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Pedunculated growth of the thigh due to *Brugia Malayi* infection in a lady

Courtesy of Professor Dato' Khirul Anuar

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Title page: The title page should contain a concise title of the article. It should identify all the authors, the name(s) of the institution(s) and their full addresses where the work was carried out. The initial and address of the corresponding author should also be indicated.

Abstract and Keywords: The second page should contain an abstract of about 150-200 words. It should state the purpose of the study, a brief description of the procedures employed, main findings and principal conclusions. Three to ten key words should also be listed below the Abstract.

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References: Number references consecutively in the order in which they are first mentioned in the text. References in the text should be indicated by a figure within parenthesis. The titles of journals in the list should be abbreviated according to the Index Medicus. Authors are responsible for the accuracy of all references. Examples of correct forms of references are given below:

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PROBLEM BASED MEDICAL CURRICULUM

The traditional Subject Centred Curriculum cannot cope with the exploding biomedical knowledge. The curriculum is not able to accommodate bits and pieces of information accumulated in biomedical sciences over the years into a basic medical curriculum. In ancient times medical students learned the practice of medicine by being apprenticed to a practising doctor. Later, with the expansion of specialists, students took part in each discipline to receive instruction. Much of what we find today, in Medical Sciences, are comparatively young disciplines. What was once Anatomy has splintered into normal Anatomy and morbid Pathology. Pathology later gave birth to Physiology, which subsequently produced Biochemistry and Pharmacology. Infectious diseases that were nonexistent a few decades ago, have now fragmented into Parasitology and Microbiology.

'The sequence of the medical curriculum which is in practice in the old schools, is thus a result of convenient arbitrary decisions, and is not based upon the needs of training physician'

The Flexner Report on 1910, which focused on university based medical education, emphasised the strengthening of departments at the expense of students' programmes. The discomfort with the Subject Centred Curriculum was perceived in the USA as early as 1956 when Case Western Reserve Medical School in Cleveland commenced its integrated curriculum. This was followed by gradual changes in more than 60 medical schools in the USA. In 1967, the General Medical Council of the U.K. recommended flexibility in curriculum planning. This resulted in the evolution of schools with completely new integrated curricula, as in Southampton and Newcastle-Upon-Tyne, in Britain. Even some of the old schools of the University of London have made major departures, as seen in the Clinical Curriculum of Royal Free School of Medicine. This trend was followed by the newer schools in Canada in McMaster and Newfoundland, the University of Newcastle in Australia and Mastricht in the Netherlands.

Medical Schools in Asia are no exception, changes in the curriculum with few adopting the integrated curricula, as in Taiwan University, Napal, Jasira in Sudan and Arabian Gulf University in Bahrain. In the Southeast Asian countries, Malaysia took the lead and introduced integrated curriculum at the Unversiti Sains Malaysia's School of Medicine in 1982. Soon other countries in this region made changes to their curriculum. To name a few, the University of Hong Kong, Tokyo Women's University in Japan, Universitas Gadja Mada in Jogjakarta, University of the East Ramon Magsaysay Memorial Medical Center in the Philippines, Prince of Songkla University and Chulalongkorn University in Thailand and finally the National University of Singapore in 1998. I have come to realize that there are many more medical schools that have such plan in their pipeline.

The Integrated Curriculum has icons that enable the students to achieve their learning objectives. The icons are that the curriculum is spiral in nature, student centred, integrated, community based, with elective programme and problem based. Problem based curriculum is based on an integrated approach to medicine, which represents an alternative to studying blocks of classified knowledge in an organised sequence. The learning process could be analytically considered to occur in three stages; acquisition of understanding, retention, and transferability to new setting.

Acquisition of understanding takes place immediately during the learning activity. Retention of the material learned is a real problem in medical education. Research has shown that the forgetting of isolated facts in anatomy is no different from that of the forgetting curve obtained with nonsense syllables. Transfer can occur most effectively if what is learned, be it a cognitive or a motor skill, is rendered meaningful to the learner; that is, that it articulates clearly with what the learner already knows and can be made to fit into his organization or structure of knowledge. If meaningful learning leads to positive transfer more effectively than rote learning, it follows that the most stable objects of instruction are concepts, principles and general strategies rather than isolated facts. To optimise transfer they must be practised well in a variety of situations. The more closely the learning condition can simulate the actual application setting, the greater the likelihood of success.

Since problems in medicine are primarily those of individual patients, most problem situations relate to an individual clinical case. In this way health professionals learn in real life. It is also argued that problem solving and clinical reasoning skills can be better achieved by this method. It comes to my mind that problem based learning can be given in the curriculum in multitudes of ways. Real patients computerised patient problems, problem simulations can be used to assist this method of learning; even the traditional lecture and discussion methods could be modified to accommodate this approach.

The problem based learning consists of more than simply learning around clinical problems. It is a fundamental intellectual process that can be applied to physiological problems in research laboratories, to problem of family dysfunction and to issues relating to health care in the community or even ethical issues.

Whatever we may say, but the next millennium would see more medical schools adopting the integrated curriculum and problem based learning would be an essential tool in the achieving of the teaching objectives. Although University of Malaya Medical centre has adopted an integrated curriculum but it is not problem based but rather Scenario Initiated Learning (SIL). Similarly many institution has moved away from total PBL curriculum (except for McMaster) due mainly to maintain enthusiasm among the teachers of medicine and meanwhile Scenario initiated learning has become more convenient. Some schools of medicine use as much as 40% PBL while Singapore adopted 20% of PBL into their infant integrated curriculum. What about University Malaya? 10%, 20%, 30% your guess would be as good as mine. Whatever percentage of PBL we adopt, the success of the programme will depend on the Commitment of the Academic staff as this programme would be labour intensive and a herculean job to sustain interest for both the academics and students. In the implementation of the integrated curriculum teachers of medicine, students need a high commitment so that the programme would be successful. What transpired at the 1st Asia Pacific Conference on Problem Based Teaching held in Singapore was loud and clear, the commitment of the institution and policy makers is utmost important to ensure the success of this innovative curriculum.

Professor Dato' Dr. Khairul Anuar b. Abdullah
Editor

STRESS AND HYPERTENSION

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Introduction

Hypertension is a major cardiovascular risk factor that directly contributes to myocardial infarction, cerebrovascular accidents, congestive cardiac failure, peripheral vascular disease and premature mortality (1). In the great majority of cases, no cause can be found and this form of hypertension is termed 'primary' or 'essential' hypertension. Various theories have been put forward and the causes can be divided broadly into genetic and environmental factors. Based on twin studies, it was estimated that about 50 percent of blood pressure variance is genetic (2). The main environmental causes are dietary and psychosocial factors. In this review, we will concentrate on the latter while noting that there is still disagreement about the role of a dietary factor, sodium chloride, in causing hypertension. A meta-analysis of trials of sodium restriction concluded that sodium intake had no influence on blood pressure in normotensive subjects and in hypertensives below 45 years of age (3). We will also discuss an interesting phenomenon known as 'white coat hypertension'.

Evidence that stress causes hypertension

While psychological factors are suspected in the development of hypertension, conclusive evidence is still lacking. There are three ways in which the roles of environmental stress or blood pressure can be assessed: animal experiments, laboratory studies and field studies.

Henry and Stephens (4) pioneered the work on stress-induced hypertension in animals. They conducted a series of experiments in mice housed in colonies which were designed to promote social interaction and conflict. It was observed that the dominant mice developed higher pressures than the subordinates. The subdominant animals were thought to show a chronic defense reaction characterised by continuous sympathetic nervous system activation. The subordinates exhibited the defeat reaction in which there was pituitary-adrenocortical axis activation. Henry and Stephens proposed two distinct response types which they referred to as the defense and defeat reactions. While negative health consequences were postulated for both, they proposed that only the defense reaction is associated with the development of hypertension.

Not surprisingly, there are only a few comparable studies in humans. A situation somewhat similar to the social interaction of mice in population cages was reported by D'Atri and Ostfeld (5) who studied men confined to prison. The dormitory occupants had an average systolic pressure of 131 mmHg whereas that of those living in single-occupancy cells was only 115 mmHg. Furthermore, transfers from single cell to a dormitory caused the blood pressure to rise (6). These changes are not thought to be due to diet as all inmates ate the same food.

Blood pressure tends to rise with age. However, this is not an invariable phenomenon and studies have shown that blood pressure can remain low throughout life. The change of blood pressure with age appears to be determined culturally rather than genetically. A good example of this is reported by Timio *et al* (7). It is a 20-year observational study of Italian nuns living in a secluded order. The nuns were compared with a control group both at entry and after 20 years. Blood pressures were the same at entry but by the end of the study the systolic blood pressure was approximately 30 mmHg in the controls than in the nuns. The differences could not be explained by changes in body weight, by diet or by childbearing. It was concluded that the differences were due to the monastic and relatively stress-free environment.

Similar observations have been made in people who migrate from a stable traditional society to a Westernised one. Studies of the bushmen of the Kalahari (8) and the nomadic Samburo of Kenya (9) have shown no increase of blood pressure with age. Bushmen who abandoned their traditional lifestyle, however, and become farm labourers, or even prisoners, have blood pressures 15 mmHg higher than the nomads (10). Samburo warriors who joined the Kenyan army also showed an increase of blood pressure (11). Many other studies confirm the effect of acculturation from traditional societies to contemporary Western life but the primary problem with almost all of them is that it is difficult to know what factors were responsible for the rise of blood pressure. While stress may be one of them, there are also major dietary and other lifestyle changes associated with the transition between cultures. Nevertheless, these studies suggest that there is something about modern society that tends to elevate blood pressure. To investigate this further, Waldron *et al.* (12) collected data from 84 different societies and concluded that higher blood pressures were associated

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with increased emphasis on a market economy, increased economic competition and decreased family ties. These associations appeared to be independent of salt intake and, in men, of obesity; but again, the way in which such cultural factors might impact on individuals to alter their blood pressures remains unclear.

A number of epidemiological studies have shown that blood pressure is related to one's position in society, with people of lower socioeconomic status and less education having higher blood pressures (13,14). However, this relationship was dismissed in other major studies such as the United States Health and Nutrition Survey (HANES III) (15) and a Copenhagen County study (16).

Stress and disease models

A convenient model, as described by Pickering TG (17), for defining the roles of psychological factors is shown in Fig. 1. The magnitude of the response is thought to be determined by the interaction of three things:The degree of arousal in the central nervous system engendered by the stimulus, the individual's ability to cope with the stressor and the physiological susceptibility.

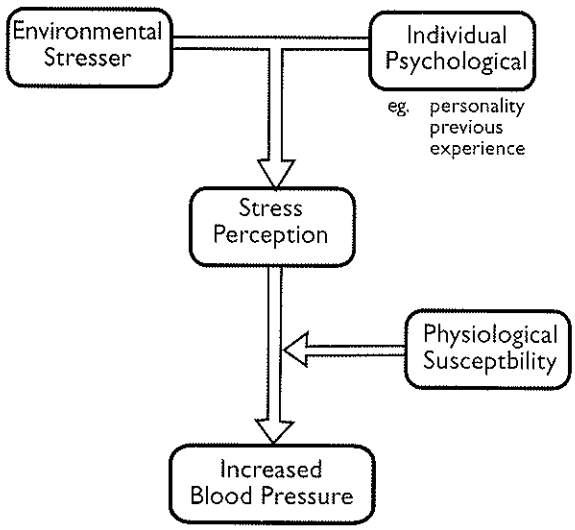

Fig. 1. A tripartite model of stress and disease

To qualify as a stressor, the stimulus must tax, exceed or threaten to exceed the adaptive capacity of the individual. The work setting is thought to be one of the most important sources of stressors in modern society. Others are the family, the site of marital discord and care-giving responsibilities. Various studies of blood pressure and specific occupations (with excessive demands) have been done (18-20) to see if there was a definite relationship but no clear picture has emerged. This is because of the difficulty in determining what characteristics of the work situation adversely affect incumbent's health either because specific characteristics are not assessed or because of a relative lack of variability within the occupation.

Karacek *et al.* (21) developed a job-strain model specifically to evaluate occupational stress. It has two orthogonal components which are psychological demands and decision latitude. The former is a measure of control while the latter is a measure of job control or autonomy. The most stressful jobs are those that are perceived to combine high demands and low decision latitude (Fig. 2). This combination is otherwise known as 'high-strain'. Studies (21-23) have shown the close association between job strain and the development of significant coronary heart disease leading to myocardial infarction. Hypertension is probably the most likely mechanism by which job strain might cause coronary heart disease (24).

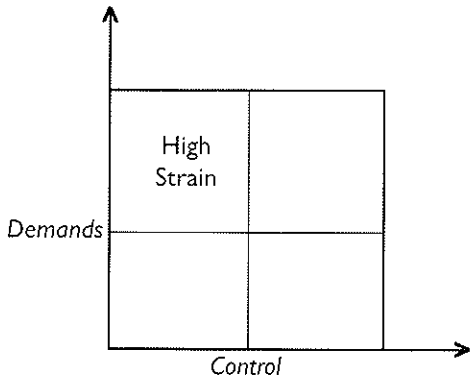

Fig. 2. The demand-control model

In a case-control study of men employed in a variety of jobs, it was found that hypertensive individuals were approximately three times as likely to be employed in high-strain jobs as the normotensive controls (25). Exposure to job strain was also associated with an increased left ventricular mass, which would be consistent with the effects of a sustained elevation of blood pressure from regularly occurring stress.

A closely related model is the effort-distress model of Frankenhaueser (26). This also has two orthogonal components (see Fig. 3), which are termed effort and distress. Effort is conceived as arousing the sympathetic

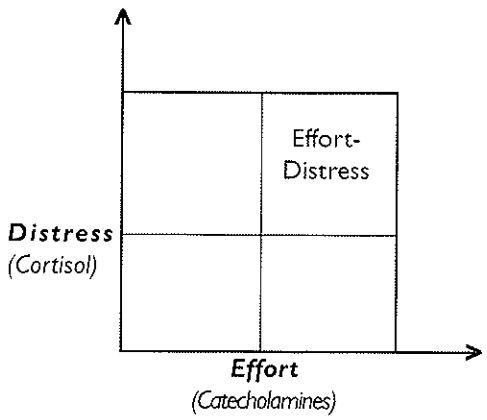

Fig. 3. The effort-distress model

nervous system, and distress is that of the adrenocortical system. While effort is needed to meet or respond to most demands, distress is experienced only when the demands are perceived as excessive, threatening or otherwise unpleasant.

Another model is the concept of lifestyle incongruity which was described by Dressler (27) as the extent to which a high-status lifestyle exceeds the norm for an individual's occupational class, which means to say living beyond one's means. It has been associated with increased blood pressure not only in developing countries (28,29), but also in American blacks (30).

Job strain and women

Pickering *et al.* (17) studied 80 women in their work site and showed that job strain had little effect on blood pressure in women. This is somewhat surprising as women are more likely than men to be employed in high-strain jobs. A study by James *et al.* (31), however, proved to the contrary. They made ambulatory blood pressure recordings in 50 normotensive working women doing technical and clerical jobs and they found that the blood pressures were elevated and were highest at work followed by at home and lowest during sleep. The most powerful behavioural predictor of systolic blood pressure was the perception that one's job is stressful, which was associated with higher pressures in all three situations: At work, at home and during sleep. An observation was made in the Framingham study that women employed in clerical jobs who also had children and were married to men with blue-collar jobs, had the highest incidence of coronary heart disease (32). A recent case-control study reported that women exposed to high job strain are at higher risk of developing preeclampsia and, to a lesser extent, gestational hypertension (33).

Pathophysiology of stress-induced hypertension

Evidence is amounting for the role of increased sympathetic nervous system activity in the early stages of essential hypertension (34,35). This raises the question of what is the main reason for this sympathetic overactivity. While it may be genetic, one has to look at the environmental aspects, and psychological stress is definitely a prime candidate, as discussed earlier. Stress can cause a transient rise of blood pressure. However, how does one explain its role in the pathogenesis of established hypertension since the latter is primarily a disorder of the tonic regulation of blood pressure rather than its short-term variability?

There are a number of theories and some of them are discussed below.

The adrenaline hypothesis

Studies have linked adrenaline with the development of stress-induced hypertension (36,37). It has been shown that infusion of adrenaline at low doses, which is equivalent to the levels seen during naturally occuring stress, can enhance noradrenaline release from sympathetic nerve terminals (38). This effect is thought to be mediated by prejunctional β2 receptors, since it can be blocked by β-blockers. Furthermore, circulating adrenaline may be taken up by sympathetic nerve terminals, stored with noradrenaline as a co-transmitter, and released with it during sympathetic nerve stimulation. There are two essential components to this mechanism. First, the release, re-uptake, and presynaptic facilitation of noradrenaline release acts as a positive feedback loop. Second, although the half-life of adrenaline in plasma is only a few minutes, if adrenaline is stored in sympathetic nerves, it may last for many hours. This is important in providing sustained effects. A schematic diagram is illustrated below (Fig. 4) .

Human studies have implicated this mechanism. An acute adrenaline infusion produces tachycardia with an increased systolic and slightly decreased diastolic pressure. When the infusion is terminated, the plasma adrenaline level returns to normal although the tachycardia and increased systolic pressure persist for an hour or two and diastolic pressure rises to above baseline levels (39). Adrenaline infusion can also enhance the pressor response to endogenous sympathetic stimulation, such as occurs during isometric exercise and the cold pressor test (40).

The delayed pressor effect of adrenaline was well demonstrated in a study conducted by Blankenstijn *et al.* (41) who infused adrenaline, noradrenaline, or dextrose for 6 hours (from 10am to 4pm) in normal volunteers, and monitored the effects on blood pressure over the next 16 hours using intra-arterial blood pressure monitoring. The pressure was first reduced by adrenaline but by the end of the infusion was above the baseline value and remained elevated throughout the night. Noradrenaline infusion produced an initial elevation of pressure but no sustained effects. The pressor effect of adrenaline was most marked during periods of increased sympathetic activity, ie. when the subject is active. This increased pressure effect was not accompanied by any changes of heart rate.

Structural changes in heart and resistance vessels

Peripheral resistance is increased in patients with sustained hypertension. This observation is thought to

be due to medial hypertrophy and can be regarded as an adaptive process in the presence of increased pressure and flow. It is unclear as to the extent to which stress can produce such changes but there is evidence that the growth of vascular smooth muscle can be influenced by a number of stress-related factors, including angiotensin, catecholamines and corticosteroids (42).

The role of glucocorticoids

The effects of glucocorticoids on blood pressure are complex and not well understood, although there is agreement that they tend to have a pressor effect (43). It plays a vital role in Frankenhaeuser's effort-distress model (26) where increased cortisol is found in the high-effort-high-distress situation. Whitworth *et al.* (44) gave four different synthetic glucocorticoids, with little or no mineralocorticoid effect, to normal subjects for five days. All four raised the blood pressure without any accompanying sodium retention.

White coat hypertension

"Doctor, why is my blood pressure always high whenever I visit you in clinic but is normal at home?"

This frequently asked question somewhat defines the phenomenon called 'white coat hypertension'. It is the finding of persistently raised blood pressure in the doctor's clinic but normal at other times. It is generally thought to be attributable to acute stress in the presence of a doctor and since the very basis for the initiation of treatment in hypertension are the clinic pressure readings, it requires more than a mere mention in this chapter. A few intriguing questions have been asked by clinicians through the years. Among others are: What is the aetiology? Is it a benign condition? Does it need to be treated?

Prevalence and demographic factors

The prevalence of white coat hypertension is perhaps higher than is generally thought. Pickering *et al.* (45) reported that 21 percent of patients with borderline hypertension (clinic diastolic pressures between 90 and 104 mmHg) studied had both systolic and diastolic pressures which were below this level during the ambulatory blood pressure monitoring. For patients with more advanced hypertension (clinic diastolic pressure above 105 mmHg), the prevalence was less at 5 percent. It has to be pointed out, however, that the criteria used to define white coat hypertension do vary between centres and as such will be a major determinant of its prevalence. Ambulatory blood pressure monitoring has been conventionally used to diagnose white coat hypertension. However, mental arithmetic is also a useful diagnostic evaluation in this condition (46).

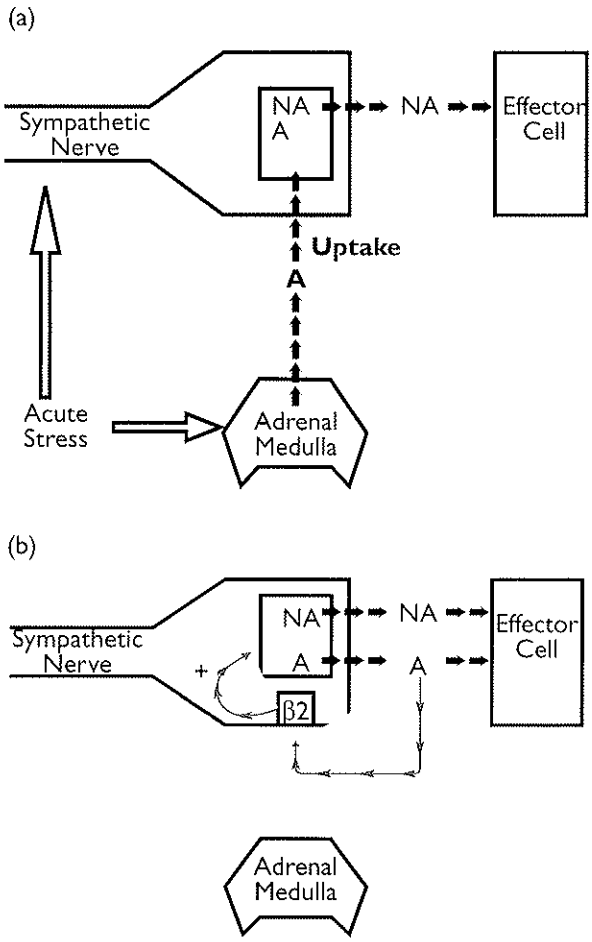

Fig. 4. Postulated mechanism for explaining the delayed pressor effects of adrenaline. **(a) Acute effect.** Adrenaline (A) is released by adrenal medulla in response to stress where it is taken up by sympathetic nerve terminals. **(b) Delayed effect.** There is gradual release of adrenaline as a co-transmitter, which enhances noradrenaline (NA) release via presynaptic β2-receptor stimulation

White coat hypertension has been found to be more common in women than men (45,47,48) although some studies have reported equal incidents in men and women (49,50).

White coat hypertension can occur at any age. It has been reported to be common in children (51) and in those over the age of 65 (52). Lerman *et al.* reported that their patients with white coat hypertension were on average nine years older than their sustained hypertensives (50). It was observed that women had white coat hypertension at a greater age than did men (53).

Psychological factors and white coat hypertension

It might be expected that white coat hypertensives would generally be more anxious but this is not necessarily the case. Laughlin *et al.* (54) reported no

correlation between the clinic and home differences. White coat hypertensives were found to have less anger than sustained hypertensives but did not differ on measures of anxiety, health worry or health stress (50).

