains to be typed. Figure 1 and Figure 2 showed the dendrogram generated from the computer cluster analysis of the DNA fingerprints for primer GEN 1-50-01 and GEN 1-50-08. With the primer GEN 1-50-01, 10 clusters and 24 single strains were observed at a similarity level of 40%. Primer GEN 1-50-08 produces 11 clusters and 20 single strains at a 40% similarity level. Some strains of *Vibrio vulnificus obtained from* cockles and shrimps were observed to cluster together, indicating their possible genetic relatedness.

Discussion

Interest in the microbiological relationship of *Vibrio vulnificus* from seafoods has been driven by the recognition that strains of these species are associated with human infections. Despite the increased interest in the *Vibrio* (11) and the rapidly expanding body of knowledge concerning the various associations of these genera with seafoods, limited studies have reported the presence of *Vibrio vulnificus* in seafoods in Malaysia (12). Biochemical characterization revealed that some of the *Vibrio vulnificus* strains isolated from cockles and shrimps in this study were of biotype1, which is known as an opportunistic human pathogen with infection resulting from the consumption of contaminated seafood or exposure to marine environment in the case of wound infections. Thus, it would obviously be of great benefit to develop rapid screening methods for seafoods for the presence of *Vibrio vulnificus* strains that are potentially pathogenic in humans.

A major obstacle in understanding the natural transmission patterns of *Vibrio vulnificus* is the lack of a simple and reliable strain typing system. Utilization of phenotypic properties of *Vibrio vulnificus* to distinguish strains from different hosts face serious limitations as the phenotypic traits of bacteria can vary under different growth conditions. In addition, recent advances in molecular biology have disclosed an enormous diversity in the microbial world, and at the same time they have pointed out the limitations of traditional typing techniques. To end this, we employed an approach that is fairly simple and which can be performed rapidly. RAPD-PCR fingerprinting has been optimized previously, primarily for environmental and cockle sources of this microorganism (12-13). Analysis of the DNA fingerprints of all the *Vibrio vulnificus* strains examined in this study with gel imaging and cluster analysis software revealed significant genetic heterogeneity among the strains. The overall grouping pattern indicated that *Vibrio vulnificus* showed a high degree of variation in its genomic organization, possibly due to transposon activity, recombination shuffling or horizontal gene transfer. This result is consistent with data from several other studies (14 -15) which demonstrated that *Vibrio vulnificus* strains isolated from environmental samples had a diverse genomic organization. Dendrogram obtained using primer GEN 1-50-01 revealed that strains VC.16, VC.23 and VS.17 were clustered together at 100% similarity level, whereas the dendrogram obtained using primer GEN 1-50-08 showed these three strains were in different clusters. Generally, clustering patterns obtained differed when different

primers were used.

Several other studies have reported on the application of various PCR-based strategies to fingerprint *Vibrio vulnificus* strains (16-19). These studies also revealed a high degree of genomic heterogeneity. Among the molecular technique used, RAPD-PCR is the most economical and has shortened the time of typing. This PCR fingerprinting method is simple and rapid, and may be a useful tool for differentiating *Vibrio vulnificus* strains in epidemiological analysis, particularly in large studies and in urgent situations. Reports suggesting that *Vibrio vulnificus* biotype 1 have pathogenic potential in humans (11) emphasizes the need for caution when dealing with seafoods contaminated with this organism.

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Malaysian Journal of Medical Sciences, Vol. 7, No. 1, January 2000 (47-53)

ORIGINAL ARTICLE

ABSORBED DOSE TO WATER DETERMINATION USING IAEA, HPA, NACP, AAPM, NCRP AND ICRU PROTOCOLS FOR 1.25 MEV GAMMA RAY 6 MV AND 10 MV X-RAYS: AN INTERCOMPARISON OF RESULTS WHEN IAEA WAS TAKEN AS A STANDARD PROTOCOL

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Absorbed dose to water was measured with ionisation chambers NE 2561 (#267), NE 2581 (#334), NE 2571 (#1028), using the IAEA standard water phantom. The ionisation chamber was inserted in the water phantom at a reference depth dependent on the type of the radiation quality used. Three radiation qualities were used namely 1.25 MeV gamma ray, 6 MV x-rays and 10 MV x-rays. The values of the absorbed dose to water were determined by the N_K- and N_X- based methods, i.e with the use of IAEA, HPA, NACP, AAPM, NCRP and ICRU protocols. The aim of this study was to make an intercomparison of the results, by taking the IAEA protocol as a standard. The largest deviation contributed by any of these protocols was recorded for each quality. It was found that AAPM, NCRP and ICRU protocols contributed 0.94% for 1.25 MeV gamma ray, NACP contributed 2.12% for the 6 MV x-rays, and NACP contributed 2.35% for 10 MV x-rays. Since the acceptable limit of deviation set by the IAEA for this absorbed dose work is ± 3%, it is clear that the overall deviations obtained were all satisfactory.

Key words : *gestational Diabetes mellitus, diagnosis, management*

Introduction

At present there are many protocols that are being used in variaus countries to determine the absorbed dose to water. In 1987, International Atomic Energy Agency (IAEA) recommended a protocol for absorbed dose to water determination for high energy photon dosimetry (1). Prior to this recommendation, several protocols have been recommended, for example; Hospital Physicists' Association in 1983 with its HPA protocol (2), American Association of Physicists in Medicine in 1983 with AAPM protocol (3), National Council on Radiation Protection and Measurement in 1981 with its NCRP protocol (4), Nordic Association of Clinical Physics in 1980 with its NACP protocol (5) and International Commission on Radiation Units and Measurement in 1973 with its ICRU protocol (6). Table 1 summarises these six protocols together with their respective formulae for calculating the absorbed dose to water. The, meanings of symbols that are used in the formulae are given in the final part of this paper.

For the purpose of dosimetry accuracy in radiotherapy, these six protocols should in practice yield a single value in the absorbed dose to water,

Table 1. Absorbed dose to water D_W *formulae according to variaus protocols*

Protocols		Equation no. in this work	References
Name	*Dw* formulas		
IAEA	$M_u N_K (1-g) K_{att} K_m (S_{w,air}) P_u P_{dis}$	1	[1]
HPA	$1.139\, M\, N_K\, C_\lambda$	2	[2]
NACP	$M_u N_K (1-g) K_{att} K_m (S_{w,air}) P_u$	3	[5]
AAPM	$M\, N_X\, k_q^{1a}\, (L/\rho)_{med,gas}\, P_{ion}\, P_{rep}\, P_{wall}$	4	[3]
NCRP	$M\, N_X\, C_\lambda$	5	[4]
ICRU	$M\, N_X\, F$	6	[6]

$$^{a}k_q = \frac{k \times (W/e) \times A_{cav} \times A_{wall} \times \beta_{wall}}{\alpha \times \left(\bar{L}/\rho\right)_{air}^{wall} \times \left(\bar{\mu}_{en}/\rho\right)_{wall}^{air} + (1-\alpha) \times \left(\bar{L}/\rho\right)_{air}^{cap} \times \left(\bar{\mu}_{en}/\rho\right)_{cap}^{air}}$$

Table 2. The radiotherapy machices that provided the three radiation qualities

Radiation quality		Machine		
No.	Energy	Name	Model	Located at
1	1.25 MeV	Co-60 teletherapy unit	Eldorado-8 (#104)	SSDL Malaysia
2	6 MV x-ray	Medical linear accelerator (linac)	Mevatron KD2	Radiotherapy and Oncology Unit, HUKM
3	10 MV x-ray	Linac	Mevatron KD2	Radiotherapy and Oncology Unit, HUKM

Table 3. Calibration factor of the three ionisation chambers

Ionisation chamber		Calibration faktor	
Model	Serial number	N_K	N_X
NE 2561	267	9.353 mGy/sd[a]	1.064 R/sd[u]
NE 2581	334	52.94 mGy/sd[b]	6.022 R/nC[d]
NE 2571	1028	41.34 mGy/sd[c]	4.703 R/nC[d]

[u]Certificate values as reference (8).

[b]Certificate values as reference (9).

[c]Certificate values as reference (10).

[d]Derived from the N_K *Value.*

for a given radiation quality and reference condition. The study acms to confirm whether the different protocols yield almost identical absorbed dose to water result, for a given radiation quality and experiment set-up.

The goalo of this study are: (1) to determine the absorbed dose to water using IAEA, HPA, NACP, AAPM, NCRP and ICRU protocols for 1.25 MeV gamma ray, 6 MV x-rays and 10 MV x-rays; and (2) to compare the results obtained by the HPA, NACP, AAPM, NCRP and ICRU with the most commonly used IAEA protocol (7).

Material and Methods

2.1 The dependence of the three elements (calibration factors, dosimeter readings and the interaction coefficients) on radiation quality and ionisation chamber in the protocol formulae.

The six formulae (given by the six protocols) for the determination of the absorbed dose to water in Table 1, mainly comprise of three elements namely:

(i) the ionisation chamber calibration factors (N_K or N_X), which depend on the type of chamber used,
(ii) the dosimeter reading (M or M_u), which depends on both the chamber and the radiation quality, and
(iii) the interaction coefficients, which depend on the ionisation chamber, the radiation quality and the protocol used.

The present work investigated the determination of the absorbed dose to water in three

Table 4. Dosimeter reading of the three ionisation chambers, in three radiation qualities

Dosimeter models		Dosimeter average reading, $M^{[a]}$ or $M_u^{[b]}$		
Ionisation chamber	Electrometer	1.25 MeV gamma ray	6 MV x-ray	10 MV x-ray
NE 2561 (#267)	NE 2560 (#151)	28.979 ± 0.040 sd/min	85.933 ± 0.047 sd/100mu	201.347 ± 0.120 sd/300mu
NE 2581 (#334)	PTW-Unidos 10005 (# 50013)	4.957 ± 0.002 nC/min	35.336 ± 0.042 nC/230mu	-
NE 2571 (#1028)	PTW-Unidos 10005 (# 50013)	6.109 ± 0.004 nC/min	15.701 ± 0.000 nC/80mu	-

[a]For HPA, AAPM, NCRP and ICRU protocols
[b]For IAEA and NACP protocols

Table 5. Values of interaction coefficients as a function of radiation quality, ionisation chamber, and protocol.