The influence of a doctor's presence on the patient's blood pressure was well demonstrated by the study of Mancia's group (55) using continuous intraarterial pressure recording in hospitalised patients. There was an immediate rise of pressure when the doctor approached the patient and put a cuff around the arm. This lasted throughout the procedure of taking a reading and only returned to baseline over a period of several minutes. Repeat visits by the same doctor did not show any habituation of this response. In another similar study, the average change of pressure evoked by the doctor was 23/18 mmHg, approximately twice as high as when the pressure was taken by a nurse (56).

Sympathetic nervous activity in white coat hypertension

Evidence is growing that sympathetic nervous activity is normal in white coat hypertensives. Saito *et al.* (57) found that the patients with white coat hypertension had normal 24-hour urine noradrenaline and adrenaline, whereas the patients with sustained hypertension had elevated levels. This finding of whole-day sympathetic overactivity in sustained hypertension was recently replicated by Pierdomenico *et al.* (58). It can, therefore, be accepted that these two conditions differ in their pathophysiologic background.

Mechanism underlying white coat hypertension: The conditioned response

A number of mechanisms explaining white coat hypertension have been postulated. The first is the exaggerated or orienting response and therefore a generalised hyperreactivity to novel or stressful stimuli. However, the bulk of evidence does not support the view that it represents a generalised hyperreactivity (59,60). The second is that it is a precursor of sustained hypertension. While this cannot be excluded, the fact that it tends to be more, rather than less common in older patients would argue against this. The third mechanism is the learned or conditioned response.

In this third phenomenon, white coat hypertension is thought to originate as part of the defense reflex which later becomes perpetuated through classical conditioning (61). For example, after a patient is told by the doctor that his pressure is a matter of concern, the patient learns to associate the doctor as the harbinger of bad news, who then becomes a conditioned stimulus that continues to elicit the pressor effect response in this patient. This is in contrast to the normal habituation, which is the diminution of the alerting response to repeat visits.

Is white coat hypertension a benign condition?

Opinions vary as to the significance of white coat hypertension. The majority of investigators believe that it represents a benign entity whereas others have suggested that the risk in this condition is similar to that of patients with sustained hypertension. Since it has important prognostic and management implications, we will discuss the evidence for the two views.

Perloff *et al.* (62) conducted a prospective study of morbidity related to ambulatory pressures. They found that patients whose ambulatory pressure was low relative to their clinic pressure were at lower risk than those with higher ambulatory pressures. In a study of patients with borderline hypertension, cardiovascular morbid events was noted in 2.1 percent of patients with white coat hypertension and 4.4 percent in those with sustained hypertension (63). Floras *et al.* (64) reported an incidence of target organ damage in 64 percent of patients with sustained hypertension but only in 19 percent of white coat hypertensives.

However, a number of studies have since emerged that contradict the assumption that white coat hypertension is a harmless condition. Muldoon *et al.* (65) found that this subset of patients had evidence of carotid artery atherosclerosis and that the number was greater than the normotensives but equal to that of the subjects with sustained hypertension. White coat hypertension also a risk factor for left ventricular hypertrophy (66). Even in the absence of structural heart disease, in contrast to normotensives, white coat hypertensives are just as likely as sustained hypertensives to get diastolic dysfunction (67). Recently, Strandberg and Salomaa (68) reported a 21-year prospective data of 536 men with cardiovascular risk factors at baseline. Blood pressure was measured both by a nurse and a doctor. The men with a white coat effect (defined here as doctor minus nurse blood pressure) more than 30 mmHg had a significantly higher mortality than other men (relative risk 2.2). Mortality was also higher in the white coat hypertensives (33.3%) than in the normotensives (9.5%), with a p value less than 0.0005.

Treatment of white coat hypertension

Two studies, using calcium channel blockers (69,70) have shown that although the effects of the medication on clinic pressure were similar in patients with both high and normal ambulatory pressure, when ambulatory pressure was normal to begin with (ie., white coat hypertension), the drug did not lower it further. Another study compared nifedipine and enalapril and confirmed that nifedipine had little effect on ambulatory pressure if it was low to begin with (ie., white coat hypertension) while enalapril lowered it whether it started out

low or high (71). This finding suggests that there may be differences between the various classes of antihypertensives in the degree to which they affect white coat hypertension.

As shown above, there is conflicting evidence as to the clinical significance of white coat hypertension. If it is a truly benign condition, then is it justified to subject this group of people with white coat hypertension to drug therapy, which is potentially life long, and incurring the possible side effects along the way? Chrysant (72), in a recent article stated that white coat hypertension was a benign condition and suggested that pharmacologic treatment should be withheld. Instead, treatment should aim at lifestyle modification, moderate salt restriction, weight reduction, regular exercise, smoking cessation and correction of glucose and lipid abnormalities. However, if one chooses to ignore the body of evidence implicating white coat hypertension as a potentially hazardous condition, then would it be right to refrain from treating this condition?

Conclusions

In this review we discussed two distinct entities: Stress-induced hypertension and white coat hypertension. The primary candidate for the development of the former is chronic exposure to stress. Job strain, which focuses on the individual's perception of his or her working conditions, has the advantage over earlier concepts of work stress. It includes not only assessment of the demands of the job, but also how well these demands can be controlled. Job strain is well recognised as a risk factor for coronary heart disease, and it is thought to exert this effect by raising the blood pressure in men but not women. Why there is a sex difference is quite intriguing, and the answer may lie in a different need for control.

The issue of white coat hypertension remains unresolved. Is it a truly benign condition? Is withholding treatment a wishful thinking or is it fully justified? As the prevalence of white coat hypertension is high and as the prognosis and clinical implications are yet uncertain, more studies need to be done to help resolve these issues.

References

1. Levy D, Larson MG, Vasan RS, Kannel WB, Ho KKL. The progression from hypertension to congestive heart failure. JAMA 1996; 275:1557-62.
2. Watt GCM, Harrap SB, Foy CJW, Holton DW, Edwards HV, Davidson RH, *et al.*: Abnormalities of glucocorticoid metabolism and the the renin-angiotensin system: a four-corners approach to the identification of the genetic determinants of blood pressure. J Hypertension 1992; 10:473-82.
3. Midgley JP, Matthew AG, Greenwood CMT, Logan AG: Effect of reduced dietary sodium on blood pressure: A meta-analysis of randomised controlled trials. JAMA 1996; 275:1590-7.
4. Henry JP, Stephens PM: Stress, health and the social environment: A sociobiologic approach to medicine. New York: Springer Verlag: 1977.
5. D'Atri DA, Ostfeld AM: Crowding: Its effects on the elevation of blood pressure in a prison setting. Behav Med 1975; 4:550-6.
6. D'Atri DA, Fitzgerald EF, Kasi SK, Ostfeld AM: Crowding in prison: The relationship between changes in housing mode and blood pressure. Psychosom Med 1981; 43:95-105.
7. Timio M, Verdecchia P, Venanzi S, Gentili S, Ronconi M, Francucci B, *et al.*: Age and blood pressure changes: A 20-year follow-up study in nuns in a secluded order. Hypertension 1988; 12:457-61.
8. Kaminer B, Lutz W. Blood pressures in Kalahari bushmen. Circulation 1968; 22:289-95.
9. Shaper AG. Cardiovascular studies in the Samburo tribe of northern Kenya. Am Heart J 1962; 63:437-42.
10. Turswell AM, Kenelly BM, Hanson JDL, Lee RB. Blood pressures of Kung bushmen in northern Botswana. Am Heart J 1972; 84:5-12.
11. Shaper AG, Leonard PJ, Jones KW, Jones M: Environment effects on the body build, blood pressure and blood chemistry of nomadic warriors serving in the army in Kenya. East Afr Med J 1969; 46:282-9.
12. Waldron I, Nowotarski M, Freimer M, Henry JP, Post N, Witten C. Cross-cultural variation in blood pressure: A quantitative analysis of the relationship of blood pressure to cultural characteristics, salt consumption, and body weight. Soc Sci Med 1982; 16:419-30.
13. Luepker RV, Rosamond WD, Murphy R, Sprafka JM, Folsom AR, McGovern PG, Blackburn H. Socioeconomic status and coronary heart disease risk factor trends. Circulation 1993; 88:2172-9.
14. Jacobsen BK, Thelle DS. Risk factors for coronary heart and level of education. Am J Epidemiol 1988; 127:923-32.
15. National Heart, Lung and Blood Institute. Chartbook on National Data on Socioeconomic Status and Cardiovascular Disease. Washington DC: NHBLI; 1995.
16. Moller L, Kristensen TS, Hollnagel H. Social-class and cardiovascular risk factors in Danish men. Scand J Soc Med 1991; 19:116-26.
17. Pickering TG, *et al.* Environmental influences on blood pressure and the role of job strain. J Hypertension 1996; 14(suppl.5):179-85.
18. Ragland DR, Winkleby MA, Schwalbe J, Holman BL, Morse L, Syme SL, Fisher JM. Prevalence of hypertension in bus drivers. Int J Epidemiol 1987; 16:208-14.
19. Theorell TPG, Ahlberg-Hulten G, Jodko M, Sigala F, Soderholm M, de la Torre B. Influence of job strain and emotion on blood pressure in female hospital personnel during working hours. Scand J Work Environ Health 1993; 19:313-38.
20. Cobb S, Rose R. Hypertension, peptic ulcer and diabetes in air-traffic controllers. JAMA 1973; 224:489-92.

21. Karasek RA, Baker D, Marxer F, Ahlbohm A, Theorell T. Job decision latitude, job demands and cardiovascular disease: A prospective study of Swedish men. Am J Publ Health 1981; 75:694-705.
22. Alfredsson L, Karasek R, Theorell T. Myocardial infarction risk and psychological work environment: An analysis of the male Swedish force. Soc Sci Med 1982; 16:463-7.
23. Karasek RA, Theorell T, Swartz JE, Schnall PL, Pieper CF, Michela JL. Job characteristics in relation to the prevalence of myocardial infarction in the US Health Examination Survey (HESS) and the Health and Nutrition Examination Survey (Haines). Am J Publ Health 1988; 78:910-18.
24. Schnall PL, Landsbergis PA, Baker D. Job strain and cardiovascular disease. Annu Rev Public Health 1994; 15:381-411.
25. Schnall PL, Pieper C, Schwartz J, Karasek R, Schlussel Y, Devereux R *et al.* The relationship between 'jobstrain', work place diastolic blood pressure and left ventricular mass index. JAMA 1990; 263:1919-35.
26. Frankenhaeuser M. The sympathetic-adrenal and pituitary-adrenal response to challenge: Comparison between the sexes. In Biobehavioral Bases of Coronary Heart Disease. Edited by Dembroski TM, Schmidt TH, Blumchen G. Basel: Karger 1983; 91-105.
27. Dressler WW. Hypertension and culture change: Acculturation and disease in the West Indies. South Salem: Redgrave 1982.
28. Dressler WW, Alfonso M, Chavez A, Viteri FE. Arterial blood pressure and individual modernisation in a Mexican community. Soc Sci Med 1987; 24:679-87.
29. Dressler WW, Santos IED, Gallagher PN, Viteri FE. Arterial blood pressure and modernisation in Brazil. Am Anthropol 1987; 89:389-409.
30. Dressler WW. Lifestyle, stress and blood pressure in a Southern black community. Psychosom Med 1991; 53:608-20.
31. James GD, Cates EM, Pickering TG, Laragh JH. Parity and perceived job stress elevate blood pressure in young normotensive working women. Am J Hypertens 1989; 2:37-39.
32. Haynes SG, Feinlieb M. Women, work and coronary heart disease: Prospective findings from the Framingham Heart Study. Am J Public Health 1980; 70:133-141.
33. Marcoux S, Berube S, Brisson C, Mondor M. Job strain and pregnancy-induced hypertension. Epidemiology 1999; 4:376-82.
34. Philipp. Sympathetic nervous activity in essential hypertension: Activity and reactivity. J Cardiosc Pharmacol 1987; 10(Suppl 4): S31-S36.
35. Goldstein DS. Plasma catecholamines in essential hypertension: An analytical review. Hypertension 1983; 86-9.
36. Brown MJ, Macquin I. Is adrenaline the cause of essential hypertension? Lancet 1981; 2:1079-81.
37. Majewski H, Rand MJ. Prejunctional b-adrenoceptor as sites of action for adrenaline in stress-linked hypertension. J Cardiovasc Pharmacol 1987; 10 (Suppl 4): S41-S44.
38. Majewski H, Hedler L, Starke X. The noradrenaline release rate I the anaesthetised rabbit: Facilitation by adrenaline. Naunyn-Schmiederberg's Arch Pharmacol 1982; 321:20-7.
39. Brown MJ, Dollery CT. Adrenaline and hypertension. Clin Exp Hypertens 1984; A6:539-49.
40. Vincent HH, Boomsoma F, Man in't Veld A, Schalekamp MADH. Stress levels of adrenaline amplify the blood pressure response to sympathetic stimulation. J Hypertens 1986; 4:255-60.
41. Blankestijn PJ, Mann in't Veld A, Tulen J, Van den Meiracker AH, Boomsma F, Moleman P *et al.* Support for adrenaline-hypertension hypothesis: 18 hour pressor effect after 6 hours adrenaline infusion. Lancet 1988; ii:1386-9.
42. Lever AF. Slow pressor mechanisms in hypertension: A role of hypertrophy of resistance vessels. J Hypertens 1986; 4:515-24.
43. Whitworth JA. Mechanisms of glucocorticoid-induced hypertension. Kidney Int 1987; 31:1213-24.
44. Whitworth JA, Gordon D, Andrews J, Scoggins BA. The hypertensive effect of synthetic glucocorticoids in man: Role of sodium and volume. J Hypertens 1989; 7:535-49.
45. Pickering TG, James GD, Boddie C, Harshfield GA, Blank S, Laragh JH. How common is white coat hypertension? JAMA 1988; 259:225-8.
46. Nakao M, Shimosawa T, Nomura S, Kuboki T, Fujita, Murata K. Am J Hypertens 1998; 11(1 pt 1):41-5.
47. O'Brien E, Murphy J, Tyndall A, Atkins N, Mee F, McCarthy G, Staessen J, Cox J, O'Malley K. Twenty-four-hour ambulatory blood pressure in men and women aged 17 to 80 years: The Allied Irish Bank Study. J Hypertens 1991; 9:355-60.
48. Khoury S, Yarows SA, O'Brien TK, Sowers JR. Ambulatory blood pressure monitoring in a nonacademic setting: Effect of age and sex. Am J Hypertens 1992; 5: 616-23.
49. Siegel WC, Blumenthal JA, Divine GW. Physiological, psychological and behavioral factors and white coat hypertension. Hypertension 1990; 16:140-6.
50. Lerman CE, Brody DS, Hui T, Lazaro C, Smith DG, Blum MJ. The white coat hypertension response: Prevalence and predictors. J Gen Int Med 1989; 4:225-31.
51. Hornsby JL, Morgan PF, Taylor AT, Treiber FA. White coat hypertension in children. J Fam Pract 1991; 33:617-23.
52. Wittenberg C, Zabludowski JR, Rosenfeld JB. Overdiagnosis of hypertension in the elderly. J Hum Hypertens 1992; 6:349-51.
53. James GD, Marion R, Pickering TG. White coat hypertension. Blood Press Monit 1998; 5:281-7.
54. Laughlin KD, Sherrard DH, Fisher L. Comparison of clinic and home blood pressure levels in essential hypertension and variables associated with clinic-home differences. J Chron Dis 1979; 33:197-206.
55. Mancia G, Bertinieri G, Grassi G, *et al.* Effects of blood pressure measurement by the doctor on patient's blood pressure and heart rate. Lancet 1983; 2:695-7.
56. Mancia G, Parati G, Pomidossi G, Grassi G, Casadei R, Zanchetti A. Altering reaction and rise in blood pressure during measurement by physician and nurse. Hypertension 1987; 9:209-15.

57. Saito I, Takeshita E, Hayashi S, Takenaka T, Murakami M,Saruta T, Nagano S, Sekihara T. Comparison of clinic and home blood pressure levels and the role of the sympathetic nervous systemin clinic-home differences. Am J Hypertens 1990; 3:219-24.

58. Pierdomenico SD, Bucci A, Costantini F, Lapenna D, Cuccurullo F, Mezzet A. Twenty-four-hour autonomic nervous function in sustained and white coat hypertension. Am Heart J 2000; 4:672-7.

59. Cardillo C, de Felice F, Campia V, Folli G. Psychophysiological reactivity and cardiac end-organ changes in white coat hypertension. Hypertension 1993; 21:836-44.

60. Pannarale G, Isea JE, Coats AJS, Conway J, Sleight P. Cardiac and blood pressure responses to mental stress in reactive hypertensives. Clin Exp Hypertens 1991;A13:1-12.

61. Pickering TG. White coat hypertension. In Hypertension: Pathophysiology, Diagnosis and Management. 2nd ed. Laragh JH, Brenner BM 1995; 1913-27.

62. Perloff D, Sokolow M, Cowan RM, Juster RP. Prognostic value of ambulatory blood pressure measurements: Further analyses. J Hypertens 1989; 7(suppl 3):S3-S10.

63. Pickering TG. Ambulatory monitoring and blood pressure variability. London: Science Press 1991.

64. Floras JS, Jones JV, Hassan MO, Osikowska B, Sever PS, Sleight P. Cuff and ambulatory blood pressure in subjects with essential hypertension. Lancet 1981; 2:107-9.

65. Muldoon MF, Nazzaro P, Sutton-Tyrrell, Manuck SB. White coat hypertension and carotid artery atherosclerosis: A matching study. Arch Intern Med 2000; 10:1507-12.

66. Owens PE, Lyons SP, Rodriguez SA, O'Brien ET. Is elevation of clinic blood pressure in patients with white coat hypertension who have normal ambulatory blood pressure associated with target organ changes? J Hum Hypertens 1998; 11:743-8.

67. Glen SK, Elliot HL, Curzio JL, Lees KR, Reid JL. White coat hypertension as a cause of cardiovascular dysfunction. Lancet 1996; 354(9028):654-7.

68. Strandberg TE, Salomaa V. White coat effect, blood pressure and mortality in men: Prospective cohort study. Eur Heart J 2000; 21(20):1714-8.

69. Weber MA, Cheung DG, Grettinger WF, Lipson JL. Characterisation of antihypertensive therapy by whole-day blood pressure monitoring. JAMA 1988; 259:3281-5.

70. Lacourciere Y, Poirier L, Dion D, Provencher P. Antihypertensive efect of isradipine administered once or twice daily on ambulatory blood pressure. Am J Cardiol 1990; 65:467-72.

71. Ashida T, Abe H, Kawano Y, Yoshida K, Kimura G, Kojina S, Yoshim H, Kawamura M, Imanishi M, Sanai T, Kuramochi M, Omae T. Effects of nifedipine SHR and enalapril on office, home and ambulatory blood pressure in white coat hypertension. Am J Cardiol 1990; 66:498-501.

72. Chrysant SG. Treatment of white coat hypertension. Curr Hypertens Rep 2000; 4:412-7.

RAPID UREASE TEST IN THE DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION

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ABSTRACT: Many tests are available for the diagnosis of *H. pylori* infection. Broadly they can be divided into invasive- endoscopy biopsy based tests and non-invasive tests. Of the endoscopy biopsy based tests the rapid urease tests (RUT) have been found to be the most convenient, accurate and inexpensive tests and they have therefore been recommended by several consensus panels and working parties as the test of choice during endoscopy.

Several RUTs are available; some are commercial: CLO test, Pyloritek, Helicobacter urease test, *H. pylori* test and others- "homemade". We strongly recommend the "homemade" 1 min rapid urease test using an unbuffered solution as originally described by Arvind *et al.* This test has been shown to be easy to prepare, inexpensive and accurate on field-testing.

Several factors affect the accuracy of the RUT. The larger the size of biopsy samples, the quicker is the postive reaction time. With the CLO test, warming the tests to 37°C has also been shown to hasten the reaction time. The effect of blood on the RUT poses an important problem in testing. It is vitally important to determine the *H. pylori* status in patients with bleeding peptic ulcers as the recurrence of bleeding has been shown to be markedly reduced or virtually abolished with *H. pylori* eradication. While the results of studies have not been entirely consistent, it is likely that presence of blood does reduce the sensitivity of the RUT. It is therefore sensible that in patients with bleeding ulcers, the RUT should not be the sole endoscopy biopsy test used and that samples should also be taken for histological examination. *(JUMMEC 2000; 1: 11-16)*

KEYWORDS: Urease test, *Helicobacter pylori.*

Introduction

Since the isolation of *Helicobacter pylori* by Warren and Marshall(1) in 1983, this bacterium is now recognised as the main aetiologic factor in peptic ulcer disease, with 90% or more of duodenal ulcers and 70% or more of gastric ulcers(2) harbouring this bacterium. It also has been shown to play an important role in the pathogenesis of cancer of stomach and gastric maltomas(3,4). *H.pylori* infection is a ubiquitous infection and is believed to be the most common bacterial infection in the world.

H. pylori infection can be detected by invasive and non-invasive tests. The established invasive endoscopy biopsy based tests include culture, histology Gram stain of a fresh tissue smear and detection of urease activity in the biopsy samples. Serology and the radiolabelled carbon urea breath tests are the non-invasive tests. These tests have become increasingly popular(4,6). The detection of antigen in the saliva(7,8) and gastric juice(9) has been used in diagnosis but these tests have not been shown to be practical nor accurate. A stool antigen test has been introduced and shown to be accurate(10,11). Of the endoscopy biopsy based tests however, the rapid urease test (RUT) is perhaps the most useful and widely used test(3,4,6).

Materials and methods

Basis of the urease test

H. pylori is the only bacterium in the upper gastrointestinal tract that produces the enzyme urease(12). This characteristic is made use of in the urease test whereby the urea substrate is hydrolyzed by the urease enzyme to produce ammonia and carbamate(13). The ammonia will equilibrates with water to form ammonium hydroxide and the presence of this compound will result in a rapid increase in pH. This change in pH will be shown up by a colorimetric

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change in the pH indicator used such as phenolphthalein(13,14).

Development of the urease test for clinical use

The first description of this phenomenon by Langenberg *et al* (14) was soon followed by its clinical application to the diagnosis of *H. pylori* in biopsy specimens by McNulty and colleagues (15). The biopsy specimen is placed in Christensen's 2% urea broth which acts as the enzyme substrate and ammonia production by hydrolysis of the urea by bacterial urease results in a change in the pH of the medium, which is detected by a color change in the pH indicator. Although there was an excellent correlation between a positive *H. pylori* culture and a positive urease reaction, this method still took several hours to produce a positive result. In the first study only 50% of biopsy specimens containing *H. pylori* were positive by six hours. Some tests required up to 24 hours to yield a positive result. Following further modifications, a commercial test – the CLO test (Delta West, Perth, Australia) became available. Using this test, Marshall(16) and Morris(17) and colleagues reported a good correlation with culture. But even then, at least 20 minutes was required for a definite positive result to be demonstrated.

Different types of urease test

Both commercial and locally produced biopsy urease tests (BUT) are in use in different countries (4,18). The CLO test is the most widely used and studied commercially RUT. Sensitivities at 24 hours are reported to be in the range of 75-99%, with specifities in most studies of approximately 95-100% [Table 1] (6,18,19,20).

Some investigators have reported excellent sensitivities after shorter periods of test observation - approximately 75% within 1 hour and about 90% at 3 hours(21). The other commercially RUT available are PyloriTek urease test which is limited by its lack of specificity compared to the CLO test(22). When the *Helicobacter* Urease Test (HUT), Polish test were compared with the CLO test, there was no difference in the reaction time, although the Polish test was more accurate and had a quicker time interval to positivity than the HUT(23).

Rapid urease test

In 1988(24), Arvin *et al* described a rapid one minute urease test was described for the diagnosis of *H. pylori* infection. This test has now been found to be so useful in the endoscopy suite and is now is the initial test of choice among patients having diagnostic upper endoscopy (4,21). As the name denotes, the one minute test gives rapid results and has practical advantages for clinical gastroenterologists as the presence of *H. pylori* can be detected in the endoscopy room sometimes even before the instrument is withdrawn. This allows the endoscopist to prescribe treatment whilst the patient is still in the endoscopy suite (6,18). Furthermore, the rapid diagnosis in the endoscopy suite has the added benefit allowing the physician to make decision to discard samples by the end of the workday rather than banking the specimens until the next day (25). The test solutions are easy to prepare even for a gastroenterologist, and in fact large batches can be prepared at one time but should be stored in a freezer and only thawed before use (18). Field-testing has shown the test to be a reliable and robust test (1,26,27,28) with high sensitivity and specificity. However, the primary reason that the RUT is perhaps preferred as the initial diagnostic test is the low cost of this test (5,15).

Factors that may affect the outcome of urease test

Several factors are though to affect the accuracy of the urease tests. These include the size of the biopsy specimens, fresh preparation of the medium, incubation temperature after the biopsy and the effect of blood on the biopsy specimens. It has been well accepted now the fact that by increasing the number or size of biopsy specimens in the Closet, it will hasten the time to a positive test but does not alter the final sensitivity or specificity (18,21). The effect of storage temperature, warming and the effect of blood on the accuracy of the RUT however remain uncertain (29,30,31,32,33).

For the homemade 1 min RUT, it is generally

Table 1. Accuracy of urease tests-published results

Test (%)	Thillainayagam *et al.* (1991)(18)	Goh *et al.* (1994)(6)	Chu *et al.* (1997)(19)	Misra *et al.* (1999)(20)
Sensitivity	89.0	96.6	92.8	92.0
Specificity	100.0	92.2	97.6	100.0
Positive predictive value	100.0	99.3	97.5	66.0
Negative predictive value	94.0	96.2	93.0	93.0

recommended that the reagent be prepared daily although this can be quite troublesome and time consuming for a gastroenterologist. The reason for this is that prolonged storage can cause spontaneous orange discoloration, due to contamination by other urease-producing bacteria. Although it has been said that the test solutions can be prepared in large batches at one time and stored in a freezer, at what temperature it should be stored and how long can be stored is unclear. Katelaris *et al.* (34) suggested that unbuffered RUT be stored at -20°C, but the accuracy of the RUT was not evaluated explicitly. Ng *et al.* (29,30) demonstrated that the unbuffered RUT is highly sensitive and specific when the reagent is stored at 4°C for fewer than 6 days, and remained accurate if stored at -20° C even at a longer storage time. However, the urea denatured with prolonged storage at 4° C and the sensitivity decreases dramatically with longer storage time. The slower reaction time of both groups stored 4° C and -20° C compared to previous study (18) may be related to the lower initial temperature of the RUT because urease activity increases dramatically with temperature and optimizes at 4° C. Therefore, the unbuffered RUT remains highly sensitive and specific when stored at 4° C for up to 5 days. When it is expected to be stored for a longer period, the bottles should be frozen at -20° C.

Incubating the CLO test at 30° to 40° C for 3 hours after the biopsy is recommended, suggesting that warming will speed the urease chemical reaction. However, this decreases simplicity and requires the purchase of additional equipment. Laine *et al.* (31) demonstrated that incubating the CLO test at 37° C hastens the time to a positive test, although the time saved is less than 1 hour in most patients. The sensitivity is improved when the test is read at 1 to 2 hours, but no improvement is seen beyond this time. Specificity is not influenced by warming. The development of occasional false-positives at the 24-hour reading may occur because of other urease-producing bacteria or may represent cases in which *H. pylori* are present in relatively small numbers and are not identified histologically (i.e."false-negative" histology). Therefore, if a final reading of the CLO test is desired within 1 to 2 hours of biopsy, CLO test incubation at 37° C should be performed. If a final reading at 3 hours or more after biopsy is acceptable, then warming of the CLO test is not necessary.

Eradication of *H. pylori* is important as it cures peptic ulcer disease and is more cost-effective to than to maintain the patient on traditional maintenance therapy (3,5,,35,36,37,38). It has also been shown that *H. pylori* eradication reduces recurrent ulcer bleeding. *Laine et al.* (39) reported that patients with bleeding caused by *H. pylori* - associated ulcer disease rarely have recurrent bleeding after *H. pylori* eradication. Perhaps the most confounding factor is the effect of blood on the accuracy of the RUT. The observation that blood may affect the RUT comes about from observation of the low prevalence of *H. pylori* in upper gastrointestinal bleeders. Indeed, several workers have shown a lower sensitivity of the RUT in the presence of blood (3,26). However, some others have reported false-positive result (40) while some have found that the test will be unaffected (33) .

Perry *et al.* (40) found that heparinized blood and alcohol will enhanced the detection of urease, and therefore improve the sensitivity. However he also reported that it will reduced the specificity results on the CLO test and *H.pylori*fast. The unexpected positive results could be due to the alkalinity of blood on the urease test alone.

On contrary, several other studies have found reduced sensitivity of the BUT in bleeding peptic ulcers. Colin *et al.* (41) demonstrated the sensitivity of the BUT (31%) was low, but at the same time histology and culture results were also low, raising concerns about the accuracy of their serology test which was used as the gold standard. Lee *et al.* (42) clearly showed a significant drop in sensitivity of the BUT, from 93% in non-bleeding ulcers to 73% in bleeding ulcers, which was consistent with earlier studies (43,44). Although high dose of omeprazole (80 mg) has been shown to interfere with the accuracy of the BUT (45), this could not explain the low sensitivity of the test as, at least in the later study (44), patients with recent use of omeprazole and antibiotics were excluded. Lee *et al.* (46) attributed that the reduced sensitivity of the BUT is merely as a result of clotted blood obscuring the color change, but it would be difficult to comprehend why all three CLO test, *H. pylori*fast and Pyloritek tests failed to detect *H. pylori* even at the high concentration of 10^9 CFU.

Although blood in the stomach is thought to interfere with the BUT, the underlying mechanism remains unknown. Leung *et al.* (1998) (32) have postulated three possible mechanisms. Firstly, the presence of anti-*H. pylori* antibody in the blood may inhibit the production of urease by *H. pylori*. Secondly, serum inhibitors such as enzymes or electrolytes may suppress the urease activity of *H. pylori*. Thirdly, various buffering systems (e.g. albumin, bicarbonate and phosphate) may interfere with the pH changes of the reagent. In Leung's *et al* study, they showed that blood adversely affects the performance of the BUT and this is mediated by the buffering effect of serum albumin on the pH indicator, rather than by a direct inhibition of the urease activity. Owing to the lack of reliability of the BUT in the presence of the blood, it is generally recommended that BUT should not be used as the only diagnostic test for *H. pylori* infection in patients with bleeding ulcers.

In a study (33) evaluating the two different types of BUT in bleeders, they found that the number of positive Pyloritek and CLO tests at three different time intervals

- 1, 4 and 24 hours were nearly identical. The small number of discordant results presumably occurred as a result of the patchy distribution of organisms in the stomach and/or the variation in individual test performance characteristics. The authors concluded that since there is no evidence of alteration in the BUT, physicians may use RUT as the initial biopsy test for the diagnosis of *H. pylori,* just as they would in patients without bleeding.

A recent study (3) using the CLO test, culture, histology, urea breath test and serology test showed that only the CLO test had better sensitivity ($p < 0.05$) in group with no blood in the tested specimens compared to the one tested with fresh blood or blood-containing material in the gastric antrum. Although this might mean that blood in the gastric antrum can interfere with the diagnosis of *H. pylori* and decrease the sensitivity of the test, but the presence of blood in the antrum had no effect on the other 4 tests. The authors suspected that blood might affect the pH value of the CLO test medium and do not favor the hypothesis that *H. pylori* migrates from the antrum to the body of the stomach when blood is present. This study also demonstrated that the non-invasive tests seemed to be more sensitive than invasive tests in detecting *H. pylori* infection in patients with bleeding peptic ulcers. As a delayed positive CLO tests were recorded, the CLO test should be observed for more than 24 hours because of the possibility of a delayed positive result in some patients with bleeding peptic ulcers.

Proton-pump inhibitors are potent antisecretory medications widely used to treat gastrointestinal disorders. When given alone, proton-pump inhibitors rarely eradicate *H. pylori*, although they do suppress the organism and this may lead to false-negative results on diagnostic testing (endoscopic biopsy or urea breath tests) for *H. pylori*. The development of false-negative breath test results in these patients may be due to decreased viability of the organisms at high intragastric pH with potent antisecretory therapy and direct inhibition of urease activity by proton-pump inhibitors. Laine et al. demonstrated that 33% of H. pylori-positive patients develop false-positive urea breath test results while taking a standard course of proton-pump inhibitor therapy. Most patients with *H. pylori* infection revert to positive urea breath test results by 1 week after the discontinuation of proton-pump inhibitor therapy, however, to ensure that a false-negative result does not occur, patients should not receive proton-pump inhibitors for 2 weeks before a urea breath test for *H. pylori* is done (47).

Discussion

With the increasing recognition of the role of *H. pylori* in gastrointestinal diseases, there is a need for a reliable, efficient and yet inexpensive diagnostic test. The ideal test for the diagnosis of *H. pylori* at endoscopy should have excellent sensitivity and specificity, be inexpensive, and provide results rapidly so patients can be informed of their *H. pylori* status and started on proper therapy at the time they leave the endoscopy unit. At present, rapid urease testing of gastric biopsy specimen can be considered the ideal test for *H.pylori* at endoscopy (18).

Compared to the other invasive and non-invasive methods, RUTs are convenient and useful tests; both the "homemade" test as well as the commercially available CLO test. This is because RUT has practical advantages for clinical gastroenterologists who require a rapid, accurate, sensitive and specific method of diagnosing *H.pylori* infection. Furthermore, preparation of the RUT does not require special laboratory skills or equipment, can be prepared in large batches at one time, to be stored in a freezer and only thawed before use. As more than 90% of the infected patients can be detected in the endoscopy room even before the instrument is withdrawn, it may help in planning therapeutic strategies, lead where appropriate to prompt introduction of treatment, and possibly reduce recall outpatients appointment. All the other invasive and non-invasive methods except for the finger-prick office-based serological tests have a major disadvantage in common which is the time required to get a definitive result for detecting *H. pylori* and thus may not be available to the clinician during the endoscopy consultation. In our local context, most importantly is the usefulness of the test in the majority of the endoscopy units in this country where laboratory facilities are not always available (3,4, 6,18,21,36).

These finger-prick tests however suffer from a lower level of accuracy. Locally validated tests show a high specificity but relatively lower sensitivity. As with all other serological tests, these tests cannot be used to assess treatment response because of the variable decline in antibody titres (48,49).

References

1. Warren JR, Marshall BJ. Unidentified curved bacilli on the gastric epithelium in active chronic gastritis 1983. Lancet 1: 1273-5 (Letter).
2. Goh KL. Prevalence of and risk factors for *Helicobacter pylori* infection in a multi-racial dyspeptic Malaysian population undergoing endoscopy. J Gastroenterol Hepatol 1997; 12: S29-S35.
3. Tu TC, Lee CL, Wu CH, Chen TK, Chan CC, Huang SH, Lee SC. Comparison of invasive and non-invasive tests for detecting *Helicobacter pylori* infection in bleeding peptic ulcers. Gastrointest Endosc 1999; 4: 302-6.
4. Lam SK, Talley NJ. *Helicobacter Pylori* Consensus. Report of the 1997 Asia Pacific Consensus Conference on the management of *Helicobacter pylori* infection. J Gastroenterol Hepatol 1998;13: 1-12.

5. Goh KL, Cutler A, Chua ABS, Ding RPH, Kandasami P, Mazlam MZ, Raj SM. *Helicobacter Pylori* And Peptic Ulcer. Optimal treatment for duodenal ulcer disease: A cost-decision analysis in Malaysian patients. J Gastroenterol Hepatol 1999; 14: 32-8.
6. Goh KL, Parasakthi N, Peh SC, Puthucheary SD, Wong NW. The rapid urease test in the diagnosis *Helicobacter pylori* infection. Singapore Med J 1994;Vol 35: 161-2.
7. Reillt TG, Poxon V, Sanders DS, Elliott TS, Walt RP. Camparison of serum, salivary and rapid whole blood diagnostic tests for *Helicobacter pylori* and their validation against endoscopy based tests. GUT 1997; 40(4): 454-8.
8. Christie JM, McNulty CA, Shepherd NA, Valori RM. Saliva serology useful for the diagnosis of *Helicobacter pylori*? GUT 1996; 39(1): 27-30.
9. Mokuolu AO, Sigal SH, Lieber CS. Gastric juice urease activity as a diagnostic test for *Helicobacter pylori* infection. Am J Gastroenterol 1997;92(4): 644-8.
10. Vaira D, Malfertheiner P, Megraud F, Axon AT, Deltenre M, Hirschl AM, Gasbarrini G, O'Morain C, Garcia JM, Quina M, Tytgat GN. Diagnosis of Helicobacter pylori infection with a new non-invasive antigen-based assay. HpSA European study group. Lancet 1999; 354(9172): 30-3.
11. Vaira D, Malfertheiner P, Megraud F, Axon AT, Deltenre M, Gasbarrini G, O'Morain C, Pajares Garcia JM, Quina M, Tytgat GN. Noninvasive antigen-based assay for assessing Helicobacter pylori eradication: a European multicenter study. The European Helicobacter pylori HpSA Study Group. Am J Gastroenterol 2000;9 5(4): 925-9.
12. Freedberg AS, Barron LE. The presence of spirochetes in human gastric mucosa. Am J Dig Dis 1940;7:443-5.
13. Sumner JB. The isolation and crystallization of the enzyme urease. J Biol Chem 1926; 69: 435-41.
14. Langenberg ML, Tytgat GN, Schipper MEI, Rietra PJGM, Zanen HC. *Campylobacter*-like organisms in the stomach of patients and healthy individuals. Lancet 1984; i: 1348.
15. McNulty CAM, Wise R. Rapid diagnosis of *Campylobacter*-associated gastritis. Lancet 1985; i: 1443-4.
16. Marshall BJ, Warren JR, FrancisGJ, Goodwin CS, Blincow ED. Rapid urease test in the management of *Campylobacter pylori*-associated gastritis. Am J Gastroenterol 1987; 82: 200-10.
17. Morris A, McIntyre D, Rose T, Nicholson G. Rapid diagnosis of *Campylobacter pylori* infection. Lancet 1986; I: 149.
18. Thillainayagam AV, Arvind AS, Cook RS, Harrison IG, Tabaqchali S, Farthing MJG. Diagnostic efficiency of an ultrarapid endoscopy room test for *Helicobacter pylori*. GUT 1991;3 2: 467-9.
19. Chu KM, Poon R, Tuen H, Law SY, Branicki FJ, Wong J. A prospective comparison of locally made rapid urease test and histology for the diagnosis of *Helicobacter pylori* infection. Gastrointestint Endosc 1997; 46: 503-6.
20. Misra SP, Misra V, Dwivedi M, Singh PA, Bhargava V, Jaiswal PK. Evaluation of the one-minute ultra-rapid urease tests for diagnosing *Helicobacter pylori*. Postgrad Med J 1999; 75(881): 154-6.
21. Laine L, Chun D, Stein C, El-Beblawi I, Sharma V, Chandrasoma P. The influence of size or number of biopsies on rapid urease test results: a prospective evaluation. Gastrointest Endosc 1996; 43: 49-53.
22. Chen YK, Godil A, Wat PJ. Comparison of two urease tests for detection of *Helicobacter pylori* infection. Dig Dis Sci 1998; 43(8): 1636-40.
23. Niv Y, Sperber AD, Scapa E, Maoz E, Fraser G, Druzd T, Zirkin H. A comparison of new rapid urease tests for detection of *Helicobacter pylori*: test characteristics and factors affecting positivity and time to positivity. J Clin Gastroenterol 1998; 27: 327-30.
24. Arvind AS, Cook RS, Tabaqchali S, Farthing MJG. One minute endoscopy room test for *Campylobacter pylori*. (letter). Lancet 1988; i: 704.
25. Man K, Poon R, Tuen HH, Law SYK, Branicki FJ, Wong J. A prospective comparison of locally made rapid urease test and histology for the diagnosis of *Helicobacter pylori* infection. Gastrointest Endosc 1997;46:503-6.
26. Yousfi MM, El-Zimaity H, Cole RA, Genta RM. Commercially available rapid urease tests vary in efficacy for detecting *Helicobacter pylori* infection. GUT 1995; 27: A51.
27. Ramirer F. A more specific rapid urease test to diagnose *Helicobacter pylori* gastritis. Gastroenterology 1995; 108: A2730.
28. Malfertheiner P, Dominguez-Munoz E, Henkenhmuller H, Neubrand M, Fisher *H.PYLORI*, Sauerbruch T. Modified rapid urease test for detection of *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol 1996; 8: 53-6.
29. Ng FH, Wong SY, Chang CM. Shelf life of unbuffered rapid urease test. Helicobacter 1997;2(2):98-9.
30. Ng FH, Wong SY, Ng WF. Storage temperature of the unbuffered rapid urease test. Am J Gastroenterol 1997; 92(12): 2230-1.
31. Laine L, Estrada R, Lewin DN, Cohen H. Influence of warming on rapid urease test results: a prospective evaluation. Gastrointest Endosc 1996;44: 429-32.
32. Leung WK, Sung JJY, Siu KL, Chan FKL, Ling TKW, Cheng AFB. False-negative biopsy urease test in bleeding ulcers caused by the buffering effects of blood. Am. J. Gastroenterol 1998; 93: 1914-8.
33. Laine L, Sidhom O, Emami S, Estrada R, Cohen H. Effect of blood on rapid urease testing of gastric mucosal biopsy speciments. Gastrointest Endosc 1998; 47(2): 141-3.
34. Kateralis PH, Lowe DG, Norbu P, *et al*. Field evaluation of a rapid simple and inexpensive urease test for detection of *Helicobacter pylori*. J Gastroenterol Hepatol 1992; 7: 567-71.
35. van der Wouden EJ, Thijs JC, van Zwet AA, Oey HB, Kleibeuker JH. Reliabilility of biopsy-based diagnostic tests for *Helicobacter pylori* after treatment aimed at its eradication. Eur J Gastroenterol Hepatol 1999; 11(11): 1255-8.
36. *Helicobacter pylori* in peptic ulcer disease. A report from a special international symposium, Brussels, September 1993 by ASTRA.
37. Goh KL, Raj SM, Parasakthi N, Kew ST, Kandasami P, Mazlam Z. Management of *Helicobacter pylori* infection – a working party report of the Malaysian Society of *Gastroenterology* and Hepatology. Med J Malaysia 1998; 53(3): 302-10.
38. Vakil N, Fennerty MB. Cost-effectiveness of treatment

regimens for the eradication of *Helicobacter pylori* in duodenal ulcer. Am J Gastroenterol 1996; 91: 239-45.

39. Laine L. *Helicobacter pylori* and complicated ulcer diseases. Am J Med 1996;100: 52S-9S.
40. Perry M, Vakil N, Cutler AF. Admixture with blood or Etoh does not explain false negative urease test in *H. pylori* positive active GI bleeders. Gastroenterology 1997;112: A257.
41. Colin R, Bigard MA, Notteghem B *et al.* Poor sensitivity of direct tests for detection of *Helicobacter pylori* on antral biopsies in bleeding ulcers. Gastroenterology 1997;112:A93.
42. Lee JM, Breslin NP, Fallon C *et al.* The biopsy urease test lacks sensitivity in bleeding peptic ulcer disease. Gastroenterology 1997;112:A195.
43. Hosking SW, Ying MY, Chung SC *et al.* Differing prevalence of *Helicobacter pylori* in bleeding and nonbleeding ulcers. Gastroenterology 1992;102:A85.
44. Lai KC, Hui WM, Lam SK. Bleeding ulcers have high false-negative rates for antral *Helicobacter pylori* when tested with urease test. Gastroenterology 1996;110:A167.
45. Stoschus B, Kalhori N, Dominguez-Munoz E *et al.* Effect of high dose omeprazole on urease activity in vivo. Gastroenterology 1995;108:A226.
46. Lee JG, Lam K Slonick J *et al.*. The effects of storage temperature, incubation conditions, and contaminants on the sensitivity of CLO, *H.pylori*fast, and Pyloritek for detection of *H. pylori*. Gastrointest Endosc 1997; 45:A95.
47. Laine L, Estrada R, Trujillo M, Knigge K, Fennerty MB. Effect of proton-pump inhibitor therapy on diagnostic testing for *H. pylori*. Ann Intern Med 1998;129:547-50.
48. Goh KL, Parsakthi, Chin SC. Validation of a rapid office-based whole blood test (Helisa^{TM}) for the diagnosis of Helicobacter pylori infection. J Gastroenterol Hepatol 1996;11:A57(abs).
49. Goh KL, Chan SH, Khoo D, Cheah PL, Parasakthi N, Manivasgar M, Chin SC. Evaluation of two finger-prick tests for the detection of Helicobacter pylori infection. J Gastroenterol Hepatol 1998;13:A98(abs).

THE *IN VITRO* HIPPOCAMPAL SLICE TECHNIQUE AS A TOOL FOR STUDYING THE CENTRAL NERVOUS SYSTEM

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ABSTRACT: The use of brain slice preparations has become increasingly popular among scientists of different disciplines in recent decades for the study of the mammalian central nervous system (CNS) in general and of synaptic phenomena in particular. The *in vitro* hippocampal slice may be the single most used preparation, among other slices of different parts of the brain areas. The use of brain slices in different experimental work offers certain advantages over the *in vivo* approaches to the study of the CNS; however, such preparations may have some limitations. This review describes the hippocampal slice technique, explores some of the different types of studies in which it was employed and points out the advantages and limitations of its use. *(JUMMEC 2000; 1: 17-23)*

KEYWORDS: Hippocampal slices, brain slices, technique, synaptic function, electrophysiology, *in vitro.*

Introduction

The brain slice preparation was first used for neurochemical studies by McIlwain *et al.* in 1951 (1). Such a preparation has developed into one of the most widely used *in vitro* techniques in neuroscience, especially after the discovery that about 0.4 mm thin slices of the hippocampal tissue that are cut in the appropriate plane maintained synaptic function (2,3). While the hippocampal slice is probably the earliest and single most used preparation, slices of olfactory cortex, neocortex, hypothalamus, caudate nucleus, amygdala and other brain areas have also been studied in the past two decades.