		1.25 MeV gamma ray			6 MV x-ray			10 MV x-ray		
		NE 2561	NE 2581	NE 2571	NE 2561	NE 2581	NE 2571	NE 2561	NE 2581	NE 2571
IAEA										
	$(1-g)$	0.997	0.997	0.997	0.997	0.997	0.997	0.997	0.997	0.997
	k_{an}	0.995	0.975	0.994	0.995	0.975	0.994	0.995	0.975	0.994
	k_m	0.984	0.99	0.991	0.984	0.99	0.991	0.984	0.99	0.991
	$(S_{w,air})$	1.133	1.133	1.133	1.119	1.119	1.119	1.105	1.105	1.105
	P_u	0.993	1.0075	0.993	0.9946	1.006	0.9946	0.9966	1.2407	0.9966
	P_{du}	0.985	0.987	0.987	0.985	0.987	0.987	0.985	0.987	0.987
HPA										
	C_2	0.951	0.951	0.951	0.95	0.95	0.95	0.943	0.943	0.943
NACP										
	$(1-g)$	0.997	0.997	0.997	0.997	0.997	0.997	0.997	0.997	0.997
	k_{an}	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
	k_m	0.991	0.963	0.991	0.991	0.963	0.991	0.991	0.963	0.991
	P_u	0.97	0.99	0.97	0.98	0.99	0.98	0.985	0.99	0.985
	$(S_{w,air})_u$	1.15	1.15	1.15	1.14	1.14	1.14	1.125	1.125	1.125
AAPM										
	k_q	0.0085[a]	0.0085	0.0086[a]	0.0085[a]	0.0085	0.0086[a]	0.0085[a]	0.0085	0.0086[a]
	$(L,\rho)_{med,air}$	1.134	1.134	1.1.34	1.127	1.127	1.127	1.117	1.117	1.117
	P_{cav}	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	P_{repl}	0.9953	0.9958	0.9958	0.9955	0.9961	0.9961	0.9925	0.9936	0.9936
	P_{wall}	0.9965	1.0099	0.9970	0.9965	1.0050	0.9965	0.9976	1.0047	0.9976
NCRP										
	C_2	0.95 rad/R	0.95 rad/R	0.95 rad/R	0.94 rad/R	0.94 rad/R	0.94 rad/R	0.93 rad/R	0.93 rad/R	0.93 rad/R
ICRU										
	F	0.95 rad/R	0.95 rad/R	0.95 rad/R	0.94 rad/R	0.94 rad/R	0.94 rad/R	0.93 rad/R	0.93 rad/R	0.93 rad/R

[a]Reference [12]

radiation qualities using three different ionisation chambers.

Table 2 shows types of machines that provided these radiation qualities and the their location.
Table 3 shows the three chambers used together with their calibration factors.
Table 4 shows the dosimeter readings obtained from the three chambers in the three radiation qualities.

The types of electrometers used with these ionisation chambers to yield the readings (charge) are given in this table. To obtain these readings, for example in the 1.25 MeV Co-60 beam, experimental set-up as shown in Fig 1 was used. Similar set up was used for the x-rays beam. Source to chamber distance (SCD) was set at 110 cm for 10 MV x-ray, and 105 cm for other radiations.

The final part of the absorbed dose to water formula is the interaction coefficients. Table 5 shows these coefficients with their values (1, 2, 3, 4, 5, 6, 11, 12). It can be seen that these values vary with the type of radiation quality, the type of ionisation chamber used for measurement and the protocol used.

2.2 Numerical examples for calculating the absorbed dose to water and the percentage deviation in absorbed dose to water

2.2.1 The absorbed dose to water

Example 1. Suppose that the absorbed dose to water in the 1.25 MeV gamma ray beam is to be determined using NE 2561 ionisation chamber based on the IAEA protocol. By looking at Table 1, eqn. 1 is the formula that should be used. For the present work, the values for eqn. 1 are: N_K = 9.353 mGy/sd (Table 3); M_u = 28.979 ± 0.040 sd/min (Table 4); $(1\text{-}g)$ = 0.997, K_{att} = 0.995, K_m = 0.984, $S_{w,air}$ = 1.133, P_u = 0.933 and P_{dis} = 0.985 (Table 5). Upon calculating, we obtained D_W = 293.24 mGy/min.

Example 2. Suppose that the absorbed dose to water in the 6 MV x-rays beam is to be determined

Table 6. D_W *and D values for three ionisation chambers, calculated using six protocols, and three radiation qualities.*

Radiation quality	Ionisation chamber	Units	D_W [Eqns. (1) to (6)]						Δ [Eqn. (7)]				
			IAEA	HPA	NACP	AAPM	NCRP	ICRU	HPA	NACP	AAPM	NCRP	ICRU
1.25 MeV gamma ray	NE 2561	mGy/min	293.24[a]	293.57	295.74	296.00	292.92	292.92	0.11[c]	0.85	0.94	-0.11	-0.11
	NE 2581	mGy/min	284.65	284.25	283.98	286.57	283.59	283.59	-0.14	-0.23	0.67	-0.37	-0.37
	NE 2571	mGy/min	275.53	273.56	275.56	275.81	272.94	272.94	-0.72	0.01	0.10	-0.94	-0.94
6 MV x-ray	NE 2561	Gy/100mu	0.860	0.870	0.878	0.869	0.859	0.859	1.12	2.12	1.05	-0.07	-0.07
	NE 2581	Gy/230mu	2.001	2.024	2.007	2.021	2.000	2.000	1.16	0.29	1.00	0.04	0.04
	NE 2571	Gy/80mu	0.701	0.702	0.712	0.709	0.694[b]	0.694	0.26	1.66	1.14	-0.92	-0.92
10 MV x-ray	NE 2561	Gy/300mu	1.994	2.022	2.041	2.014	1.992	1.992	1.42	2.35	1.00	-0.10	-0.10

Table 7. Comparison of absorbed dose to water let the present study and previous students

Two protocol that are being compared	Study		Previous student			Comments on the results of present study
	Condition	Results of deviation (%)	Condition	Results of deviation %	Reference	
HPA, in comparison with AIEA	Radiation quality Co-60, 6 MV and 10 MV using NE 2561 chamber	0.11% to 1.42%	Same as present work	-1.29% to -0.22%	13	It is in a good agreement
AAPM, in comparison with AIEA	Radiation quality Co-60, 6 MV and 10 MV using NE 2561, NE 2581and NE 2571 chambers	0.10% to 1.14%	Different from present study: This other study uses radiation quality Co-60, 4 MV and 25 MV using PTW, Capintec and Farmer chambers	-0.40% to-1.20%	14	One to one comparison cannot be done as conditions are different

with NE 2571 ionisation chamber using the NCRP protocol. By looking at Table 1, eqn. 5 is the formula that should be used. For the present work, the values for eqn. 5 are: N_X = 4.703 R/nC (Table 3); M_u = 15.701 ± 0.000 nC/80mu (Table 4); C_l = 0.94 rad/R (Table 5). Upon calculating, we obtained D_W = 0.694 Gy/80mu

Similar methods as shown in examples 1 and 2 were used to calculate D_W for other radiation qualities using the three chambers for the six protocols. The results are shown in Table 6.

2.2.2 The percentage deviation in absorbed dose to water

The percent age deviation in the absorbed dose when we compare with the IAEA protocol is

$$\frac{D_W \text{ (Other protocol)} - D_W \text{ (IAEA protocol)}}{D_W \text{ (IAEA protocol)}} \times 100\%$$

Example 3. Suppose the deviation of the absorbed dose to water in the HPA protocol results (obtained in the determination of absorbed dose to water in 1.25 MeV gamma-ray beam using an NE 2561 chamber) is to be calculated. By looking at Table 6, we have $ = 100% x (293.57 – 293.24)/ 293.24 = 0.11 %.

Similar method as shown in example 3 was used for the other protocols to calculate other $ for the three radiation qualities using the three chambers. The results are shown in Table 6.

Result and Discussion

Column 4 of Table 6 shows the values of the absorbed dose to water determined by the IAEA protocol for three radiation qualities using the three types of ionisation chambers. Columns 5, 6, 7, 8 and 9 show the absorbed dose to water calculated by the HPA, NACP, AAPM, NCRP and ICRU protocols respectively. Columns 10, 11, 12, 13 and 14 show how the other protocols deviate from the IAEA results.

In this study the NCRP and ICRU protocols yielded results which are in good agreement with the IAEA protocols, followed by the HPA, AAPM and NACP protocols. From Table 6, it can be seen that all the deviation values given by these two (NCRP and ICRU) protocols are lower than ±1%.

Table 7 shows the comparison between the present study and present studies. For the HPA

Figure 1. Experimental set-up for the absorbed dose to water determination in a 60 Co beam at the SSDL.

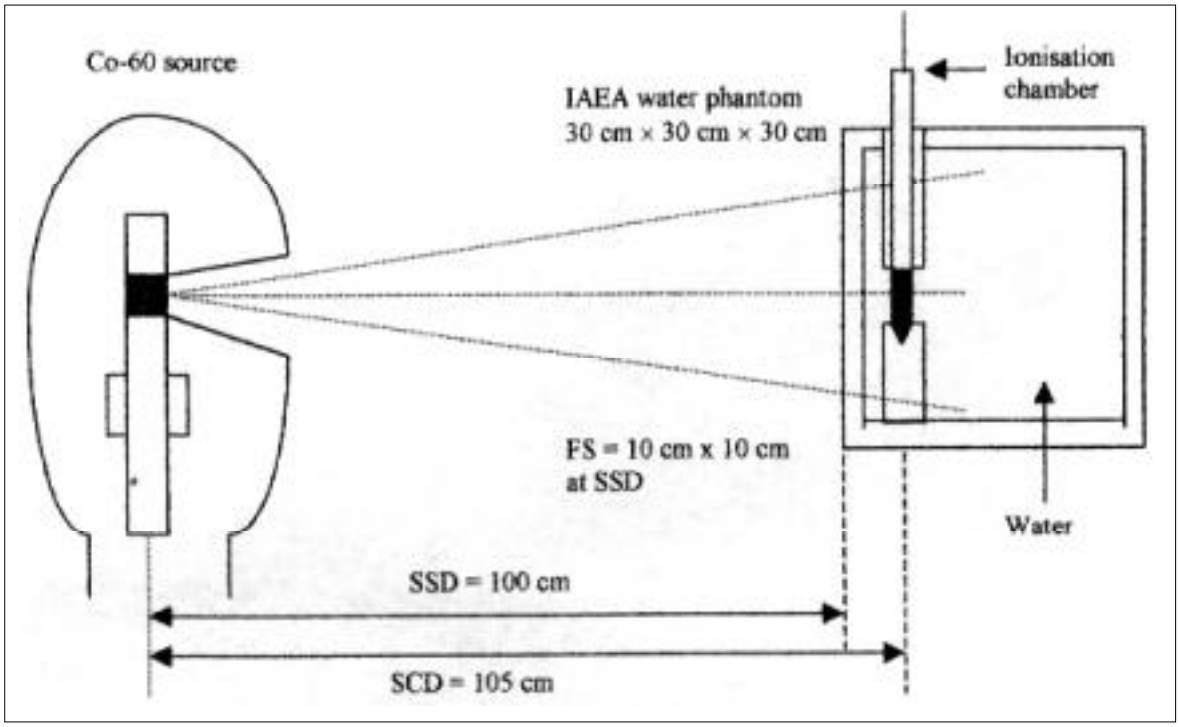

Figure 2. List of symbols and meaning.