Slices are taken from various parts of the brain, maintained in a chamber in the presence of a bathing medium and used for many purposes (4). The available literature pertaining to this subject is very vast and diverse. The aim of this review is to briefly describe the technique, explores the many different types of studies in which it was employed and discusses its advantages and limitations. For further details about the different aspects of this technique and its uses are found in the references provided herein and in many other resources.

Preparation of slices

In general, rodents are the animals of choice for the preparation of hippocampal and other brain slices. The rat and the guinea pig are the most used. After decapitation, the brain is removed rapidly from the skull and rinsed with cold artificial cerebrospinal fluid (ACSF) which has been equilibrated with 95% O_2/ 5% CO_2 gas mixture. The composition of ACSF may vary (5); however, it contains the following basic ingredients: NaCl, KCl, NaH_2PO_4, $CaCl_2$, $MgSO_4$, $NaHCO_3$ and glucose (6-8). In some studies, NaH_2PO_4 is substituted for KH_2PO_4 (9). The brain area under study is then dissected out and sliced into slices of 100-500 µm. Tissue choppers of different designs can be used for slicing; most of them utilize a razor blade for the actual cut. Slices are then collected in a small beaker containing oxygenated ACSF and transferred with a pipette to the incubating/ recording chamber. The detailed procedure for the preparation of the rat hippocampal slices has been described (4). A schematic representation of the rat hippocampal slice, with its CA1 and CA3 regions and connecting synapses identified, is shown in Figure 1.

Although some investigators recommend keeping the time from decapitation until placement of slices in the chamber (Figure 2) to a maximum of 8 min, it has been shown that even when hippocampal slices were prepared 30 min after decapitation, CA1 excitatory synaptic function completely recovered (10).

Incubating/recording chambers

Slices require an environment that enables the maintenance of their metabolic activity and electrophysiological function. The minimum

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requirements for this purpose are: a suitable ionic environment, provision of oxygen and glucose, an appropriate temperature and removal of metabolic wastes. This environment is normally provided by incubation chambers, many of which have been described (3,11–13).

Three types of chambers are used: the interface, the submerged and static - each with its own advantages and limitations (5). In the interface chamber (3,12), the slices are placed on a fine mesh through which ACSF is flowing (Figures 2 and 3) and a thin layer of a humidified 95% O_2/ 5% CO_2 gas mixture. Those who were not satisfied with the limitations of the interface chamber, for their experimental purposes, developed the submerged chamber (14–16). In such a submerged chamber, the slices are held between two mesh grids and are totally submerged in an oxygenated bathing medium. The static chamber was developed for the purpose of studying metabolic products of the slice (13). It can be regarded as a variant of the interface chamber in which the flowing medium is replaced by a static pool (5). The pool must be flushed at regular intervals to provide more glucose and remove waste products. Oxygenation is almost entirely via the upper surface. Whether to use the static type, in which the bathing medium volume is large compared to the volume of the slice, or the continuous perfusion type, depends upon the plan of the experiment. Oxygenation and heating could be problematic in the static chamber. Addition, and later washout, of drugs or ions is easy in the continuously perfused chamber but difficult in the static one.

In these two basic chamber types, slices can be either submerged below the bathing fluid surface or maintained at the fluid-gas interface. These two approaches, each has it own advantages and limitations. The composition of the bathing medium (ACSF) of a fully submerged slice may be changed quickly, movement artifacts are minimized and dryness is avoided. However, field potentials are difficult to record because of current shunting by the ACSF, stimulus artifacts may be difficult to control and oxygenation can be inadequate. The partially submerged (interface) slice has a better oxygen supply and much less fluid shunting which gives rise to larger (1-20 mV) extracellular evoked field potentials. However, high humidity must be kept in the chamber atmosphere to prevent dryness and changing the composition of the ACSF for drug studies is much slower due to the low flow rate than with submerged slices. Several designs are available commercially; however, those who wish to construct such chambers can find instructions and guidance (17-19). Besides providing basic mechanical support and the necessary ingredients for the maintenance of viable tissue slices, chambers must be equipped with temperature control devices and provide clear and easy visualization of, and access to, the slices for accurate positioning of the recording and stimulating electrodes.

Recording and stimulating electrodes

Since, by its nature, the brain slice is a compromised system, any recording equipment must be of high quality. At the center of that equipment are the recording and stimulating electrodes. For extracellular recording of field potentials (population spike), field post-synaptic potentials (EPSP) and pre-volley; borosilicone glass-micropipettes filled with either 4 M NaCl or ACSF (with impedance of 1-3 meghoms) are most suitable. Impedance higher than 10 meghoms makes background noise bothersome. For intracellular recordings, electrodes are pulled from fiber-filled borosilicate micropipettes filled with a solution of 4 M potassium citrate, potassium acetate or potassium chloride with impedance of 70-150 meghoms. Lower impedance electrodes tend to seal poorly so recordings tend to be less suitable while electrodes with impedance above 150 meghoms are both noisy and prone to clog.

Several types of stimulating electrodes can be used (9). Bipolar electrodes made of insulated tungsten needles with tips of about 50 μm and resistance of 0.5-2.0 meghoms are quite satisfying. Concentric bipolar stimulating electrodes are not recommended because their relatively large tip diameter damages the tissue. Other recommended metals for making of stimulating electrodes are silver and platinum. Stimulation can also be achieved with a glass micropipette filled with wood's metal and plated at the tip with gold and platinum. In an interface chamber, healthy slices produce evoked responses (CA1 population spike in hippocampal slice) of 10 mV or more in amplitude with stimulation currents of 50-100 μA and a duration of 0.1 msec (Figures 4 and 5).

Data acquisition system

Responses to the electrical stimulation of slices are recorded, stored and retrieved for future analysis and plotting. The data acquisition system being commonly in use consists of a digital oscilloscope, which is connected to a personal computer (7,8). Positioning of both the stimulating and recording electrodes is done manually and with precision (Figure 3). However, recording and storing of responses can be done manually if many slices are checked or automatically when responses within a single slice are followed on a continuous basis for a long duration. Using appropriate software computer programs (20), stored responses can be analyzed to produce parameters such as population spike amplitude and latency and further characterize them (8,9).

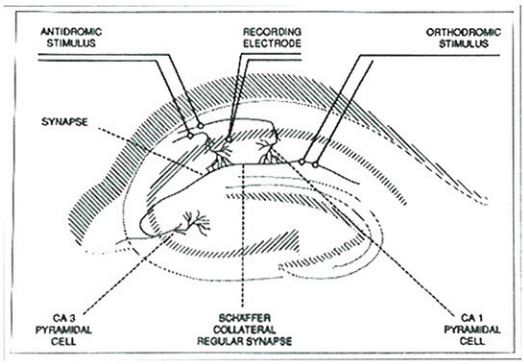

Figure 1. A schematic representation of the rat hippocampal slice, with its distinct CA1 and CA3 regions identified. Locations for placements of electrodes for orthodromic and antidromic stimulation and the site for the placement of the recording electrode are shown.

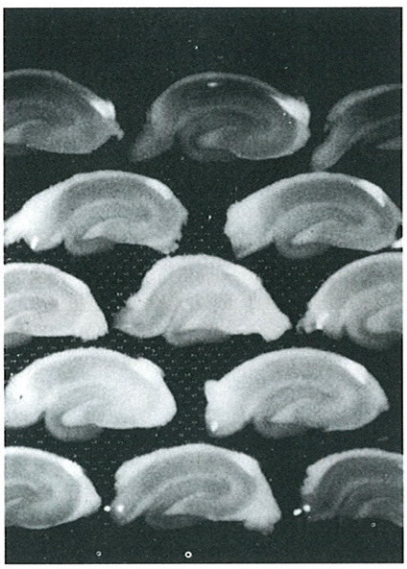

Figure 2. Arrangement of actual rat hippocampal slices in an interface incubating/recording chamber. Slices are placed on a fine mesh through which oxygenated ACSF flows gently.

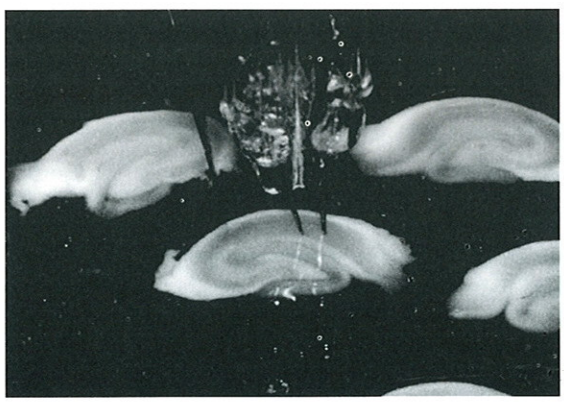

Figure 3. A photograph showing an arrangement of actual rat hippocampal slices on the mesh of the *in vitro* incubation chamber. Placement of the stimulating electrode (double metal bars) and the recording electrode (fine tipped micropipette) are also shown.

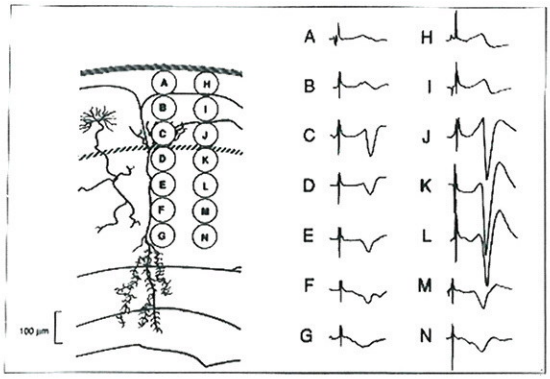

Figure 4. Variation of CA1 population spike potential (PSP). The stimulating electrode is placed at a point slightly above the measure scale (100 µm, in the lower left corner) and the recording electrode is positioned in CA1. There is a characteristic change in the shape of PSP related to the position of the recording site. For comparative responses to treatment(s), consistency in placing such a recording electrode is of significance - especially at the sites that produce the largest PSP's (positions J, K and L).

Figure 5. Three actual single recordings of PSP from the CA1 region of 3 different rat hippocampal slices. Placement of the recording electrode was around the J, K and L sites (shown in Figure 4).

Different electrophysiological recordings

Depending on the brain structure under study, placement of electrodes and the type of recording (extracellular or intracellular), different responses can be produced. Different types of responses can be evoked in, and recorded from the hippocampal slice preparation. The most common response is the population spike (Figure 5). This response is recorded extracellularly and is obtained from the pyrimidal cell layer by applying an orthodromic stimulation to afferent fibers in the stratum radiatum or oriens (Figures 1 and 3). It is a summation of single action potentials of many

neurons in the vicinity of the recording electrode. Population responses with shorter latency can be produced by stimulating pyrimidal cell axons in the alveus. These are also population spikes that result from an antidromic stimulus (Figure 1). Another extracellular response recorded frequently from the hippocampal slice is the field excitatory post synaptic potential (EPSP). This negative potential is obtained by stimulating the same sites stimulated to induce an orthodromic population spike response. However, the recording electrode is placed at the site of the afferent input to the pyramidal cell dendrites. The EPSP response is believed to be the current flowing into the dendrites at, and near, the electrode site. The synchronous firing of the afferent fibers, appearing as a biphasic deflection preceding the EPSP, is known as the pre-synaptic volley or pre-volley (9). Intracellular recordings of either spontaneously active or stimulated neurons are not different from those made *in vivo*, though pulsations due to heart beat and respiration are absent.

Use of slices in different studies

The use of the hippocampal slice technique in experimental work has been increasing rapidly over the last three decades. At present, the available literature pertaining to the use of this technique is very vast and diverse, thus attempting to cover all would be almost impossible. A brief mention of the major types of studies in which this *in vitro* method was employed would be appropriate for the purpose of this review. For further details, the reader should investigate the particular field or research problem of interest. Although most of the studies mentioned herein use electrophysiological techniques, as this preparation proved to be a tool in the study of the fundamentals of neurophysiology at the cellular and simple neuronal circuit level, others may not employ electrophysiology. More of the major research fronts in which the slice preparation has been used as the appropriate technique are still forthcoming, such as endocrinological and pharmacological studies as well as those which involve pathological situations of the CNS.

While the neuronal membrane properties can be studied using intracellular recordings, including the study of ion channels and putative neurotransmitters, the study of synaptic activity can be performed with extracellular recordings and specific stimuli. The nature of different synaptic connections has been studied by evoking excitatory and inhibitory post-synaptic potentials (EPSPs and IPSPs). For such studies, other electrophysiological techniques such as voltage clamping and ionophoresis can be very useful. Electrophysiological measurements can also be combined with morphological and biochemical correlates (9).

The hippocampal slice preparation has been widely used in studying the physiology and pharmacology of many aspects about neurons. Such studies were performed either entirely with the slice preparation or by inducing changes or treatments in the intact animal and then studying the affected brain area as a slice. Many studies endeavored to understand the cellular mechanisms underlying modulation of electrophysiological activity in neurons (21–26), to study the effects of temperature on excitatory transmission (27,28), to understand cerebral energy metabolism (29–31) and study the physiological and molecular mechanisms of age-related memory loss (32). The hippocampal slice preparation was also used extensively in plasticity (the strengthening or weakening of synaptic connections due to internal and/or external stimuli) and in neurotoxicity studies (33-35). Pharmacological studies involving neurotransmission (36-40), investigating the effects of various classes of antidepressant drugs on the electrically evoked release of neurotransmitters (41-45) and the effects of certain drugs on neuronal activity (46,47) utilized this slice preparation.

The utilization of the slice technique in understanding the mechanisms involved in certain pathological situations and in searching for means to alleviate or prevent them has been extensive. The slice preparation provided a model for studying epileptiform activity (4,6,48,49). This proved valuable in understanding the mechanisms and effects of anticonvulsant drugs, those which increase inhibitory synaptic transmission in the CNS and may be used in the treatment of seizures. Theoretically, substances that block the uptake of inhibitory transmitters such as gamma-aminobutyric acid (GABA) into intracellular compartments should increase inhibition and thus have potential value as antiepileptic drugs. The hippocampal slice preparation has been employed in studies geared to understand the different cellular mechanisms involved in anticonvulsant effects of these drugs (50-53). The hippocampal slice has also been extensively used as a model for investigating the effects of hypoxia, the physiopathology of cerebral ischemia and for finding possible protective remedies for such (8, 54-71).

Advantages and limitations of the slice technique

Brain slices are used because they offer certain advantages over the *in vivo* approaches to the study of the CNS. Such advantages include:

1. Rapid preparation, using rather relatively inexpensive animals (mouse, rat, guinea pigs...etc.) where anesthetics are not necessary.
2. Mechanical stability of the preparation, due to lack of heart beat and respiratory pulsation. Such conditions permit intracellular recordings for long periods.

3. Simple control over the condition of the preparation, where PO_2, PCO_2, pH and temperature can be controlled and maintained as desired.

4. Direct visualization of the slice structure, thus allowing the accurate placement of both recording and stimulating electrodes in desired sites.

5. Slices have no blood-brain barrier, thus their extracellular space is accessible to the perfusion medium and its content (ions, transmitters, drugs...etc.).

6. While simplified, the brain slice preparation maintains structural integrity, unlike cell cultures or tissue homogenates.

On the other hand, there are some limitations for these preparations, which include:

1. Lack of certain inputs and outputs which normally exist in the intact brain.

2. Certain portions of the sliced tissue, especially the top and bottom surfaces of the slice, are damaged by the slicing action itself.

3. The brain slice deteriorates with the passage of time and the tissue gets "older" at a much faster rate than that of the brain in the intact animal.

4. The effects of decapitation ischemia on the viability of the slice are not yet well understood.

5. Since blood-borne factors may be missing from the artificial bathing medium of the brain slice, they can not benefit the preparation and thus the optimal composition of the bathing solution has not been fully assessed.

References

1. McIlwain H, Buchel L and Chesire JD. The inorganic phosphate and phosphocreatine of brain especially during metabolism *in vitro*. Biochem J 1951; 48: 12-20.
2. Yamamoto C and McIlwain H. Electrical activities in thin sections from the mammalian brain maintained in chemically-defined media *in vitro*. J Neurochem 1966; 13: 1333-43.
3. Skerde KK and Westgaard RH. The transverse hippocampal slice: a well-defined cortical structure maintained *in vitro*. Brain Res 1971; 35: 589-93.
4. El-Sabban F, Reid KH and Edmonds HL Jr. High potassium induced epileptiform bursting in rat hippocampal slices. J Univ Malaya Med Cent 1997; 2: 95-98.
5. Reid KH, Edmonds HL Jr, Schurr A, Tseng M and West C. Pitfalls in the use of brain slices. Prog Neurobiol 1988; 31: 1-18.
6. Reid KH, Young C, Schurr A, Tseng M, Payne RS, Keelen P, Miller J and Iyer V. Audiogenic seizures following global ischemia induced by chest compression in Long-Evans rats. Epilepsy Res 1996; 23: 195-209.
7. El-Sabban F, Reid KH, Zhang YP and Edmonds HL Jr. Influence of flunarizine on photochemically-induced synaptic dysfunction in the *in vitro* rat hippocampal slices. Asia Pacific J Pharmacol 1998; 13: 1-7.
8. El-Sabban F, Reid KH and Edmonds HL Jr. Responses of rat hippocampal slices in a high-K^+ medium following *in vivo* global ischaemia. Clin Exp Pharmacol Physiol 1998; 25: 612-6.
9. Schurr A. Brain slice preparation in electrophysiology. Kopf Carrier, David Kopf Instruments 1986; No. 15: 1-5.
10. Schurr A, Reid KH, Tseng M and Edmonds HL Jr. The stability of the hippocampal slice preparation: an electrophysiological and ultrastructural analysis. Brain Res 1984; 297: 357-62.
11. Gibson IM and McIlwain H. Continuous recording of changes in membrane potential in mammalian cerebral tissues *in vitro*: recovery after depolarization by added substances. J Physiol 1965; 176: 261-83.
12. Haas HL, Schaerer B and Vosmansky M. A simple perfusion chamber for the study of nervous tissue slices *in vitro*. J Neurosci Meth 1979; 1: 323-25.
13. Teyler TJ. Brain slices preparation: hippocampus. Brain Res Bull 1980; 5: 391-403.
14. Nicoll RA and Alger BE. A simple chamber for recording from submerged brain slices. J Neurosci. Meth 1981; 4: 153-6.
15. Lipton P and Whittingham T. The effect of hypoxia on evoked potentials in the *in vitro* hippocampus. J Physiol 1979; 287: 427-38.
16. Pandanaboina MM and Sastry BR. Rat neocortical slice preparation for electrophysiological and pharmacological studies. J Pharmac Meth 1984; 11: 177-86.
17. Langmoen IA and Anderson P. The hippocampal slice *in vitro*. A description of the technique and some examples of the opportunities it offers. In: Kerkut GA, Wheal HV, Eds. Electrophysiology of Isolated Mammalian CNS Preparations. New York: Academic Press; 1981; 51-105.
18. Richard CD. The preparation of brain tissue slices for electrophysiological studies. In: Kerkut GA, Wheal HV, Eds. Electrophysiology of Isolated Mammalian CNS Preparations. New York: Academic Press; 1981; 107-32.
19. Schurr A, Reid KH, Tseng MT, Edmonds HL Jr and Rigor BM. A dual chamber for comparative studies using brain slice preparation. Comp Biochem Physiol 1985; 82A: 701-4.
20. Reid KH. BASIC programs with machine-language interpolations: a versatile tool for clinician programmer. Microcomputers in Anesthesia IV, Ashville, North Carolina, October 13-14, 1983.
21. Fisher R, Gray R and Johnston D. Properties and distribution of single voltage-gated calcium channels in adult hippocampal neurons. J Neurophysiol 1990; 64: 91-104.
22. Gray R, Rajan AS, Radcliffe KA, Yakehiro M and Dani JA. Nicotine increases presynaptic calcium and enhances glutamate release in the hippocampus. Nature 1996; 383: 713-6.
23. Luthi A, Gahwiler BH and Gerber U. A slowly inactivating potassium current in CA3 pyramidal cells of rat hippoc-

ampus *in vitro*. J Neurosci 1996; 16: 586-94.