List of symbols

(l-g)	Value for fragtion energy of secondary charges particles that is lost to bremsstrahlung
α	Fraction of ionization due to electron from buildup cap
A*ion*	Ion collection efficiency
A*wall*	Wall corection factor
β*wall*	Absorbed dose/collision fraction of kerma
C_{λ}	Exposure to absorbed dose conversion factor
D_w	Absorbed dose to water
F	Coefficient relating the exposure in roentgens to absorbed dose in water expressed in cGy
k	Charge per unit mass of air per unit exposure
K_{att}	Attenuation factor
K_m	Non-air
k_q	Chamber correction factor
(L/ρ)	Stopping power ratio-medium to gas
M or M_u	Electrometer reading
N_K	Air kerma calibration faktor
N_X	Exposure calibration factor
P_{dis}	Correction for displacement correction
P_{repl}	Replacement correction
P_u	Perturbation factor

protocol, a study that was newformed by Kadni [13] shawed a good agreement with the present study. The other study done by Huq and Nath [14] however can not be compared to this study as different conditions were used.

The largest deviation contributed by any of these protocols was recorded for each quality. It was found that AAPM, NCRP and ICRU contributed 0.94% for 1.25 MeV gamma ray, NACP contributed 2.12% for the 6 MV x-rays, and NACP contributed 2.35% for 10 MV x-rays. Since the acceptable limit of deviations set by the IAEA for this absorbed dose work is ± 3% [15], it is clear that the overall deviations obtained were all satisfactory.

Conclusions

HPA, NACP, AAPM, NCRP and ICRU protocols have not yielded significant differences in absorbed dose to water value when compared with the recent IAEA protocol. The differences in data for interaction coefficients have minor influences on the final results. It can be concluded that, despite the many differences in the values of these protocols, the final results were almost identical. Therefore, not suprisingly, the five protocols are still being used in the western countries. In Malaysia, a preliminary survey [16] showed that four out of thirteen health institutions use the HPA protocol while two health institutions use the ICRU and AAPM protocols.

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ORIGINAL ARTICLE

SPLIT-COURSE RADIOTHERAPY IN STAGE IV HEAD & NECK CANCER

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Short course hypo-fractionated radiotherapy is a standard regime for the palliation of stage IV head and neck cancers. However few patients respond favorably and require further radiotherapy in curative intent. We have used split-course radiotherapy technique to find out this conversion rate from palliative to curative intent. This was a prospective study conducted from November 1998 to October 1999; twenty-six (26) patients with stage IV head & neck cancers were treated with a hypofractionated regime of radiotherapy. A tumor dose of 30 Gy in 10 fractions [time dose fraction (TDF) 62] over 2 weeks was delivered using a 6 MV linear accelerator. A conventional 2 field or 3 field technique was used. Patients were assessed for the regression of tumor on fifth day, tenth day of radiotherapy and 4 weeks after the completion of radiotherapy. Patients showing complete response and good partial response were allowed to receive further radiotherapy of 30 Gy in 15 fractions [TDF 49]. There were 21 males and 5 females in the study with a median age of 44 years (range 19-77 years). All patients completed the initial regime. Complete responses were observed among 14 patients (54%); partial response in 6 patients (23%), and no response was seen among 6 patients (23%). Sixteen patients (61%) were suitable for radical radiotherapy after phase-I course of the above schedule. Seventeen patients (65%) showed an improvement in the general well being with a better quality of life. One year actuarial survival was (76%), with a median survival time of 12 months. Split-course technique is a useful radiotherapy treatment in stage IV head and neck cancers to distinguish between the subset of patients who would require curative treatment and who would not.

Key words : *Split-course radiotherapy, head and neck cancers.*

Introduction

Head and neck cancers account for 5% of all malignancies world over, but the incidence is very high in some part of Asia which may be as high as 20% (1). The management options of cancers in these sites are surgery, radiotherapy or combination of surgery and radiotherapy. The 5-year cure rates by surgery or radiotherapy alone in their early stages (stage I & II) range from 70% to 90%. In late stages (stage-IV), the 5-year cure rate drops significantly to less than 10% (2). The poor results of head and neck cancers at the late stages are basically due to the large tumor volume, high intra-tumoral hypoxic cell fraction, and associated co-morbid medical illness, which complicate tissue tolerance. In general, palliative radiotherapy is recommended to treat majority of stage IV head and neck cancers. The aim of palliative radiotherapy is to relieve symptoms due to large tumor, infiltration to other structures, and control of discharge and bleeding. The palliative radiotherapy induced local control should be maintained for at least a reasonable period of time.

The standard radiotherapy dose schedule for palliation is 30 Gy in 10 fractions over a 2-week period. The above dose of radiation is supposed to

Table-1: Characteristics of study patients

Number of patients	***26***
Male : Female	***21:5***
Age	***44 years (19-77 years)***
Sites of primary (numbers)	***nasopharynx (11)***
	metastatic nodes (4)
	laryngopharynx (5)
	maxilla (2)
	tongue(2)
	palatine tonsil(1)
	cheek(1)
RT dose	***Phase-I 30Gy/10#/2 wks***
	Phase-II 30 Gy/15#/3 weeks
Response rate	***CR 54%***
	PR 23%
	NR 23%
Tumor regression rate	***mean 4 weeks***
Actuarial 12-month survival	***76%***

give durable tumor control at the local site. The response to radiation in squamous cell cancers of the head and neck varies due to many tumor related factors. Split-course radiotherapy is basically used in advanced cancers to differentiate between well-responding tumors from poorly responding tumors in various regimes and intervals. Previous studies in the past had tried split-course radiotherapy in lung cancer patients to differentiate between well-responding tumors from poorly responding tumors (3-4). Subsequently split-course technique has been used in head and neck cancer patients as an alternative to conventional fractionation (5-6). This technique of radiotherapy was found to be useful in bladder cancer, lung cancer and glioma of the brain (7-9). There are various fractionation schemes utilized for split-course radiotherapy including multiple doses a day schemes (10). Despite criticism for the use of split-course technique, current evidence shows that split-course technique is radiobiologically sound and produce similar results as conventional radiotherapy with less number of fractions and increased patient compliance (6,11). Here we would like to present our results of split-course radiotherapy in head and neck cancers.

Materials and Methods

The study was carried out between November 1998 to October 1999 in the division of Radiotherapy and Oncology of Hospital Universiti Sains Malaysia. Patients were subjected to selection criteria. Inclusion criteria were: patients with squamous cell carcinoma, age between 15 to 75 years, and Eastern Cooperative Oncology Group (ECOG) performance status between 1-3 were entered into the study. Patients with co-morbid illness like diabetes mellitus, connective vascular diseases, prior radiotherapy and chemotherapy were excluded from this study. A detailed demographic profile was recorded in a special form. The primary tumor was evaluated by either clinical estimation of the largest tumor extent in three dimensions or by the help of endoscope procedures. The neck nodes were measured in three dimensions before starting radiotherapy.

Classical two-field or three-field techniques was used to plan patients for radiotherapy. Radiotherapy was delivered in two phases. The phase-I consisted of 30 Gy in 10 fractions over 2 weeks (Time Dose Fraction [TDF] 62) and phase-II consisted of 30 Gy in 15 fractions over 3 weeks (Time Dose Fraction [TDF] 49). The dose was calculated at mid-plane for parallel-opposed fields and at 3-cms depth for direct anterior port to the lower neck. Wherever possible thermoplastic immobilization cast was used for accurate dose delivery and day-to-day reproducibility. In multifield techniques, treatment-planning computer was used to obtain optimal dose homogeneity. Measurable tumor volume was recorded in three dimensions on day-5 and day-10 of the phase-I radiotherapy course. After the completion of phase-I radiotherapy, the tumor volume (response) was again recorded at the

4th week. Patients achieving good response (complete response and some partial response with favorable performance status) were advised further radiation in phase-II. The response was classified as complete response (CR) if the regression was complete, partial response (PR) if the response was between 50-90%, and no response (NR) if the response was sub-optimal. The treatment related parameters and response to radiotherapy were analyzed and survival estimated using Kaplan-Meier survival analysis.

Results

Twenty-six head and neck cancer cases were eligible for this technique of radiotherapy. There were 21 men and 5 women with a median age of 44-years (range 19-77 years). All patients received the phase-I schedule of radiotherapy. Following phase-I radiotherapy, 16 (61%) patients achieved favorable response (all CR plus good PR cases) and recommended for further radiotherapy (phase-II) up to a radical dose (Fig 1a & Ib). There was a gradual trend of regression of tumor with a median regression period of 4-weeks (Fig-2). Complete response [CR] was observed among 14 (54%) patients, partial response [PR] in 6 (23%) patients, and no response [NR] in 6 (23%) patients (Fig-3). Seventeen (65%) patients showed improvement in the general well being with better Eastern Cooperative Oncology Group (ECOG) performance status. The follow-up period ranged between 4 to 20 months with a median survival time of 12 months (95% CI 8.22). Patients achieving CR showed better local control duration than PR or NR (Fig-3). Initial bulky tumor volume predicted a poor outcome. The 12-month actuarial survival rate was 76 % (Fig-4).

Discussion

In our study we observed a favorable outcome in split-course radiotherapy technique. From the 26 patients who were subjected to phase-I radiotherapy, 61% patients showed good response and subsequently received further radiotherapy up to radical dose. The response rate of tumors was 54% CR, 23% PR and 23% NR. Patients with no response developed disease progression or death by other intercurrent illness. The survival of stage IV head and neck cancers following full course of

Fig.1a: A case of advanced head and neck cancer with ulcerated right cervical lymph node