24. Avoli M, Kohling R and Barbarosie M. Anoxia blocks the presynaptic control of GABA release at inhibitory terminals in the rat hippocampus. Neuroscience 1996; 75: 999-1002.
25. Schmitz D, Empson RM and Heinemann U. Serotonin reduces inhibition via 5-HT1A receptors in area CA1 of rat hippocampal slices *in vitro*. J Neurosci 1995; 15: 7217-25.
26. Steinhauser C, Jabs R and Kettenmann HP. Properties of GABA and glutamate responses in identified glial cells of the mouse hippocampal slice. Hippocampus 1994; 4: 19-35.
27. Masino SA and Dunwiddie TV. Temperature-dependent modulation of excitatory transmission in hippocampal slices is mediated by extracellular adenosine. J Neurosci 1999; 19: 1932-9.
28. Buldakova S, Dutova E, Ivlev S and Weiss M. Temperature change-induced potentiation: a comparative study of facilitatory mechanisms in aged and young rat hippocampal slices. Neuroscience 1995; 68: 395-7.
29. Schurr A, Payne RS, Miller JJ and Rigor BM. Study of cerebral energy metabolism using the rat hippocampal slice preparation. Methods 1999; 18: 117-26.
30. Gasparovic C, King D and Feeney DM. Metabolism in single rat brain slices measured by magnetic resonance spectroscopy. Brain Res Brain Res Protoc 1999; 4: 97-102.
31. Siciliano JC, Gelman M and Girault JA. Depolarization and neurotransmitters increase neuronal protein tyrosine phosphorylation. J Neurochem 1994; 62: 950-9.
32. Bach ME, Barad M, Son H, Zhuo M, Lu YF, Shih R, Mansuy I, Hawkins RD and Kandel ER. Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation *in vitro* and are attenuated by drugs that enhance the cAMP signaling pathway. Proc Nat Acad Sci USA 1999; 96: 5280-5.
33. Wiegand H and Altmann L. Neurophysiological aspects of hippocampal neurotoxicity. Neurotoxicology 1994; 15: 451-8.
34. Ashton D, Willems R, Marrannes R and Janssen PA. Extracellular ions during veratridine-induced neurotoxicity in hippocampal slices: neuroprotective effects of flunarizine and tetrodotoxin. Brain Res 1990; 528: 212-22.
35. Miguel-Hidalgo JJ and Cacabelos R. Beta-amyloid (1-40)-induced neurodegeneration in the rat hippocampal neurons of the CA1 subfield. Acta Neuropathol (Berlin) 1998; 95: 455-65.
36. Imperato A, Obinu MC and Gessa GL. Stimulation of both dopamine D1 and D2 receptors facilitates in vivo acetylcholine release in the hippocampus. Brain Res 1993; 618: 341-5.
37. Stillman MJ, Shukitt-Hale B, Galli R, Levy A and Lieberman HR. Effects of M2 antagonists on *in vivo* hippocampal acetylcholine levels. Brain Res Bull 1996; 41: 221-6.
38. Woodward JJ. A comparison of the effects of ethanol and the competitive glycine antagonist 7-chlorokynurenic acid on N-methyl-D-aspartic acid-induced neurotransmitter release from rat hippocampal slices. J Neurochem 1994; 62: 987-91.
39. Scuvee-Moreau J, Seutin V and Dresse A. A quantitative pharmacological study of the cholinergic depolarization of hippocampal pyramidal cells in rat brain slices. Arch Physiol Biochem 1998; 105: 365-72.
40. McKinney M, Miller JH and Aagaard PJ. Pharmacological characterization of the rat hippocampal muscarinic autoreceptor. J Pharmacol Exp Therap 1993; 264: 74-8.
41. Mongeau R, De-Montigny C and Blier P. Effect of long-term administration of antidepressant drugs on the 5-HT3 receptors that enhance the electrically evoked release of [3H] noradrenaline in the rat hippocampus. Eur J Pharmacol 1994; 271: 121-9.
42. Gur E, Dremencov E, Lerer B and Newman ME. Venlafaxine: acute and chronic effects on 5-hydroxytryptamine levels in rat brain *in vivo*. Eur J Pharmacol 1999; 372: 17-24.
43. Johansson IM, Bjartmar L, Marcusson J, Ross SB, Seckl JR and Olsson T. Chronic amitriptyline treatment induces hippocampal NGFI-A, glucocorticoid receptor and mineralocorticoid receptor mRNA expression in rats. Brain Res Mol Brain Res 1998; 62: 92-5.
44. Langosch JM, Repp M, Berger M and Walden J. Effects of the atypical antidepressant trimipramine on field potentials in the low $Mg2^{+}$-model in guinea pig hippocampal slices. Eur Neuropsychopharmacol 1998; 8: 209-12.
45. Uz T and Manev H. Chronic fluoxetine administration increases the serotonin N-acetyltransferase messenger RNA content in rat hippocampus. Biol Psych 1999; 45: 175-9.
46. Ameri A. Effects of the alkaloids 6-benzoylheteratisine and heteratisine on neuronal activity in rat hippocampal slices. Neuropharmacol 1997; 36: 1039-46.
47. Ameri A. The effects of cannabinoids on the brain. Prog Neurobiol 1999; 58: 315-48.
48. Otoom S, Tian LM and Alkadhi KA. Veratridine-treated brain slices: a cellular model for epileptiform activity. Brain Res 1998; 789: 150-6.
49. Polc P, Jahromi SS, Facciponte G, Pelletier MR, Zhang L and Carlen PL. Benzodiazepine antagonists reduce epileptiform discharges in rat hippocampal slices. Epilepsia 1996; 37: 1007-14.
50. Dailey JW, Reith ME, Steidley KR, Milbrandt JC and Jobe PC. Carbamazepine-induced release of serotonin from rat hippocampus *in vitro*. Epilepsia 1998; 39: 1054-63.
51. Sayin Ü and Rutecki PA. Effects of pilocarpine on paired pulse inhibition in the CA3 region of the rat hippocampus. Brain Res 1997; 758: 136-40.
52. Sagratella S. Characterization of the *in vitro* antiepileptic activity of new and old anticonvulsant drugs. Gen Pharmacol 1998; 30: 153-60.
53. Albus H and Williamson R. Electrophysiologic analysis of the actions of valproate on pyramidal neurons in the rat hippocampal slice. Epilepsia 1998; 39: 124-39.
54. Li MM, Payne RS, Reid KH, Tseng MT, Rigor BM and Schurr A. Correlates of delayed neuronal damage and neuroprotection in a rat model of cardiac-arrest-induced cerebral ischemia. Brain Res 1999; 826: 44-52.
55. Charchaflieh J, Cottrell JE and Kass IS. The effect of fentanyl on electrophysiologic recovery of CA1 pyramidal cells

from anoxia in the rat hippocampal slice. Anesth Analg 1998; 87: 68-71.

56. Onitsuka M, Mihara S, Yamamoto S, Shigemori M and Higashi H. Nitric oxide contributes to irreversible membrane dysfunction caused by experimental ischemia in rat hippocampal CA1 neurons. Neurosci Res 1998; 30: 7-12.
57. Obeidat AS and Andrew RD. Spreading depression determines acute cellular damage in the hippocampal slice during oxygen/glucose deprivation. Eur J Neurosci 1998; 10: 3451-61.
58. Onitsuka M, Mihara S, Inokuchi H, Shigemori M and Higashi H. Mild hypothermia protects rat hippocampal CA1 neurons from irreversible membrane dysfunction induced by experimental ischemia. Neurosci Res 1998; 30: 1-6.
59. Zapater P, Moreno J and Horga JF. Neuroprotection by the novel calcium antagonist PCA50938, nimodipine and flunarizine, in gerbil global brain ischemia. Brain Res 1997; 772: 57-62.
60. Okada M, Amamoto T, Tomonaga M, Kawachi A, Yazawa K, Mine K and Fujiwara M. The chronic administration of docosahexaenoic acid reduces the spatial cognitive deficit following transient forebrain ischemia in rats. Neuroscience 1996; 71: 17-25.
61. Taylor CP, Burke SP and Weber ML. Hippocampal slices: glutamate overflow and cellular damage from ischemia are reduced by sodium-channel blockade. J Neurosci Meth 1995; 59: 121-8.
62. Kolb SJ, Hudmon A and Waxham MN. Ca2+/calmodulin kinase II translocates in a hippocampal slice model of ischemia. J Neurochem 1995; 64: 2147-56.
63. Raley-Susman KM and Murata J. Time course of protein changes following *in vitro* ischemia in the rat hippocampal slice. Brain Res 1995; 694: 94-102.
64. Djuricic B, Berger R and Paschen W. Protein synthesis and energy metabolism in hippocampal slices during extended (24 hours) recovery following different periods of ischemia. Metab Brain Dis 1994; 9: 377-89.
65. Yasui M and Kawasaki K. Vulnerability of CA1 neurons in SHRSP hippocampal slices to ischemia, and its protection by $Ca2^{+}$ channel blockers. Brain Res 1994; 642: 146-52.
66. Lobner D and Lipton P. Intracellular calcium levels and calcium fluxes in the CA1 region of the rat hippocampal slice during *in vitro* ischemia: relationship to electrophysiological cell damage. J Neurosci 1993; 13: 4861-71.
67. Vornov JJ and Coyle JT. Enhancement of NMDA receptor-mediated neurotoxicity in the hippocampal slice by depolarization and ischemia. Brain Res 1991; 555: 99-106.
68. Cherici G, Alesiani M, Pellegrini-Giampietro DE and Moroni F. Ischemia does not induce the release of excitotoxic amino acids from the hippocampus of newborn rats. Brain Res Devl 1991; 60: 235-40.
69. Jensen MS, Lambert JD and Johansen FF. Electrophysiological recordings from rat hippocampus slices following *in vivo* brain ischemia. Brain Res 1991; 554: 166-75.
70. Raley-Susman KM and Lipton P. *In vitro* ischemia and protein synthesis in the rat hippocampal slice: the role of calcium and NMDA receptor activation. Brain Res 1990; 515: 27-38.
71. Amagasa M, Ogawa A and Yoshimoto T. Effects of calcium and calcium antagonists against deprivation of glucose and oxygen in guinea pig hippocampal slices. Brain Res 1990; 526: 1-7.

FACTORS AFFECTING SEVERITY OF NEONATAL JAUNDICE IN PATIENTS PRESENTING FROM HOME

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ABSTRACT: A descriptive study of full- term neonates with jaundice was carried out to determine factors affecting severity of neonatal jaundice for those infants presenting to the hospital from their homes. Severe jaundice (serum bilirubin ≥250 μmol/l) was significantly more likely in infants whose mothers consumed traditional herbs during the postpartum period ($p<0.001$) and if the jaundice was first detected by the parents or relatives rather than by medical personnel ($p<0.05$). In addition, the interval between detection of jaundice and presentation to hospital was significantly longer in jaundice that was first detected by parents compared to those detected by medical personnel. In conclusion, factors affecting severity of neonatal jaundice for infants who present from home could be influenced by the socio-cultural practices of maternal postpartum use of herbs, the ability of parents to detect jaundice and the urgency of the parents in seeking medical treatment once the jaundice was detected. Further studies need to be done to explore the association of these factors with neonatal jaundice more specifically. *(JUMMEC 2000; 1: 24-27)*

KEYWORDS: Hyperbilirubinaemia, socio-cultural practices, herbs.

Introduction

Neonatal jaundice is a very commonly encountered problem, which may affect up to 60% of newborn infants (1). Causes of neonatal jaundice include Rhesus and ABO incompatibility, erythrocytic Glucose-6-Phosphate Dehydrognase (G6PD) deficiency, prematurity and sepsis (2). Recent studies have demonstrated several other factors associated with neonatal jaundice. These include breast- feeding, weight loss after birth, oriental race, male sex and low gestational age (3). However, up to 55-68% of infants with jaundice have no apparent cause found (3, 4).

With the common practice of early postpartum discharge from hospital, very much is dependent on the ability of the parents to detect jaundice at home. Furthermore, socio- cultural practices by nature of the different ethnic groups may also contribute to the severity of neonatal jaundice at presentation to hospital.

An understanding of these factors would assist doctors in determining which infants are at risk of presenting with more severe jaundice if discharged during the early postpartum period and thus provide closer supervision and education to the parents of these at-risk infants.

This study therefore sets out to determine whether socio- cultural factors and the ability of parents to detect jaundice at home have any influence on the severity of neonatal jaundice at presentation.

Materials and methods

This study was conducted in the Paediatric Institute, Kuala Lumpur Hospital, Malaysia, a tertiary referral centre for paediatric patients in the country. The hospital however, also caters for the general health needs of the middle and lower income groups in the city. The community is made up of three main ethnic groups, namely the Malays, Chinese and Indians.

Newborn infants that were admitted to the ward for clinical neonatal jaundice were identified by daily ward review over a one- month period. Babies who were found to have clinical jaundice in the out- patient department were generally admitted to the ward and started empirically on phototherapy while awaiting the serum bilirubin level results. Thus, the majority of infants who presented with neonatal jaundice to the hospital were seen in the ward.

Information on the birth, nutrition, socio-cultural

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practices and the detection of jaundice was obtained from direct interview of the parents. The information was also supplemented by data in the patients' medical records as well as from the baby's growth and development card given to the parents following birth.

G6PD deficiency screening is routinely performed in all infants at birth and is normally recorded in the child development card. With regard to the detection of jaundice, parents were asked about the person who first detected the jaundice in their child. The detection of jaundice could be made by the parents themselves, relatives or medical personnel. In our health system, newborn babies who were discharged from the hospital were normally followed up with home visits by the public health nurse or midwife. Jaundice might be first detected by the nurses during such home visits or coincidentally by doctors when parents brought the infant to the doctor for other medical complaints.

These neonates were included in the study if they have been born at home or have been earlier discharged home from the maternity ward. Only infants with unconjugated jaundice and without any defined cause to account for the jaundice were selected. Infants were excluded from the study if an apparent cause of the jaundice was found. These causes included Rhesus and ABO incompatibility, G6PD deficiency, sepsis or suspected sepsis, prematurity (gestation< 37 weeks) or low birth weight (weight< 2.5 kg), polycythaemia and cephalohaematoma. Infants who developed jaundice while still in the maternity ward were also excluded from the study. Informed verbal parental consent was obtained for each of these selected infants before the investigator conducted the interview or extracted information from the patient's medical records.

Data from a total of 98 patients with neonatal jaundice were collected. From these patients, 16 patients were born premature or of low birth weight, 12 were diagnosed as having sepsis or suspected sepsis, 5 had G6PD deficiency, one had ABO incompatibility while another one was an infant of diabetic mother with polycythaemia and suspected sepsis. These 35 patients had a cause to account for the jaundice and were excluded, while the remaining 63 patients formed the study population.

A total serum bilirubin (SB) level on admission of 250 μmol/l (15 mg/dl) or more is regarded as significant (5), and forms the threshold level where phototherapy would be required (1, 6).

The patients were divided into the high bilirubin group (SB>=250μmol/l) and the low bilirubin group (SB<250μmol/l). The analysis of data was then done using the chi- square test and the Fisher's exact test for qualitative data and the student- t test for quantitative data to determine factors contributing to the high bilirubin level, with significance level at 0.05.

Table 1. Characteristics of infants with neonatal jaundice (n=63).

Age, days	
Mean ± S.D.	5.7 ± 3.1
Range	2 – 17
Gender (Male/ Female)	34/29
Birth Weight, kg	
Mean ± SD.	3.2 ± 0.4
Range	2.6 – 4.2
Ethnic Group (%)	
Malay	44 (69.8)
Chinese	13 (20.6)
Indian	5 (6.4)
Others	1 (1.3)
Feeding (%)	
Fully breast-fed	46 (73.0)
Mixed breast/ bottle	15 (23.8)
Fully bottle-fed	2 (3.2)
Total serum bilirubin, μmol/l	
Mean ± SD.	250 ± 56
Range	124 – 401

Results

The characteristics of the 63 infants included in the study are as outlined in Table 1. Out of the 63 patients, 32 (50.8%) of them had a SB level of 250 μmol/l or more while the remaining 31 infants had a SB level of less than 250 μmol/l.

On analysis of qualitative data, factors significantly associated with high SB levels were consumption of traditional herbs in the immediate postpartum period ($p<0.001$) and the initial detection of jaundice by parents or relatives rather than by medical personnel ($p<0.05$). No significant association was found to relate high SB levels with gender and ethnic group, neither was there any association with whether the infant was fully breast-fed or not (Table 2).

There was significantly longer lag time from detection of jaundice to admission in the group where the jaundice was first detected by parents/relatives compared to the group whose jaundice was first detected by medical personnel. However, no significant difference in this interval was found when comparing the group where there was maternal consumption of traditional herbs with the group where there was not. (Table 3).

On analysis of quantitative data, factors such as the interval from the detection of jaundice to admission, age on admission, maternal age, parity and birth weight have not been found to be significantly associated with high SB levels. This is shown in Table 4.

Table 2. Analysis of Qualitative Data For Factors Associated with Severe Jaundice (Bilirubin level ≥ 250 μmol/l).

Factors	Total number Of patients	Number with severe jaundice	Rate ratio (95% CI)	p value
Feeding				
Fully breast-fed	46	21	0.7(0.4-1.1)	NS
Mixed/ Bottle-fed	17	11		
Gender				
Male	34	15	0.8(0.5-1.2)	NS
Female	29	17		
Ethnic group				
Malay	44	20		
Chinese	13	9	-	NS
Indian	5	3		
Maternal postpartum consumption of herbs				
Yes	22	18	2.4(1.4-3.8)	<0.001
No	41	14		
First person to detect jaundice*				
Parents/ Relatives	33	21	1.7(1.1-2.5)	0.04
Medical personnel	29	11		

* Data missing from one patient
NS = Not significant

Discussion

Previous studies on factors contributing to neonatal jaundice have mainly focused on antenatal, birth and feeding factors, as well as factors associated with the infant (3, 7, 8, 9). Furthermore, in these studies, all the infants stayed in the ward throughout the duration of the study. However, the variables that may affect severity of jaundice at presentation can become much more complicated in situations where the infant was brought in for jaundice from home, having had early postpartum discharge from the hospital or having been born at home.

Besides the contributory factors enumerated above, the severity of neonatal jaundice on admission in such situations might also be determined by several other factors. These factors included firstly, the socio-cultural practices in the homes, secondly, the ability of the parents or health personnel in detecting jaundice in the infant, and thirdly, the response of the parents in seeking medical help once the jaundice was detected.

This study has demonstrated that the socio-cultural practice of consuming traditional herbs amongst mothers in the postpartum period had significant association with more severe neonatal jaundice. This association can be interpreted from different angles. The association could mean that the practice of using traditional herbs might be more prevalent among those who would try traditional remedies initially in treating neonatal jaundice and thus present late to the hospital.

Table 3. Analysis of variables associated with prolonged interval between detection of jaundice and presentation to hospital.

Variables	Interval, days ± S.D.	p value
Postpartum maternal consumption of herbs		
Yes (n=22)	1.7 ± 1.5	NS
No (n=41)	2.0 ± 3.7	
First person to detect jaundice *		
Parent /relative (n=33)	2.8 ± 3.3	0.03
Medical personnel (n=29)	1.0 ± 2.8	

* Data missing from one patient
NS = not significant

Table 4. Analysis of Quantitative Data of Factors Associated with Severe Jaundice (Serum Bilirubin, SB level ≥ 250 μmol/l).

Variables	SB<250 μmol/l (n=31)	SB≥250 μmol/l (n=32)	p value
Interval, days*	1.5±2.9	2.3±3.3	NS
Parity	2.5±1.7	2.7±1.5	NS
Maternal Age, years	27.6±4.8	28.7±5.3	NS
Birth weight, kg	3.1±0.4	3.2±0.3	NS
Age of infant, days	5.1±2.9	6.3±3.1	NS

* Interval between detection of jaundice and presentation to hospital
NS = Not significant

However, this had not been shown to be the case in this study as the interval between detection of jaundice and admission did not differ between those who used herbs compared to those who did not.

Another more plausible explanation would be the direct effects of the herbs in causing severe jaundice in infants. As almost all infants in our study were still being breast-fed (either fully or partially) on admission, the herbs consumed by the mother might had been excreted via breast milk and transmitted to the infant. Literature had shown that some Chinese herbal medicines could displace the bilirubin molecule from their serum protein binding sites and increase the risk of neonatal jaundice (10, 11). Traditional herbs from the Malays and Indians might also have similar effects. In the light of the findings in this study, the contribution of traditional herbs in causing severe neonatal jaundice would warrant deeper analysis and review.

The ability of the parents in detecting neonatal jaundice has also been demonstrated in this study to be an important factor in determining the severity of neonatal jaundice at presentation to hospital. A higher proportion of jaundice that were detected by parents or relatives presented with higher bilirubin levels compared to jaundice that were detected by medical personnel. With the practice of early postpartum discharge of mother and infant, the responsibility to detect jaundice would fall on the parents. It would therefore be important to train parents to accurately detect and assess progression of jaundice in their infant. Parents who were trained had been shown in one study to be able to detect and estimate the degree of jaundice with high correlation with actual bilirubin levels (12).

In this study, it has been shown that there was a shorter interval between the time of detection and admission if the jaundice was detected by medical personnel. This might imply that parents tend to respond more promptly in bringing their infant to hospital if the jaundice was first detected by medical personnel, compared to jaundice that was detected by the parents themselves. Parents might experience indecision whether to bring the infant in for further assessment if they themselves detected the jaundice. On the other hand, parents might be more inclined to promptly follow the instructions of the medical personnel who first detected the jaundice, as the medical personnel might be perceived by the parents to be more experienced in jaundice detection.

In conclusion, the severity of neonatal jaundice for infants that were brought from home to hospital could be related to socio- cultural practices in the homes, particularly with regard to postpartum maternal consumption of traditional herbs, the ability of the parents to detect jaundice and the response of the parents once the jaundice was detected. Larger, detailed studies would be required to confirm these findings and explore each of the three factors more specifically.

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References

1. Provisional Committee for Quality Improvement and Subcommittee on Hyperbilirubinemia, American Academy of Pediatrics. Practice parameter: Management of hyperbilirubinemia in the healthy term newborn. Pediatrics 1994; 94: 558-65.
2. Wong HB. Neonatal hyperbilirubinaemia. The Bulletin of the Kandang Kerbau Hospital Singapore 1964; 3: 1-7.
3. Maisels MJ, Gifford K, Antle CE, Leib GR. Jaundice in the healthy newborn infant: A new approach to an old problem. Pediatrics 1988; 81: 505-11.
4. Guaran RL, Drew JH, Watkins AM. Jaundice: clinical practice in 88000 liveborn infants. Aust N Z J Obstet Gynaecol 1992; 32: 186-92.
5. Tan KL. Glucose-6-phosphate dehydrogenase status and neonatal jaundice. Arch Dis Child 1981; 56: 874-7.
6. Tan KL, Boey KW. Clinical experience with phototherapy. Annals Acad Med Singapore 1989; 18: 43-8.
7. Bracci R, Buonocore G, Garosi G, Bruchi S, Berni S. Epidemiologic study of neonatal jaundice. Survey of contributing factors. Acta Pediatr Scand 1989; 360: 87-92.
8. Fok TF, Lau SP, Hui CW. Neonatal jaundice: Its prevalence in Chinese babies and associating factors. Aust Paediatr J 1986; 22: 215-9.
9. Tudehope D, Bayley G, Munro D, Townsend S. Breast feeding practices and severe hyperbilirubinaemia. J Paediatr Child Health 1991; 27: 240-4.
10. Chan TY. The prevalence use and harmful potential of some Chinese herbal medicines in babies and children. Vet Hum Toxicol 1994; 36: 238-40.
11. Yeung CY, Leung CS, Chen YZ. An old traditional herbal remedy for neonatal jaundice with a newly identified risk. J Paediatr Child Health 1993; 29: 292-4.
12. Madlon- Kay DJ. Recognition of the presence and severity of newborn jaundice by parents, nurses, physicians and icterometer. Pediatrics 1997; 100: E3.

THE USE OF STEROID THERAPY AND ITS IMPACT ON CHILDREN HOSPITALISED WITH VIRAL CROUP

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ABSTRACT: The use of steroid therapy and its treatment impact on children hospitalised with viral croup between 1994 - 1998 were reviewed by comparing 30 patients who received steroid therapy with 65 patients who did not. There was no difference in the socio-demographic and clinical profile between the two groups. The hospital stay (2.8 ± 1.3 vs 3.5 ± 1.2 days, $p = 0.02$) and duration of documented stridor (1.3 ± 0.5 vs 2.8 ± 1.0 days, $p = 0.001$) were shorter for patients who received steroid therapy although they appeared to have more severe respiratory distress. There were no complications associated with steroid therapy use. Despite reluctance in administering steroid therapy for viral croup, patients who received the treatment had a shorter hospital stay and duration of stridor. *(JUMMEC 2000; 1: 28-32)*

KEYWORDS: Croup, steroid therapy, hospital stay.

Introduction

Viral croup usually occurs in the second year of life and presents with respiratory symptoms over a few days followed by bark-like coughing, stridor and respiratory distress of varying severity. Although viral croup is essentially a benign self-limiting illness, the stridor associated with respiratory distress and feeding difficulties is uncomfortable for the child and worry most parents prompting them to seek medical attention.

Viral croup does not appear to be a major problem in the tropical region where it accounts for less than 5% of all childhood respiratory tract infections (1, 2). Although this observation markedly contrasts that found in the temperate region (3), it does not underscore the need for an effective treatment for viral croup in children in this region. Steroid therapy has in the last few years been shown to be effective in reducing the severity of symptoms and shortening the duration of illness in viral croup. As steroid therapy favourably alters the course of illness with very little side-effects encountered, its use is now recommended as routine treatment for viral croup (4, 5).

Steroid therapy has been used in our unit for children with viral croup since late 1997. We therefore set out to evaluate the impact of steroid therapy on the outcome of viral croup by comparing patients admitted for viral croup who received steroid therapy and those who did not receive steroid therapy.

Materials and methods

Patient population

We reviewed the medical records of all children admitted with a diagnosis of viral croup according to Court's criteria (6) to the Department of Paediatrics, University Malaya Medical Centre between 1st January 1994 and 31st December 1998.

Patient characteristics

Demographic and clinical data was extracted from the medical records. The severity of illness at admission was divided into mild (stridor when agitated only with no subcostal/intercostal recession), moderate (stridor at rest with subcostal/intercostal recession) and severe (stridor with severe respiratory distress and cyanosis or altered level of consciousness). In addition, the croup score (Table I) was calculated for each patient to reflect the degree of respiratory distress (7). The degree of respiratory distress was proportional to the croup score.

Treatment and outcome

The attending physician that managed the individual patient determined the treatment strategy including the

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use of steroid therapy. Steroid therapy for the treatment of viral croup was only routinely used in our unit since November 1997. Patients were then divided into Group I (patients who received steroid therapy) and Group II (patients who did not receive steroid therapy). Patients in Group I either received dexamethasone or nebulised budesonide (Figure I). Information concerning additional treatment given to both groups such as nebulised adrenaline, oxygen therapy and intravenous fluids were also reviewed.

Statistical analysis

The students' t test was done to compare quantitative data and dichotomous variables were compared using the Fishers exact test. A p value of less than 0.05 was considered as significant.

Results

A total of 95 patients with a diagnosis of viral croup were admitted during the study period of which 30 patients (32%) received steroid therapy (22 patients received dexamethasone, 4 patient received necbulised budesonide and 4 patients received both). The majority of patients (70%) who received steroid therapy were admitted in 1998 (Figure II). There was no difference in the socio-demographic and clinical profile of both groups except that patients in Group I appeared to have more severe respiratory distress as they had a higher croup score and respiratory rate (Table II).

There were no deaths encountered during the study period but 5 patients developed pneumonia, 2 patients had bacterial tracheitis and 2 patient required mechanical ventilation for severe upper airway obstruction. However, there was no difference in the development of these complications between the two groups (10% vs 9%, p = 0.38).

The duration of hospital stay of patients in Group I was significantly shorter (2.8 ± 1.3 vs 3.5 ± 1.2 days, p value = 0.02) and these patients also recovered faster as the period of stridor documented in the ward was shorter (1.3 ± 0.5 vs 2.8 ± 1.0 days, p value = 0.001). As expected, patients in Group I were more likely to require O_2 supplement as they had more severe respiratory distress. The use of nebulised adrenaline was low in this study population as only 7 patients (7%) received it. There was no significant difference in the additional treatment strategy provided between the two groups (Table II). No patient in Group I developed side effects from the use of steroids. None of the patients returned with stridor after discharge.

Discussion

The management strategy adopted for viral croup in view of its benign self-limiting nature has essentially been

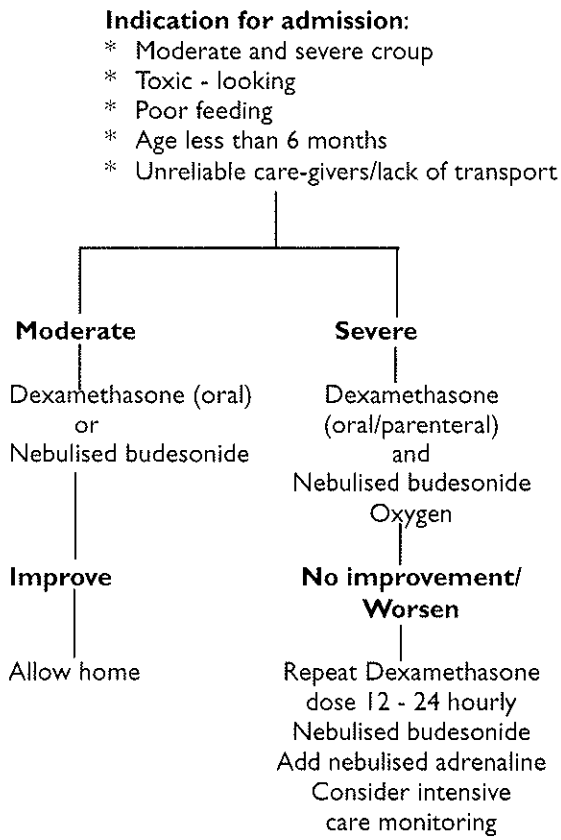

Figure I. Treatment protocol algorithm for patients hospitalised with viral croup.

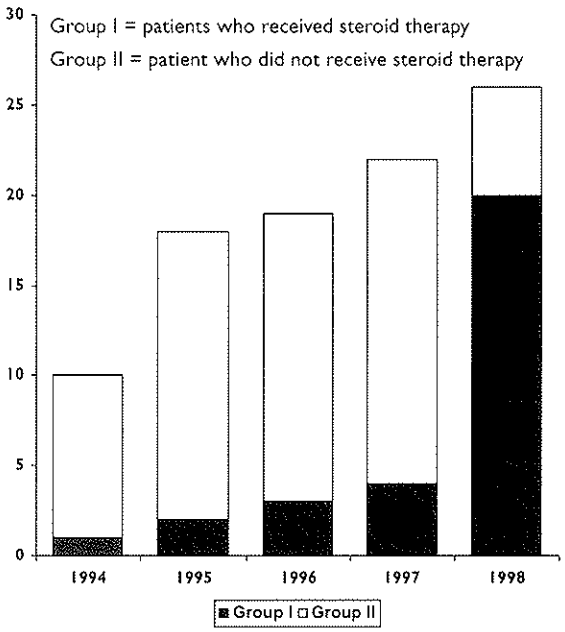

Figure 2. The use of steroid therapy between 1994-1998 (n=95)

of clinical vigilance and symptom relief with the use of mist tents, oxygen supplementation and nebulised adrenaline. Nonetheless this view has changed over the years with the recognition of the efficacy of steroid therapy in viral croup as illustrated by the trend of its use in our unit. In addition, a hospitalisation rate of up to 20% and intubation rate of up to 3% documented in two large case series of viral croup warrants a more aggressive approach in its treatment (8, 9).

There has been ample debate and scepticism over the use of steroid therapy in viral croup since it was first advocated in the 1950s and evidence for its use has been convincing only since the 1990s. Several studies have demonstrated that steroid therapy when compared to a placebo resulted in a faster clinical improvement, shorter hospital stay and a reduced need for intubation (10, 11, 12, 13). Although our observational study was not specifically meant to evaluate the efficacy of steroid therapy, its results re-affirm these findings and provide additional evidence for its routine use. Nonetheless the actual mechanism of action of steroids in viral croup is largely unknown, but believed to be the reduction of subglottic oedema by decreasing local blood flow and vascular permeability.

The concern that the use of steroid therapy may render the patient vulnerable to systemic bacteria or viral infection has instilled reluctance for its wide routine use. Our study population was no exception as despite the introduction of a clinical practice guideline (CPG) for the use of steroid therapy in viral croup, CPG non-adherence of 23% was still encountered in 1998. Practically all studies done to evaluate the use of steroid therapy in viral croup demonstrated an excellent safety profile (14). One patient in each group of our study population developed bacterial tracheitis (*Eschericia coli and Staphylococcus aureus* respectively) that was evident at admission and not likely associated with steroid therapy. There has been only three reported cases of bacterial tracheitis associated with the use of steroid therapy of whom two of the cases had an occult neutropenia (15, 16).

There is still difficulty in recommending the best steroid regiment as the trials that evaluated the efficacy of steroid therapy in viral croup used different routes of administration and doses. Smaller doses of dexamethasone have been shown to be just as effective as larger doses and there is no difference in the efficacy between nebulised budesonide and systemic steroids (10, 17). Interestingly, the onset of action with the use of nebulised budesonide is associated with more rapid onset of action of within 2 - 4 hours compared with systemic steroids (18). A combination of nebulised budesonide and dexamethasone has been shown to be even more effective (19) and is perhaps the ideal combination for the more severe cases. In our unit, dexamethasone is the preferred steroid therapy of choice as it is cheaper, easy to administer and well tolerated by the patient (Figure 1). Nebulisation therapy may be quite distressing for the patient and can worsen the upper airway obstruction in an already uncomfortable anxious child.

Table I. Croup Severity Scoring System (7).

Symptom	Score	Total Cumulative score
Stridor		2
None	0	
When agitated	1	
At rest	2	
Retraction		5
None	0	
Mild	1	
Moderate	2	
Severe	3	
Air entry		7
Normal	0	
Decreased	1	
Markedly decreased	2	
Cyanosis in room air		12
None	0	
With agitation	4	
At rest	5	
Level of consciousness		17
Normal	0	
Disorientated	5	

It is apparent that steroid therapy should be advocated in patients hospitalised with viral croup in view of the strong clinical evidence to support its use. However, it is more difficult to advocate its routine use in mild cases that are not distressed, feeding well and are expected to recover spontaneously. Recent studies have shown that when steroids given to patients with mild croup in the emergency department, its use was associated with a reduced need for hospitalisation and a shorter duration of illness (20, 21). This dilemma usually faced in the out-patient and emergency unit can perhaps be best addressed by prescribing steroid therapy to a selected group of patients who upon discharge may not seek medical attention should the respiratory distress worsen. This selected group of patients may include those with a lack of transport, stay in homes with difficult access to a health-provider facility, poor socio-economic circumstances and unreliable caretakers.

Nebulised racemic adrenaline has been to shown to offer effective relief in patients with moderate to severe croup as it reduces laryngeal mucosal oedema by causing constriction of the mucosal capillaries (22, 23). However, very few patients received nebulised adrenaline in our

Table 2. Demographic, clinical profile and severity of illness between Group and Group II (n=95)

	Group I (n =30)	Group II (n=65)	*p* value
Socio-demograhic Profile			
Mean age (months)	14.3 ± 8.4	12.7 ± 6.4	0.24
Sex distribution (M:F)	2.1 : 1	3.6 : 1	0.28
Ethnic distribution			
Malay	43%	37%	
Chinese	40%	49%	0.90
Indian	14%	14%	
Social class IV and V **	17%	15%	0.92
Family monthly income (RM)	1691 ± 752	1889 ± 689	0.54
Weight less than 3rd centile	7%	10%	0.13
Clinical Profile			
Duration of symptoms (days)	2.5 ± 1.6	2.2 ± 1.3	0.27
Fever	87%	86%	0.57
Cough	93%	95%	0.61
Feeding difficulty	73%	57%	0.08
SpO_2	94 ± 8	97 ± 3	0.09
Heart rate (per minute)	154 ± 21	153 ± 16	0.49
Respiratory rate (per minute)	52 ± 10	49 ± 9	0.02*
Severity of illness			
Severe illness	10%	6%	0.07
Croup Score	4.7 ± 2.0	3.9 ± 1.5	0.04*
PICU admission	20%	12%	0.57
Treatment required			
O_2 supplement	25%	12%	0.03*
Intravenous fluid	27%	21%	0.46
Nebulised adrenaline	5%	9%	0.21

* significant p value

**Social class was determined according to the UK General Registrars occupational classification based on the father's occupation.

unit as the racemic from of adrenaline is not available here and concern of worsening of the initial clinical picture described as the "rebound phenomenon". Nebulised adrenaline may be nonetheless very useful in conjunction with steroid therapy as it can provide temporary relief for a distressed patient and provide the time necessary for the steroids to take effect.

There is little doubt that the benefit of steroid therapy use has had a great impact in developed nations where treatment of viral croup cost up to USD 60 million a year (24), the majority attributable to in-patient care. In tropical nations like Malaysia, viral croup appears less prevalent as it accounts for only 2% of all patient admitted with lower respiratory tract infection (25); an observation that possibly reduces the cost benefit impact of steroid therapy compared to our more developed counterparts. Nonetheless, the benefit and safety evident from its use should not be denied to patients admitted with viral croup in this region and despite concerns about the best route and optimal dose of steroid therapy, there is little reason not to advocate its routine use.

References

1. Suwanjutha S, Chantarojanasiri T, Watthana-Kasetr S *et al.* A study of nonbacterial agents of lower respiratory tract infection in Thai children. Rev Infect Dis 1990; 12: S923–8.
2. Huq F, Rahman M, Nahar N *et al.* Acute lower respiratory tract infection due to virus among hospitalised children in Dhaka, Bangladesh. Rev Infect Dis 1990; 12 : S982–6.
3. Wright AL, Taussig LM, Ray CG *et al.* The Tuscon children's respiratory study II: Lower respiratory illness in the first year of life. Am J Epidemiol 1989; 129 : 1232-46.
4. Macdonald WBG, Geelhoed GC. Management of child-

hood croup. Thorax 1997; 52 : 757-9.

5. Kaditi AG, Wald ER. Viral croup: current diagnosis and treatment. Pediatr Infect Dis J 1998; 17 : 827-34.
6. Court SDM. The definition of acute respiratory illnesses in children. Postgrad Med J 1973; 49: 771-6.
7. Westley CR, Cotton EK, Brooks JG. Nebulised recemic epinephrine by intermittent positive pressure breaths (IPPB) for the treatment of croup : a double-blind study. Am J Dis Child 1978; 132 : 484-7.
8. Sendi K, Srysdale WS, Yoo J. Tracheitis: outcome of 1700 cases presenting to the emergency department during two years. J Otolaryngol 1992; 21 : 20-4.
9. Geelhoed GC. Sixteen years of croup in a western Australian teaching hospital: effects of routine steroid treatment. Ann Emerg Med 1996; 28 : 621-6.
10. Geelhoed GC, Macdonald WBG. Oral and inhaled steroids in croup: a randomised, placebo-controlled trial. Pediatr Pulmonol 1995; 20 : 355-61.
11. Kuusela A, Vesikari T. A randomised, double blind, placebo-controlled trial of dexamethasone and racemic epinephrine in the treatment of croup. Acta Paediatr Scand 1988; 77 : 99-104.
12. Godden CW, Campbell MJ, Hussey M, Cogswell JJ. Double blind placebo controlled trial of nebulised budesonide for croup. Arch Dis Child 1997; 76 : 155-8.
13. Geelhoed GC, Macdonald WBG. Oral dexamethasone in the treatment of croup: 0.15 mg/kg versus 0.3 mg/kg versus 0.6 mg/kg. Pediatr Pulmonol 1995; 20 : 362-8.
14. Yates RW, Doull IJ. A risk benefit assessment of corticosteroids in the management of croup. Drug Saf 1997; 16 : 48-55.
15. Johnson DW, Schuh S, Koren G, Jaffe DM. Out-patient treatment of croup with nebulised dexamethasone. Arch Pediatr Adolesc Med 1996; 150 : 349-55.
16. Burton DM, Seid AB, Kerns DB, Pransky SM. *Candida* laryngotracheitis : a complication of combined steroid and antibiotic use in croup. Int J Pediatr Otolaryngol 1992; 23 : 171-5.
17. Geelhoed GC, Macdonald WBG. Oral and inhaled steroids in croup: a randomised, placebo-controlled trial. Pediatr Pulmonol 1995; 20 : 355-61.
18. Klassen TP, Feldman ME, Watters LK, Sutcliffe T, Rowe PC. Nebulised budesonide for children with mild-moderate croup. N Eng J Med 1994; 331 : 285-9.
19. Klassen TP, Watters LK, Feldman ME, Sutcliffe T, Rowe PC. The efficacy of nebulised budesonide in dexamethasone treated outpatients with croup. Pediatrics 1996; 97 : 463-6.
20. Geelhoed GC, Turner J, Macdonald WBG. Efficacy of small single dose of oral dexamethasone for out-patient croup: a double blind placebo controlled clinical trial. Bri Med J 1996; 313 : 140-2.
21. Cruz MN, Stewart G, Rosenberg N. Use of dexamethasone in the out patient management of acute laryngotracheitis. Pediatrics 1995; 96 : 220-3.
22. Fogel JHM, Berg IJ, Gereber MA, Sherter CB. Racemic epinephrine in the treatment of croup: nebulisation alone versus nebulisation with intermittent positive pressure breathing. J Pediatr 1982; 101 : 1028-31.
23. Corneli HM, Bolte RG. Outpatient use of racemic epinephrine in croup. Am Fam Physician 1992; 46 : 683-4.
24. Henrickson KJ, Kuhn SM, Savatski LL. Epidemiology and cost of infection with human parainfluenzae virus types 1 and 2 in young children. Clin Infect Dis 1997; 18: 770–9.
25. Chan PWK, Goh AYT, Chua KB, Khairullah NS, Hooi PS. Viral aetiology of lower respiratory tract infection in young Malaysian children. J Pediatr Child Health 1999; 35: 287-90.

PATTERN OF HEAD INJURY IN MOTORCYCLE FATALITIES

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ABSTRACT: Head injury contributes to a significant proportion of motorcycle fatalities. The site and pattern of head injury in victims of fatal motorcycle crashes in 1995 were studied retrospectively. The site of impact on the head was determined based on the injuries on the scalp, pattern of skull fractures and injuries of the brain. Of 54 cases, 9 had massive crush injuries. Of the remaining 45 cases, the site of impact was as follows: frontal 21, lateral 14, posterior 4, vertex 1, chin 1 and unascertainable in 4 cases. The majority of injuries occurred on the front and lateral aspects of the skull. Motorcycle helmets are currently tested for impact energy attenuation and penetration test at the vertex. Helmet standards need to take into account site and nature of injury. *(JUMMEC 2000; 1: 33-35)*

KEYWORDS: Head injury, motorcycle fatalities, helmet standards.

Introduction

With control of communicable diseases, injuries are emerging as an important problem in developing countries. Malaysia is no exception and injuries of all types are a major health problem (1). The investigation and reporting of road traffic crashes is carried out by the traffic unit of the Royal Malaysia Police using an adapted software of the Microcomputer Accident Analysis Package of Transport and Road Research Laboratory, United Kingdom. According to police data motorcyclists (riders and passengers) constituted 59.3% of road fatalities in 1998 (2). Head and "multiple" injuries were observed in 49.7% and 33% of victims of motorcycle fatalities respectively (2). "Multiple" injuries includes head injuries in this classification by the police. An earlier autopsy study of motorcycle fatalities at this centre showed that head injuries were present in 60% of victims (3).

The site and pattern of head injuries in motorcycle fatalities was studied to correlate the common sites of injuries with existing standard requirements for motorcycle helmets.

Materials and methods

In Malaysia, all fatalities due to traffic crashes require a post-mortem examination under the provisions of the Criminal Procedure Code (F.M.S. cap. 45). The University of Malaya Medical Centre, Kuala Lumpur serves as a referral centre for almost all victims of road crashes occurring in the adjacent district of Petaling Jaya. From the autopsy register for 1995, reports of motorcyclists were studied and only those with head injuries were included. The injuries on the scalp, skull and brain were charted and tabulated. The site of impact on the head was determined based on the injuries on the scalp, pattern of skull fractures and injuries on the brain. On the face and scalp, the injuries are usually localized at the site on impact. However, in some cases, there were extensive internal head injuries with little or no external injuries. In these cases, the pattern of skull fractures was used to determine the direction of force upon the head. According to Spitz, the fractures of skull usually follow the rule of thumb (4):-

- impact of the face causes fractures of the facial skeleton
- impact of the forehead causes a sagittal fracture of the base of the skull
- impact of the chin may transmit the force through the temporomandibular joints to the base of the skull and cause a "hinge" fracture.
- A side or lateral impact of the head causes similar "hinge" fracture of the base of the skull

The pattern of skull fractures with the scalp and facial injuries were evaluated together with brain injuries. Site of impact was substantiated by internal haemorrhages (extradural, subdural or subarachnoid) and lacerations or contusions on the brain (coup or contrecoup).

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Results

A total of 54 cases of head injuries were extracted from the records. Of these, 9 cases were due to massive crush injuries (e.g. head being run over by a car). The sites of impact in the remaining 45 cases is given in Table 1.

The front of the head and face was by far the most common site of impact. The next most common site was the lateral aspect (including both right and left sides). Only 4 cases were due to impact on the back of the head. Impact on the vertex was observed in only one case and in 4 cases, the direction of the impact could not be ascertained.

In 4 cases of lateral impact, the side of the impact i.e. whether right or left could not be inferred. In these instances, the victims had the classical sagittal ("hinge") fracture of the skull with an apparently uninjured scalp. The internal injuries to the brain were not specific enough. Hence, lateral impact were charted as such without giving the direction of laterality (right or left).

Case studies

Case 1. A 19 year adult male motorcyclist, collided with a stationary lorry parked at the side of the road. He died on the spot. Autopsy examination showed deep lacerations on the face with underlying comminuted fractures of the facial bones and anterior cranial fossae. The frontal lobes of the brain were lacerated. In addition, he had a closed fracture of left humerus.

The impact site was determined to be frontal.

Case 2. A 22 year old adult male motorcyclist, skidded off the road and hit the ground. He was admitted to University Hospital with a serious head injury (Glasgow Coma Scale of 4/15). In view of his condition, he was treated conservatively. He succumbed to his injuries 7 hours later. Autopsy showed laceration of the right occipital region of the head, fracture of the right temporal bone of the skull radiating to the across the middle cranial fossa with contusion of the right temporal lobe of the brain. There was also subdural haemorrhage over the left temporo-parietal lobes of the brain. In addition, there was fracture of the right humerus.

The impact site was determined to be right lateral.

Case 3. A 18 year old motorcyclist, crashed into a car parked at the side of the road and then thrown off onto the road where he was ran over by an army truck. He died on the spot. He sustained extensive comminuted fractures of the skull with the brain grossly lacerated. In addition, he sustained multiple injuries including rupture of the heart.

The mechanism of injury was due to run-over by the truck.

Table 1. Site of impact to the head in 45 motorcycle fatalities, University of Malaya Medical Centre, Kuala Lumpur, 1995.

Site of impact	Number of cases
Frontal	21
Lateral	14
Posterior	4
Vertex	1
Chin	1
Unascertained	4
Total	45

The three cases illustrated the usual mode and pattern of fatal head injuries in motorcyclists.

Discussion

The common sites of impact were the front and sides of the head. Only one case showed evidence of impact to the vertex. Unfortunately, we could not correlate these injuries with the markings on the helmet as the helmet was either not brought with the victim to the hospital or not described in the autopsy report. There was also no mention as to whether the helmet remained in place i.e. on the head or dislodged from the head. Theoretically, the helmet can dislodge if the straps are not tied or are loosely tied or if there is massive force. In a few cases, we could infer that the helmet was worn properly based on the injury (or non-injury) on the scalp and strap marks on the chin. An earlier study showed that a significant proportion of motorcyclists either do not strap their helmets or strap them loosely (5). In these instances, the helmet does not confer any protection.