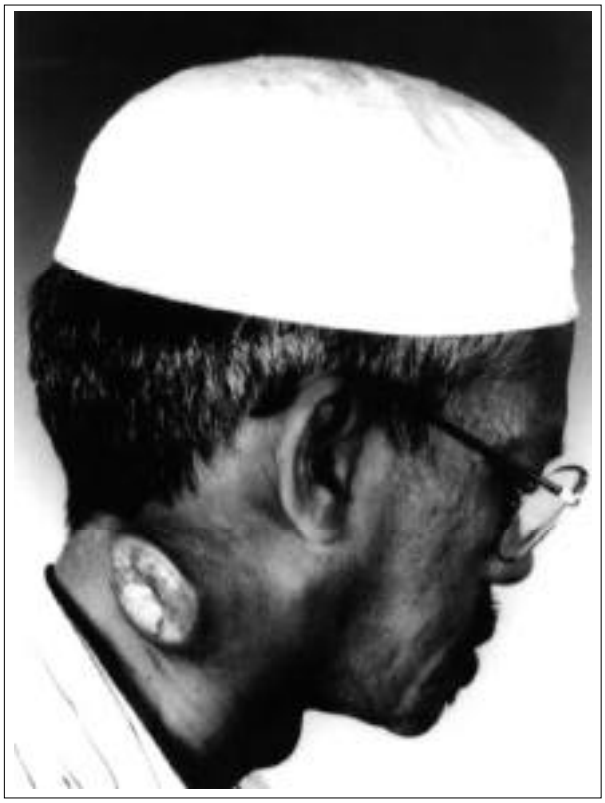
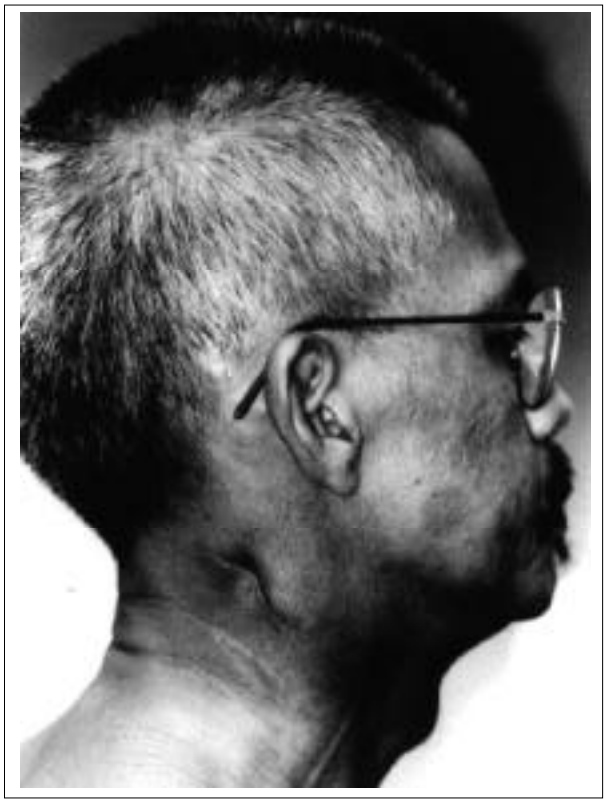


Fig.1b: Eight months following split-course radiotherapy patient achieved good local control of disease with healing of the ulcer

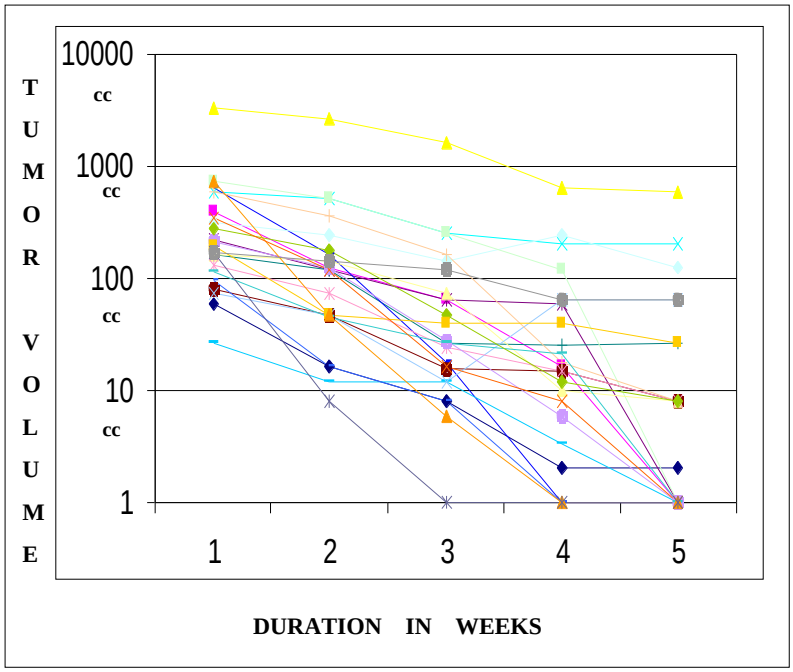


radiotherapy vary considerably from site to site and range from 10% to 30% at 5-years(2). The maximum number of treatment failures were encountered within first two years of follow-up. Better 5-year results were achieved with combined modality methods (12).

Fletcher et al introduced the principle of split-course radiotherapy for the treatment of advanced lung cancers (13). High-risk patients with non-small cell lung cancers (NSCLC) were recommended short-course of hypofractionated radiotherapy (30 Gy in 10 fractions over 2 weeks) and radiation response were reassessed after 4-8 weeks. Patients showing favorable response were subjected to further radiotherapy (30 Gy in 10 fractions) (8,13). This principle has been used in other tumor sites such as carcinoma of esophagus, anal canal cancer and brain gliomas (9,14-15). The conversion rate that proceeds from palliative to radical intent was up to 50%. In our study we found a conversion to radical intent was 61%, which allowed us to deliver radical dose of radiation.

In head and neck cancers, split-course radiotherapy is delivered in conventional fraction size (i.e. 180-200 cGy/fraction/day) with a mid-course split, or a rest period of 2-3 weeks. To reduce acute reactions, the overall treatment time of split-course radiotherapy is lengthened. Another split-course approach includes rapid fractionation of 3 Gy/fraction/day for 10 days with a 2 week split, followed by 3 Gy/fraction/day for another 10 days. The overall treatment course is approximately the same as conventional fractionated radiation therapy. Clinical experience indicates a lower control from split-course approach compared with continuous conventional fractionation radiotherapy (3-4). The inferior results are probably due to excessive repopulation and regeneration of the tumors, as the treatment interval was considerably prolonged. Rapid split-course radiotherapy may produce comparable local control rate as conventional fractionation, but at the cost of severe complications (16). The initial split-course trials were conducted with conventional fractionation and the inter-radiotherapy interval was about 4 weeks, which probably could give rise to increase in the number of resistant clonogens. Regarding treatment with high dose per fraction, it is very clear from the Christie Hospital, Manchester experience that radiation in higher dose per fraction delivered in conservative field techniques can result in good local control rate with minimal complications. Other studies by Barton et al showed the overall interval more than 4-6 weeks were compatible with tumor resistance (17). In split-course technique, the radiation was delivered immediately after establishing complete or good partial response within 4-weeks. Hence if we consider appropriate interval, field plan for radiation, and dose per fraction properly, then split-course radiotherapy technique could prove most effective in advanced head and neck cancers.

Fig.2: Graph showing pattern of tumor regression following radiotherapy



The principles of split-course radiotherapy in head and neck cancers have been blamed for the development of resistant clonogens due to increase in the hypoxic cell component. A recent study by Jund et al (11) showed a tendency of decrease in the hypoxic cell fraction after the respite. The main factor for resistance is the interval between two phases of radiotherapy course (17-18). Recently split-course radiotherapy is being used along with chemotherapy and altered fractionations (19).

The main aim of split-course radiotherapy in stage IV epidermoid cancers of the head and neck is to achieve good palliation, which was noticed among 65% of our patients. Secondly the conversion of intent of radiotherapy from palliative to radical intent was up to 61%. With this experience we suggest that split-course radiotherapy is a simple therapeutic test which should be tried in advanced head and neck cancers selected for radiotherapy. However in future, a combination of concurrent chemotherapy and altered fractionation might improve the outcome of this poor prognostic group of patients.

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Fig.3: Relation between response and survival in months

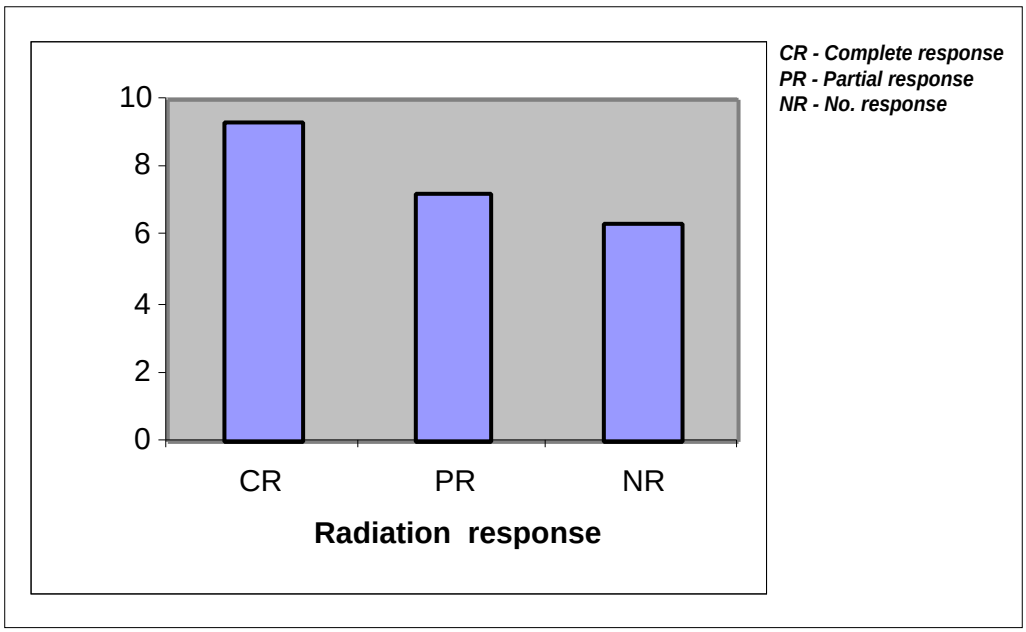
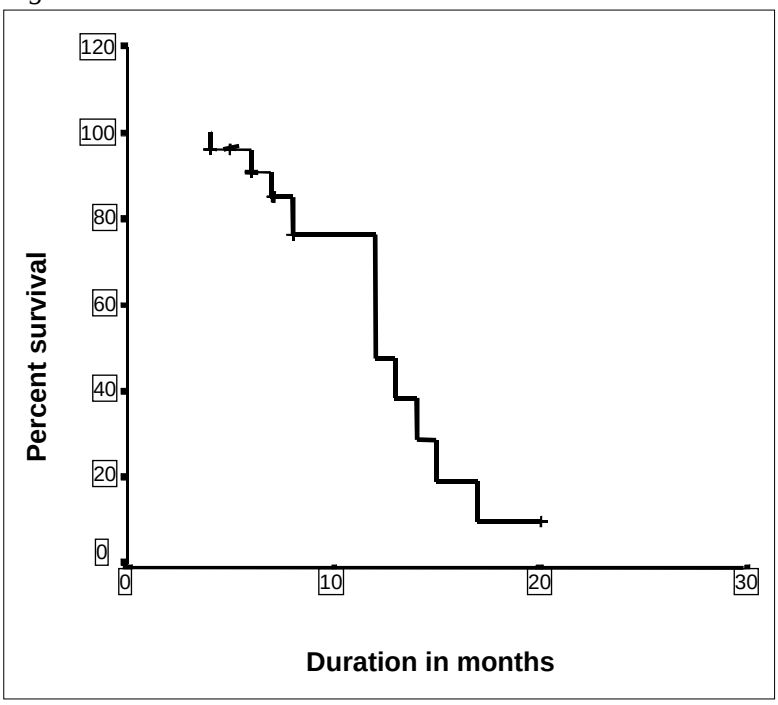


Fig.4: Actuarial survival curve



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