It is therefore concluded that available evidence points to a need for the frontal and lateral aspects of the head to be protected by the helmet.

Helmets typically have a rigid covering consisting of a stiff outer shell and a crushable liner. The stiff outer shell protects by its ability to spread a concentrated load of energy at its outer surface over a larger area. The crushable liner is a protective padding protects by its capacity to manage impact energy (6).

In some subjects who displayed no scalp injuries but had severe intracranial injuries, it is postulated that the helmet was able to withstand the impact without destruction but transmitted the energy of the impact to the skull resulting in fractures and brain injuries. It can be speculated that the protective liner in such cases was unable to manage the impact energy because it was either too soft (thus becoming flattened during the impact), too hard (thus transmitting energy without adequate absorption)or because it was too thin (thus 'bottoming out' before the energy was adequately absorbed).

Testing of helmets essentially comprises impact energy attenuation, penetration resistance, strength of retention system and effectiveness of retention system ('roll-off') (7). Of these, the two of most interest to this study are the impact energy attenuation test and the penetration resistance test.

Impact energy attenuation testing utilizes an accelerometer to measure the rate of acceleration to the head imparted by the impact. The lower the acceleration reading the better the job being performed by the helmet.

Penetration resistance testing primarily assesses the structural integrity of the helmet. As a secondary benefit, the penetration resistance test assesses the ability of the helmet to protect the head against sharp or pointed objects that might impact the head during an accident. The penetration resistance test utilizes a falling dart to create a very high point loading on the outer surface of the shell. Penetration resistance testing does not assess the ability of the helmet to resist the crushing effects of a vehicle running over the helmet but clearly a helmet that affords penetration resistance would be expected to be somewhat more rigid in this respect than one which offered no penetration resistance.

It is normally the intention of Standards (the Malaysian Standard MS-1-1996 included) that the impact energy attenuating and penetration resisting properties of a helmet be consistent over all parts of the helmet above the test line (a boundary delineating critical areas of the head that are to be protected).

Laboratories in most countries are permitted to vary the location of impact energy attenuation sites anywhere above the test line (provided that minimum distances are observed from previous impact sites on the same specimen). However, testing of helmets in a number of countries, including Malaysia, has typically concentrated penetration resistance testing within the crown area of the helmet (6). This has often been imposed by limitations on the ability of test equipment to reach the sides of the helmet. What this means is that critical areas towards the front and sides of the helmet are in some countries not assessed (8).

Impacts to the vertex of the head are rare while impacts to the front and sides form the bulk of injuries. It is inconsistent therefore that physical testing of helmets be omitted from these areas of the helmet. A good helmet should provide protection to the entire region of the front and sides of the head. Laboratory testing should as far as possible take into account the mechanism of injuries in real crash situations.

References

1. Injury Prevention and Control Unit, Disease Control Division, Public Health Department, Ministry of Health."Epidemiology of Injury in Malaysia", December 1997.
2. Polis Diraja Malaysia. Statistical Report of Road Crash. 1998.
3. Nazarina AR, Sarvesvaran R. Krishnan R. A study of motorcycle fatalities. 14th World Congress of the International Association for Accident and Traffic Medicine, Singapore, August 1995.
4. Spitz WU. The road traffic victim. In Spitz WU Ed. Medicolegal investigation of death. 3rd Edition. Springfiel; 1993: 528-66.
5. Krishnan R, Karim H. Research to prevent injuries.World Health Forum 1998;19: 159-60.
6. Standard for protective headgear. North Highlands. Snell Memorial Foundation, 1995.
7. Malaysian Standard MS 1:1988, Specification for protective helmets for vehicle users (First revision), Standards and Industrial Research Institute of Malaysia.
8. Mohan D, Kothiyal KP, Mistral BK et al. Helmet and head injury study of crash involved motorcyclists in Delhi. Proceedings of the International IRCOBI Conference on the biomechanics of impacts 1984, Netherlands September 1984. International Research Committee on Biokinetics of Impacts (IRCOBI).

DEVELOPING A WEBSITE – THE DEPARTMENT OF SOCIAL AND PREVENTIVE MEDICINE EXPERIENCE

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ABSTRACT: In 1999, the Department of Social and Preventive Medicine set up its website on the Internet (http://ummc.med.um.edu.my/spm/). Setting up the website was a whole new experience for the author and many new things were learnt along the way. This paper describes how the website was constructed, the problems, and solutions to all these problems. The experience of the author in setting up the SPM website may be useful to others wishing to set up their own department websites. *(JUMMEC 2000; 1: 36-40)*

KEYWORDS: Internet, SPM, website, web page

Introduction

The World Wide Web is barely a decade old, which in the design of most things seems such a short time. Yet in that short space of time, its growth has been nothing short of spectacular. Before its existence, many people did not even know that there was such a thing as the Internet. Today, many Malaysians surf the Web (as it is popularly called) and terms such as e-commerce, chat rooms and e-mail is no longer alien to many of us. To many of us, the Web (short for the World Wide Web) is the Internet and anything associated with the Internet has to do with the Web. Malaysia came into the Web with the setting up of its first Internet Service Provider called JARING (Joint Advanced Research in Networking) by MIMOS Berhad; a body set up at the time to promote the Internet among Malaysians. Since the introduction of JARING and the push by the government to introduce the Multimedia Super Corridor, Malaysian websites have mushroomed. Where there were no portals, there are several today all designed by and targeted at Malaysians. Examples include JARING itself, Catcha.com, Cari, NewMalaysia etc. Educational institutions were not to be left behind with many jumping on the bandwagon and setting up websites to promote themselves. Sad to say, the initial euphoria following the setting up and launching of these websites died down after the website was launched.

In the year 2000, we find many types of websites today. Newspapers are increasingly available online. Even medical journals are now published online. The mushrooming of the online edition of the British Medical Journal is one such example (1). Among the many advantages of setting up websites is the instant updating of information (2) which suits many types of information providers. In Malaysia the government's push for the construction of the Multimedia Super Corridor for example, is partly derived from the realisation that developing countries stand to be left behind in the Internet revolution if they do not act quickly. As aptly pointed out by Arunachalam (3) the Internet will eventually reduce the gap between developed and developing countries but first it will increase the gulf between the haves and the have-nots.

In 1998, no department in the University of Malaya Medical Centre (UMMC) except the Department of Physiology had a website of its own (and that was not known to many in the faculty and fewer still to those outside the faculty). Thus the Department of Social and Preventive Medicine (SPM) was among the first few departments in the faculty to actually set up a fully functional website called SPMNet.

Objectives of the website

One has to be quite clear as to the objective (4) of setting up a website and balance the questions of cost and expertise. For many institutions the main objective is to publicise oneself. Secondly, it can be used to educate and inform others. Thirdly it can be used for business or the so-called e-commerce. As a teaching department, the SPM Department depends on a regular supply of clients for its programs to ensure its survival. Publicity

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is thus considered an important thing and a website would thus help to publicise the department and its programs internationally. Thus the first objective was considered the most important followed by the second objective.

Cost of setting up a website

The cost of setting up an independent website (unique domain name and separate line) within Malaysia can be quite prohibitive. Aside from the cost of a server and related software, a digital leased line (bandwidth 64 kbps) costs more than RM 20,000 per year to maintain. This plus an Internet account (leased line) adds up to something like RM37,000 per year to maintain. Few departments can afford this sort of expense and after weighing the cost, an independent website is too expensive to start and maintain. In reality, one does not have to do this. If the institution already has a leased line and a functioning web server with a domain name, one could always ride on the web server. This avoids the problems of applying for a separate line and domain name, although domain names are generally not expensive (RM 100 per year to maintain with MYNIC). In the case of the SPM Department, there was a choice. The first choice was to use a separate web server and have a URL (Uniform Resource Locator) like http://spm.med.um.edu.my . The second choice was to use the faculty's existing web server and publish the SPM website on a sub-folder on the web server. Thus the URL would read http://ummc.med.um.edu.my/spm/ . The first choice would have been ideal if there was a working web server. The SPM Department has a server in working condition. Unfortunately, the server was not equipped with the necessary software to act as a web server and money was hard to come by in 1998 due to the economic recession. In the initial phase of setting up the website, it was not necessary to have the website up all the time so the website was hosted on a PC for testing. This method does have certain disadvantages as a PC running Windows 95 is simply not a very secure server and having a website running on a PC tends to slow down the general running of the computer. Towards the end of designing the website, it was decided that the second option i.e. of publishing the SPM website on the faculty's web server would be a better option. The second option does have the advantage of leaving the maintenance of the server out of the hands of the department, making it easier to manage the website.

HTML and HTML editors

Web pages are basically written in HTML (Hypertext Markup Language). This is a relatively simple programming language to learn and one does not need a compiler to design web pages. All one needs is a text editor like Notepad or WordPad in order to write HTML. In recent years, the advent of powerful HTML editors like Microsoft FrontPage has made HTML writing using text editors obsolete and hardly anyone writes HTML in this way. Even word processors like Microsoft Word 97 can create HTML pages without any fuss and without the author knowing a word of HTML. The SPM website was created using the Microsoft FrontPage 98 software, a powerful and fairly sophisticated tool for creating web pages. There are a few advantages of using such software as FrontPage. Firstly, there is no need to learn HTML. The mechanics of making web pages can be learnt within minutes. Secondly, the author can see the web page being created as it would appear, the so-called WYSIWYG (What You See Is What You Get). In other words, it is akin to using a word processor. Thirdly, one can insert images, music, video, animation (using Dynamic HTML), Java applets at will and can test out the effects immediately. It thus provides a very user-friendly interface for creating web pages for the beginner.

Layout of web page

There are two ways to create a web page. The first way (not to be recommended) is to download someone else's web pages and use those as templates. The second way is to build it from scratch. Creating a web page from scratch would definitely be recommended, as many things gained from the experience cannot be duplicated. It is certainly a more tedious way of doing things but certainly more enjoyable and rewarding as the satisfaction at the end of it all is worth every bit of the effort.

The layout of the web page needs considerable thinking. Adopting or copying someone's layout (however good it looks) may not be right for a department's web page. A commercial website may be filled with banners and all sorts of advertisements which may not serve any purpose for a department website and will just slow down the person browsing the website. If the objective of the website is to publicise the department and to ensure that information is presented well, then the layout of the web page must be tailored to fulfil that objective.

In the case of SPMNet, because the objective was to publicise and inform rather than to attract business, information is presented in a simple but meaningful way. To achieve this, two templates were drawn up. The two templates are really quite similar and only differ in the number of columns used in the main table. One uses a two-column approach while the other uses a three-column approach. The two-column approach is used for top-level pages (except the very first page) while lower-level pages apply the three-column approach. The use of tables in designing web pages is not new and is used extensively all over the world. The primary reason for using tables is to ensure precise placing of information and objects. Certainly it is possible not to use tables for creating web pages but control is difficult and one can never be too sure how it will turn out.

Site layout

Site layout is important to ensure that the website does not become cluttered. A cluttered site that is not organised makes editing pages difficult. To avoid clutter, it is advisable to create folders and sub-folders wherever necessary. What was intended to be a simple website may well turn out to have a hundred pages and this makes editing difficult as one has to remember many names. Organising by the use of folders is thus advantageous in this respect. What folders to use is up to the Webmaster and depends on what is presented on the website. In the case of SPMNet for example, each unit within the department is given a separate folder. Images are centralised in a separate folder with sub-folders to indicate which images are animated GIFs, which images are logos and so on. Staff profiles organised in a separate folder from the units to make it easy to locate the staff profile page.

Navigation

A good website must be easily navigated. Some websites use navigation bars to aid the user in going through its site. Others use a simple column approach to provide easy navigation through. In the case of the SPM website, the entire website can be navigated using the left-most column which contains links to other main pages within the website. To further ease navigation, a hover bar is provided at the bottom of every page to help the user get to certain critical pages quickly.

Sophistication

Technology used on the web moves very quickly. What is today current and state-of the art browser may well be obsolete tomorrow and this can be seen by the speed of improvements in browsers. Having said that, it would be a mistake on the part of the Webmaster to assume that everyone has the latest generation browser and plug-ins. If the idea is to reach as many people as possible, then the Webmaster must consider many Internet users still using third or even second-generation browsers. The message is the web page must not be so sophisticated that older browsers cannot read the important information that the Webmaster is trying to get across. Because of this, if the Webmaster wishes to use Java or ActiveX for example to enhance the web page, it should not be such that the entire website depends on it. The use of these technologies should be judicious and should used only where deemed necessary. It is with this in mind that SPMNet was constructed. The use of Java has been kept to a minimum and it only appears as navigation bars at the bottom of the page. These navigation bars are not absolutely essential as hyperlinks to the same pages can be found on every page.

Problems in setting up the website

The biggest problem in setting up the website (assuming that there is a web server ready to host the web pages) is getting the information. In any case, what goes into the website is determined by whether the information is readily obtainable or not more than anything else. If the aim of the website is to promote the organisation (and it frequently is), then the correct type of information must be available on the website. Getting this information can be a problem as some information although known to everyone is not in any written form. Getting personal profiles can be a headache as not everyone may share the Webmaster's enthusiasm for creating the web page. A compromise would be to only publish information that the person deems fit to appear on the website. In the case of SPMNet, getting the information was initially a problem as many documents were either not updated or difficult to find. Personal profiles were initially a headache as not everyone would like to publish the same sort of things online for the whole world to see. A compromise was reached whereby only information regarded as publishable by the staff was put on the website.

SPMNet contains a lot of hyperlinks to interesting sites like journals, medical organisations and health-related sites. As with any website or web page, there is the problem of link rot. Link rot basically means that the links provided on the web pages are no longer live. Checking for links has to be done regularly to prevent this from happening. Fortunately, present day HTML editors can check all these hyperlinks without one having to do these manually. Verifying these hyperlinks are best done in the early morning so as to capitalise on available bandwidth as it may take a while to do particularly when there are a lot of links. Even when a broken link is found, it is usually not wise to delete the link without checking out the site manually. Checking involves either pinging (sending a stream of data from one computer to another on the Internet to determine the response) or using a browser to manually check out the links. Pinging will establish whether such a site is registered as a domain while a browser will check out the actual page. Occasionally a link is reported as broken when it actually is not because of the way the website handles verification requests. Even when a link is reported as broken, it should not be deleted or edited unless it has been confirmed to be no longer active. For SPMNet, the usual practice is to ping and browse the website. If both methods report a non-existent website, then a search is performed using at least 3 major search engines. Sometimes the search engine will turn up an alternative site that is more recent. Should such a thing happen, then the recent website is checked out and if found to be correct, the link is corrected. Links are only deleted if several verifications prove that the website no longer exists. One has to regularly do this to prevent link rot from setting in. The more links present

in a website, the more time-consuming it becomes to check for link rot.

Testing

Any new website cannot be assumed to be ready without extensive testing. It is not uncommon to find websites that are not properly tested and pose many problems for the user. What test you want to carry out depends basically on the objectives of setting up the website in the first place and who the website is aimed at. Basically any website should be tested for accessibility and browser compatibility. Not everyone has very fast computers and connections (T1,T3 lines) with sophisticated browsers and plug-ins. The majority still use dial-up connections from home (28-56 kbps modems) and pre-Pentium era PCs. Though the dominant browsers at the present moment is Microsoft's Internet Explorer and Netscape Navigator, this does not mean that other browsers can be ignored if the objective is to reach as many people as possible.

Testing for accessibility should be done at home using real-world connections rather than the Ethernet connection at the office. Ethernet in most office networks typically offers speeds between 10 to 100 Mbps (although higher speeds are possible, it is not typical) and this is virtually unheard of at home at the present moment. It is then that it becomes painfully obvious that what appears to snap onto the screen at the office takes forever to load at home. Efforts must be made to find the source of the delays and solutions found. Some solutions require only simple measures such as removing unnecessary music and movie files which tend to slow down loading. Others may not be so simple such as optimising images so that they are reduced in size without compromising quality.

Compatibility with commonly used browsers such as Internet Explorer, Netscape Navigator and Opera must be done to avoid errors in the display of web pages. Fortunately there are free tools on the Internet like NetMechanic which assesses the HTML code written to see whether it conflicts with certain browsers. This takes the hassle out of going through each page with different types of browsers (with their different versions) to see whether the code is compatible or not.

SPMNet was tested using real-world connections from home at various times of the day. As a result of the testing, it was found that certain pages took a long time to load because of the size of those files and appropriate measures were taken to reduce the access time to a more reasonable amount. Browser compatibility checks were carried out using software such as NetMechanic to determine compatibility with the different versions of different browsers. Testing took several weeks and this was a good learning period for the Webmaster involved.

Publishing the website

SPMNet was published on July 1, 1999. Publishing a website means setting it up on a web server so that the whole world can access the information. Publishing to one's own web server is the most ideal situation because it is easier to remember a domain name but there are many problems one must content with. The web server has to be a dedicated server that is it has to function as a web server all the time. Normal desktop PCs can be used as a web servers but problems will surely be encountered. Firstly most PCs are loaded with the Windows 9x operating system (OS) which is not the most stable operating system in the world. Secondly, although it would be possible to use the personal web server (PWS) available with the Windows 9x OS, this is not to be recommended as the PWS is not a very secure server software. Having considered these things and considering that SPM is after all a part of the Faculty of Medicine, it was decided that the website would be hosted by the faculty's own server. There are inherent advantages in this. The faculty's web server is a dedicated web server running the Internet Information Server (IIS) on top of Windows NT making it less likely to crash as it would not be burdened with other work at the same time. Secondly, the use of the faculty's web server would enable a mirror copy available on the originating PC as backup should the web server crash, or the website was compromised. Thus a speedy return to operations is possible with the backup being ready to take over at a moment's notice. This has proved necessary on a couple of occasions, both being occasioned by the web server crashing causing the website not to be available. On both occasions, the entire content of the website was wiped out and had to be restored from the backup copy.

Publicising the website

Once SPMNet was published, there were not many hits initially. Hits are simply visits to the website. A lack of hits would imply that few people know about the website, which would defeat the purpose of the website in the first place. To get visitors to the website, the Webmaster must be prepared to spend more time publicising the website. The simplest way is simply to inform as many people as possible, either through the e-mail or more traditional mail system. This can be done quite easily and was done in the case of SPMNet. A second and better method is to get the website listed on other sites where one believes one's target audience is likely to come from. For example one could get one's website listed on the Ministry of Health's website

and whoever was visiting that website may come across the link to one's website, and be tempted to visit it. Other sites where the SPM website is listed include health-related sites, counterpart sites and major search engines and portals. There are many search engines available on the web and listing is free on most. Because it is time-consuming to go to each and every search engine, it is sometimes wiser to use search engine listing sites to help. An example of such a site is the Add-me.com which will assist in listing the website of one's choice on 25 major international search engines. In this manner a lot of publicity is gained with minimal effort. One should not forget the local search engines too and has can be seen nowadays, there are many local search engines concentrating on Malaysian websites.

Maintaining the website

Very often websites are set up at great expense and launched with great fanfare. Because these websites were constructed by professional web developers, they are frequently very well designed. The problem starts when information on the website becomes outdated. Usually, by commissioning web developers to do the job of creating the website, there is no technology transfer and as such no one in the organisation knows how to perform maintenance. It would be better for the organisation to send someone for training and create the web pages by themselves. The resulting web pages may not be as good as those created by professionals but certainly updating will be a lot easier. In the case of the SPMNet, these web pages were created in-house using available expertise. As such, while it may not be the best that can be done, updating is certainly very easy and can be done at any time.

Benefits of the website

Since the website was launched in 1999, there have a number of benefits. Apart from the usual congratulatory messages (and some criticisms) from people who have viewed the website, it has certainly made the department more visible to the outside world. A number of prospective students have been in touch with the department staff inquiring about studies in the faculty, postgraduate and undergraduate. Some inquiries have been received about jobs in the University of Malaya Medical Centre (UMMC), which were promptly directed to the appropriate persons. It is still too early to say what proportion of students coming to study in the UMMC has surfed through the SPM website as the site is barely a year old but a number of inquiries have been received from prospective international students. It is envisaged that the website will be utilised as a means of conducting long-distance learning in the future and efforts are underway to make this a reality. Eventually it is hoped that part of the teaching of undergraduate and postgraduate students be conducted through the Internet. With that in mind, it is the author's personal opinion that all departments in the UMMC should move towards adopting this new media in order to remain relevant in the Information Age.

References

1. Delamothe, T., Smith, R. The BMJ's website scales up. British Medical Journal 1998; 316:1109-1110.
2. Sellapan P. Internet – A Guide to the Information Age. Sejana Publishing 1998. ISBN 983-2017-05-X.
3. Smith R. The Internet and the developing world. British Medical Journal 1998; 316: 1116.
4. Craig D. Webbery and Nettery at http://www.strum.co.uk/webbery/ 9 May 2000.

PARASITOLOGICAL AND SEROLOGICAL INVESTIGATION INTO LYMPHATIC FILARIASIS AMONG IMMIGRANTS AT SEMENYIH DETENTION CENTRE, SELANGOR, WEST MALAYSIA

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ABSTRACT: Parasitological and serological investigations for lymphatic filariasis were performed on 450 immigrants detained at the Immigration Centre at Semenyih, Selangor, West Malaysia. The country of origin of these immigrants were Indonesia, The Philippines, Myanmar, Bangladesh, India and Pakistan. *Brugia malayi* adult worm homogenate (BmAH) antigen was used for the detection of antifilarial IgG. A monoclonal antibody-based ELISA (MAb.XC3-ELISA) specific for filarial circulating antigens and non-phosphorylcholine reactive was used to detect antigenemia in these immigrants. Parasitologically 67 (14.89 %) were positive for *W. bancrofti* and 54 (12.0%) for *Brugia malayi.* Serologically 63 % had antifilarial IgG titre to the BmAH antigen. While Bancroftian filariasis is now unknown in Peninsular Malaysia, the potential of it to be reintroduced into Peninsular Malaysia by the immigrant population is discussed. *(JUMMEC 2000; 1: 41-44)*

KEYWORDS: Lymphatic filariasis, immigrants, antifilarial IgG, antigenemia

Introduction

During the last few years before the economic slow down, there has been an influx of immigrants into Malaysia. Due to better economic prospects thousands of immigrants have entered Malaysia in search for a better life. The immigrants are employed in various economic sectors such as domestic maids, agriculture, building and construction. The government of Malaysia has tightened the immigration policy. Those seeking employment must obtain work permit in addition to proper and valid travelling documents. Failture to produce these documents lead to deportation. Pending deportation they will be detained at Imigration Dentention Center. There is need to ensure that Malaysia is not burdened by the need for care of immigrant with health problems as well as the increased risk posed to the local population from exposure to communicable disease.

Lymphatic filariasis is endemic in Asia. The infection persist as a major cause of clinical morbidity and a significant impediment to socioeconomic development. Its prevalence is increasing world wide, largely because of rapid unplaned urbanization in many endemic areas. It is estimated that at least 120 million people are infected. The sub-periodic form of *Brugia malayi* occurs mostly in the swamp forest areas of Malaysia and has a wide animal reservoir which includes monkeys. The periodic form is endemic mainly in the coastal rice field regions of Malaysia (3, 4). In Malaysia *W. bancrofti*, especially in the cities have been eliminated. However their vectors especially those responsible for the trasmission of *W. bancrofti* breeds in abundance in the cities. With the influx of immigrants and in relation to their occupational nature, the whole facet of lymphatic filariasis in Malaysia may change.

We report in this paper our parasitological and serological findings on the prevalence of lymphatic filariasis among immigrants detained at the Semenyih Immigration center. If the trend described in this paper persist, one would be able to predict the forthcoming

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health problems pose to this cpuntry by the migrant population. This will help the design and planning of health services for migrants to be undertaken on a more rational basis.

Materials and methods

Subjects

The sample population consisted of 450 male immigrants and their age range from 12 years to 60 years majority being in the 21 to 33 years age group. The country of origin of these immigrants are Indonesia,The Phillipines, Bangladesh, Myanmar, India and Pakistan.

Sera

Blood samples were taken by veinpuncture between 8 to 12 P.M. Sera were seperated from blood samples and stored at –20° C until used. The presence of microfilaremia was detected by microscopic examination of stained thick smears of blood (60 µl) obtained at night. Controls were residents of Perak Tengah, an endemic area for *Brugia malayi*. These individuals were with symptoms of chronic disease and of acute filariasis (adenitis, lymphangitis, or pulmonary eosinophilia), and also were microfilaremic. In addition normal individuals from non-endemic area were also included as negative controls.

Antigens

Brugia malayi adult homogenate antigen (B.m.AdH) was used to titrate the antifilarial IgG antibodies. B.m.AdH was prepared from 50 adult worms recovered from infected gerbils by peritoneal lavage. Worms were homogenized in sterile normal saline and were sonicated using Dynatech Sonic Dismembrator at 6 kilocycles MHZ for 3 times on a 3 minutes off and 3 minutes on pattern in ice. To ensure proper fragmentation, a drop of suspension was examined under light microscope. Crude mixture was kept at 4° C for overnight and again centrifuge at 2000 rpm for 10 minutes. The supernatant was removed and centrifuged at 1300 rpm for 20 minutes at 4° C. Finally the supernatant was lyophilized, aliquotted and stored at –2° C until use. Protein concentration was determined by Lowry's method (7).

Enzyme immunoassay (EIA)

A monoclonal antibody designated as MAbXC3 was used in EIA for the detection of antigenemia in these subjects. MAbXC3 is a non-phosphorylcholine reactive mouse IgG 1 (1-2; 5-6). Briefly, serum specimens were treated to release antigens from immune complexes and to remove interfering proteins prior to performing the monoclonal-based EIA (MAbXC3-EIA). Serum specimens were diluted with an equal volume of 0.1 M disodium EDTA (pH 7.5), heated 5 min on a boiling water bath and microcentrifuged at 16,000 x g for 7 min before testing in the assay. Preliminary studies demonstrated that the procedure provides more than 90 % recovery of antigen activity. The optimal dilutions of B.m.AdH antigens and the monoclonal antibody (MAbXC3) were determined by chequer board titration. Polyvinyl microtiter plates (Dynatech Lab.,Alexandria,VA, USA) were sensitized by overnight incubation at 37° C with 100 µl/well MAbXC3 (20 µg/ml in 0.1 M Na HCO3, pH 8.0). Serum specimens that have been serially diluted were added to the sensitized microplates in triplicates (50 µl/well).After two hour, the plates were washed with PBS with 0.05% Tween 80 (Sigma Chem. Co.) and 100 µl/well MAb.XC3 was again added. The plates were incubated at 37 °C for 1 hr. After washing, 50 µl of an appropriate dilution of peroxidase conjugated goat antisera against mouse IgG1 (KPL, Gaithesburg, MD) was added. After washing the plates were developed with 2-azino-di-(3-ethylbenzthiazoline sulfonic acid) substrate for 30 min. The reaction was stopped with 50 µl 4 M H_2SO_4. Optical density was read *vs* a PBS blank at 405 nm with an ELISA reader (Titertek Multiscan, Linbro, Hamden, CT, USA). Since 3 of 20 non-endemic normal sera gave positive reaction at serum dilution of 1:150 when sceened for antigen, we considered 1:300 titre as positive for filarial antigen and the sera were screened at 1:300 dilution.

Direct EIA was performed to detect antifilarial IgG antibodies to B.m.AdH. Binding of antifilrial IgG in the serum samples from these immigrants was detected using peroxidase labelled goat anti-human IgG (KPL, Gaithesburg, VA, USA). Since 3 of 20 non-endemic normal sera gave a positive reaction at serum dilution of 1:80 when screened for antifilarial IgG, we considered 1: 160 as positive reaction for filarial antigen and the sera were sceened at 1:160 dilution.

Results and Discussion

Parasitologically, microfilaremia with *W. bancrofti* were detected in 67 (14.89 %) of the 450 immigrants (Table 1). Microfilaremia with *B. malayi* were detected in 54 (12.0%). Microfilaremic cases were detected the highest among the Indonesian immigrants (77 cases) followed by Bangladeshi (32 cases) and their total numbers were 180 and 120 respectively which were also higher than others.

The antifilarial IgG titers of these immigrants to B.m. AdH are shown in Table 2. A panel of 20 well-characterized sera of local Malaysians from Perak Tengah an endemic area and normal individuals from non-endemic area were included as positive and negative controls respectively. The highest titer (5120) was seen among the Indonesians (n=15).

Table 3 shows the profile of filarial circulating antigen titer. 155 (34.44 %) were antigenemic. Antigenemic cases were high among the Indonesians and the Bangladeshi. The highest titer (9600) were seen among

Table 1. Microfilaremic cases detected among immigrants according to country of origin.

Country of Origin	No. tested	No. positive (%)	No. of microfilaremic cases (%) W. bancrofti	B. malayi
Indonesia	180	77 (17.1)	42 (9.3)	35 (7.8)
Philippines	45	3 (0.7)	2 (0.4)	1 (0.2)
Myanmar	60	5 (1.1)	3 (0.7)	2 (0.4)
India	30	2 (0.4)	1 (0.2)	1 (0.2)
Bangladesh	120	32 (7.1)	18 (4.0)	14 (3.1)
Pakistan	15	2 (0.4)	1 (0.2)	1 (0.2)
Total	450	121 (26.9)	67 (14.9)	54 (12.0)

Table 2. Detection of antifilarial IgG employing BmAdHom antigen in direct EIA.

Country of origin	No. tested	No. positive (%)	Reciprocal of antifilarial IgG 160	320	640	1280	2560	5120
Indonesia	180	18 (40.0)	15	21	36	21	33	54
Philippines	45	27 (6.0)	-	-	3	9	3	12
Myanmar	60	37 (8.22)	-	1	2	10	9	15
India	30	5 (1.11)	-	-	-	-	-	5
Bangladesh	120	81 (19.33)	2	4	6	15	21	33
Pakistan	15	1 (0.22)	-	-	-	-	-	1
Total	450	331 (74.89)	17	26	47	55	66	120

Table 3. Detection of filarial circulating antigen employing MabXC3-EIA.

Country of Origin	No. tested	No. positive (%)	Reciprocal antigen titre 300	600	1200	2400	4800	9600
Indonesia	180	81 (18.0)	33	24	9	6	6	3
Phillipines	45	12 (2.7)	6	3	2	1	-	-
Myanmar	60	15 (3.3)	7	6	1	1	-	-
India	30	3 (0.7)	2	1	-	-	-	-
Bangladesh	120	42 (9.3)	15	9	7	6	3	2
Pakistan	15	2 (0.4)	1	1	-	-	-	-
Total	450	155 (34.4)	64	44	19	14	9	5

3 Indonesians and 2 Bangladeshi. All these immigrants come from countries which are endemic for lymphatic filariasis. In an endemic population microfilaremia and clinical manifestations may be absent, such as during early infections. Therefore the diagnosis of lymphatic filariais is then extremely difficult. Nevertherless most of them may exhibit positive skin tests should they are subjected to challenge with antigenic extracts of human and animal filariids since most of them invariably contain antifilarial IgG. Similarly, the antifilarial antibodies can be detected by a variety of serological assays (8-10). However, these reactions persist for many years even when control programs are effective in interrupting transmission (11- 12). Sharing of antigens between different filarial species and between filariae and other nematodes further limits the usefulness of antibody assays for filariasis. (5, 6).

Diagnostic assay based on the detection of parasite antigen in biological specimens with highly specific monoclonal antibodies would alleviate some of these shortcomings (10- 12). The MAbXC3-EIA that has been developed and used in this study should serve the purpose. To be applicable in the field setting of rural Malaysia where laboratory facilities are inadequate, the

assay protocol can be modified to make it more simpler to perform. With these considerations in mind, a dipstick assay in which the MabXC3 (which is specific to serum filarial antigen) retained on nitrocellulose membrane would be a suitable format. Patients serum can be dotted directly to such portable dipstick which has potential for field application and in this case immigrants entering Malaysia.

Finally the findings from this study appear to show that foreign workers may pose a sizeable amount of health problems especially with regard to *W. bancrofti* transmission. There is potential of Bancroftian filariasisis may eventually be reintroduced into Peninsular Malaysia through these immigrants. Screening can be conducted on every immigrant entering Peninsular Malaysia and screening for lymphatic filariasis should be included together with other communicable diseases. If these are not address quickly it may endanger the health of this country, while we readily acknowledge their contribution towards national development.

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References

1. Wan Omar A, Zahedi M, Sulaiman O, Ismail S, Hashim Y. Serological and entomological investigation into lymphatic filariasis at a Malaysian Aboriginal settlement, Negeri Sembilan, West Malaysia. J Mal Soc Hlth 1994; 12 (No. 1):1-4.
2. Wan Omar, A., Zahedi, M., Hashim, Y., Sulaiman, O. 1994. Detection of Brugia malayi antigens in serum samples of humans living at Perak Tengah, Peninsular Malaysia: Field evaluation. Jpn J Sanit Zool 1994; 45(No. 2): 147-50.
3. Mak J. W. Filariasis. Bulletin Institute Medical Research. 1985; 35: 5-8.
4. Mak JW, Yen PKF, Lim PKC, Ramiah N. Zoonotic implications of cats and dogs in filarial transmission in Peninsular Malaysia. Trop. Geog. Med 1980; 32: 259-64.
5. Wan Omar A, Zahedi M, Hashim, Y. Vaccination of jirds with immunogens isolated by monoclonal antibody affinity chromatography protects against Brugia pahangi homologous challenge. Asia Pacific J. Mol. Biol. Biotech 1993; 1 (1): 117 – 21.
6. Wan Omar A, Hashim, Y, Pakeer, O. Eosinophilia and antigenemia at the onset of patency and parasite specific antibody in lymphatic filariasis. Mosquito-Borne Dis. Bull 1992; 9: 47-54.
7. Lowry O, Rosenberg N, Farr A. Protein measurement with Folin-phenol reagent. J. Biol. Chem. 1951; 103: 265-7.
8. Chanteau S, Moulia-Pelat JP, Glaziou P, Nguyen NL, Luquiaud P, Pichart C, Martin PMV, Cartel JL. Og7C3 circulating antigen: a marker of infection and adult worm burden in Wuchereria bancrofti filariasis. J Infect Dis 1994; 170: 247-50.
9. Lammie PJ, Hightower AW, Eberhard ML. Age-specific prevalence of antigenemia in a Wuchereria bancrofti-exposed population. American J Trop Med Hyg 1994; 51: 348-55.
10. Weil GJ, Ramzy RMR, Chandrashekar R, Gad AM, Lowrie RC Jr, Faris R. 1996. Parasite antigenemia without microfilaremia in bancroftian filariasis. American J Trop Med Hyg 1996; 55: 333-7.
11. Weil GJ, Lammie PJ, Weiss, N. The ICT filariasis test: a rapid-format antigen test for diagnosis of bancroftian filariasis. Parasitology Today 1997; 13:401-4.
12. Aheng HJ, Tao ZH, Cheng WF, Xu M, Fang RL, Piessens WF. Parasite antigenemia in untreated and treated lymphatic filariasis filarial infections. American J Trop Med Hyg 1990; 43:481-8.

TRANSCATHETER CLOSURE OF PATENT DUCTUS ARTERIOSUS: OUR INITIAL EXPERIENCE IN UNIVERSITY OF MALAYA MEDICAL CENTRE

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ABSTRACT: Six patients underwent transcatheter closure of patent ductus arteriosus (PDA) using either conventional Gianturco coils or the Amplatzer Ductal Occluder (ADO) device. All patients were females with a median age of 23.3 (range 4 to 26 years). The mean PDA size measured on the lateral aortogram was 3.81mm (range 2.3-5.83mm). Complete closure of the PDA was achieved in all patients. *(JUMMEC 2000; 1: 45-47)*

KEYWORDS: Patent ductus arteriosus, transcatheter device closure

Introduction

The role of devices to close the patent ductus arteriosus has been popular the last decade due to the ease of deployment and excellent results (1). Although the first device for transcatheter PDA closure was described by Porstmann in 1967, the evolution of the device types and improvement in techniques was limited by too large delivery system especially for children and the complexity in procedural techniques. Furthermore, the success rate was not encouraging and there were significant post-procedure complications.

Earlier reports of transcatheter device closure for PDA using the Rashkind, Botalloccluder, buttoned devices and coils have variable degrees of success with an incidence of residual shunting in 3-38% of patients.

However, the use of conventional coils and the Amplatzer Ductal Occluder (AGA Medical Corporation, Golden Valley, MN) in major paediatric cardiac centres including the National Heart Institute in Kuala Lumpur has shown excellent closure rates with ease of deployment and minimal complications (1,2).

We present our initial experience in transcatheter closure of PDA in six patients between October 1999 and March 2000.

Materials and methods

Patients

From October 1999 to March 2000, 6 patients (all females) underwent cardiac catheterization and device closure of a PDA as an alternative to the standard surgical ligation. Written consent was obtained from all patients or guardians of paediatric patients. All patients had clinical and echocardiographic findings of a PDA.

All patients were females with a median age of 23.3 (range 4 to 26 years). The median weight was 30.5kg (range 15-47 kg)

Four patients were asymptomatic with good effort tolerance. Two patients were symptomatic and were on diuretics and inotropes to control heart failure. One patient had associated severe mitral and aortic regurgitation due to rheumatic heart disease but remained in heart failure despite the disease being in remission due to a large PDA (10-mm diameter). She was in NYHA functional class I. Another patient with symptomatic heart failure had severe pulmonary hypertension and was in NYHA functional class IV. Both patients underwent surgical ligation of PDA but still had a large residual shunt following surgery.

Device option and procedure

The device of choice for closure of the PDA in these patients was decided by the diameter from the lateral descending aortogram. PDA with a diameter of less then 3.5-mm was closed by coils and PDA with a diameter of more than 3.5 mm was closed by the Amplatzer device.

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All patients underwent a right and left heart catheterization using a 4F or 5F sheath in the femoral artery and 5F to 6F sheaths in the femoral vein. Heparin was given at a dose of 50units/kg in all patients. The two paediatric patients (aged 4 years and 11 years) were under general anaesthesia for the whole duration of the procedure.

Transcatheter deployment of the device was achieved antegradely (from the venous side) in all patients. In one patient, the leading guidewire had to be snared from the arterial side due to difficulty in maneuvering entry into the PDA from the main pulmonary artery.

Deployment of coils was achieved using size 4F multipurpose catheters and each coil is delivered via an individual catheter. This is done by inserting more than one catheter sheath per femoral vein.

Delivery of the Amplatzer Ductal Occluder (ADO) is achieved by using a size 7F or 8F Mullin's catheter and released from its steel wire cable.

A check lateral aortogram is performed before the release of the devices to ensure position and occlusion status. Cineangiogram is taken during the release of the device.

A chest X-ray and 2D echocardiogram is done at 24 hours post procedure for reassessment.

Results

Three patients underwent closure using the Gianturco coils and three others was closed using the Amplatzer Ductal Occluder. The mean PDA size measured on the lateral aortogram was 3.81 mm (range 2.3-5.83 mm). The number of coils used to close the PDA depended on the size of the PDA. Three coils (5 cm length X 8 mm helical diameter) were used to close the PDAs sizes 3.5mm and 2.5 mm. Two coils (5cm X 8 mm) were used to close the 2.3 mm PDA.

Complete closure with no residual shunt was achieved immediately after deployment of the coils in all these patients. 2D echocardiogram with Doppler at 24 hours post-procedure reconfirmed closure status.

The larger PDAs were closed with ADO depending on the size of the PDA and the morphology of ductus. In two patients (PDA size 4.8 mm and 5.83 mm), this was achieved by using the size 8/10 (8 mm diameter with 10mm retention disc) ADO, whereas a size 10/12 was needed to close in one other patient (PDA size 4.2 mm).

Complete immediate closure was achieved in two patients following ADO release. One patient with a trace shunt upon deployment of the ADO showed very minimal shunt on Doppler echo at 24 hours of procedure and none on reassessment at 1 month.

The mean pulmonary artery pressures before closure was 27 mmHg (range 10-54 mmHg). There was no significant gradient across the ascending and descending aorta after the procedure.

There was no evidence of left pulmonary artery or aortic obstruction from 2D echocardiogram on the following day and follow-up at 1 month and 3 months respectively. There was no femoral arterial or venous complication.

All patients were discharged home by 48 hours after the procedure. One patient with symptomatic heart failure who was bedridden in the ward before the procedure achieved quite a drastic improvement in symptoms after the procedure when she was able to ambulate within 24 hours of the procedure.

All patients were reassessed with 2DE at 1 and 3 months post procedure.

Table 1. Clinical and Closure Data for Patients

No	Wt (Kg)	Qp/Qs	PDA dia (mm)	Device Type/size	Results		
					Immediate	24h	1 month
1	25	1.4	5.8	ADO 8/10	TS	TS	C
2	35	1.6	4.8	ADO 8/10	C	C	C
3	47	1.3*	4.2	ADO 10/12	C	C	C
4	32	1.1	2.3	5cm X 8mm (2)	C	C	C
5	29	1.3	3.5	5cm X 8mm (3)	C	C	C
6	15	1.2	2.5	5cm X 8mm (3)	C	C	C

Abbreviations:
ADO: Amplatzer Ductal Occluder
TS: Trace shunt
C: Complete closure

*The shunt and PDA diameter was underestimated due to significant pulmonary hypertension and distorted anatomy of the pulmonary artery

Discussion

Transcatheter closure of PDA is now widely practiced in all major medical institutions with cardiology services especially paediatric cardiology. The National Heart Institute in Kuala Lumpur has been performing these procedures since 1994 with good results using both the conventional coils and the ADO.

After having exposure and hands on experience with these procedures, we report our initial experience on our own patients from the UMMC.

We performed all closures antegradely i.e. deployment of the device from the venous side, using the smaller size 4F catheters for the coils and size 7F or 8F Mullin's catheter for the ADO. The deployment of coils proved to be a more challenging procedure as all the multi-purpose catheters are passed through the ductus simultaneously until tight and almost occluding the ductal blood flow and then only each coil is released individually.

The lack of a controlled release mechanism seems to be the major disadvantage of the Gianturco coil as it carries the risk of peripheral embolization if not positioned securely. The inability to reposition the coils once extruded also gives the operator a low comfort level. Controlled release coils are being developed to overcome this but is expensive with no added benefit being described (3). The incidence of peripheral pulmonary artery embolization remains between 6.8-8.7% (1,2).

The use of conventional Gianturco coils is favoured for the smaller ductuses i.e. less than 3.5 mm in size in view of the ease of deployment using small catheters (Size 4F and 5F) which is suitable for children reduced costs and excellent closure rates.

The Amplatzer Ductal Occluder requires the use of a Mullin's sheath and a steel wire cable for release. Short term results have showed 100% closure rates at 3 months with very minimal complications in PDA diameters of up to 6mm (1). Report from the National Heart Institute (soon to be published) in over 200 patients have also showed > 95% closure rates in PDAs closed by the ADO.

This self-expandable, mushroom shaped device, made of 0.004 inch thick Nitinol wire has an excellent memory upon expansion and is also delivered via the transvenous route. The rim of the device on the arterial side has a 2mm skirt (retention disk) to ensure secure positioning in the ampulla of the PDA.

Deployment of this device proved to be with ease in all our patients but can be difficult the uncommon types of PDA morphology (5).

The use of transcatheter closure of residual PDA after surgery has also shown to be complimentary as in our patients and also described elsewhere (4). In conclusion, transcatheter closure of PDA is a safe and effective procedure in both the paediatric adult age group and is an alternative to surgery in selected group of patients.

References

1. Masura J, Walsh KP, Thanapoulous B, Chan C, Bass J, Goussous Y, Gavora P, Hijazi ZM. Catheter Closure of Moderate to Large-Sized Patent Ductus Arteriosus Using the New Amplatzer Duct Occluder: Immediate and Short term Results. J Am Coll Cardiol 1998; 15(4): 878-82.
2. Alwi M, Kang LM, Samion H, Latiff HA, Kandavel G, Zambahari R. Transcatheter Occlussion of native patent ductus arteriosus using conventional Gianturco coils. Am J Cardiol 1997; 79: 1430-2.
3. Wolfgang AK. Current therapy of the patent ductus arteriosus. Current Opinion in Cardiology 1998, 13: 59-65.
4. Podnar T, Masura J. Transcatheter Occlusion of Residual Patent Ductus Arteriosus After Surgical Ligation. Pediatr cardiol 199; 20:126-30.
5. Krichenko A, Benson LN, Burrows P, Moes CAF, McLaughlin P, Freedom RM. Angiographic classification of the isolated, persistently patent ductus arteriosus and implications for percutaneous catheter occlusion. Am J Cardiol 1989; 67: 877-80.

ANTI-STAPHYLOCOCCAL ACTIVITY OF EW1, A TRADITIONAL HERBAL REMEDY FOR CARBUNCLES

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ABSTRACT: A powder (EW1) made from a mixture of herbs used for the treatment of carbuncles by traditional medicine practitioners in China was investigated for anti-staphylococcal activity by agar diffusion, time-kill studies and M.I.C. determinations performed on 17 clinical isolates and a reference strain ATCC 29213. It was found that EW1 had little demonstrable *in vitro* activity against the clinical isolates tested but inhibited the growth of the ATCC strain at 10 mg/l and retarded its growth in broth culture by an average of 1.5 log reduction in colony count. *(JUMMEC 2000; 1: 48-50)*

KEYWORDS: Traditional medicine, anti-staphylococcal activity.

Introduction

Although modern, evidence-based medicine is regarded as the most advanced form of curative practice, in many populations, traditional, alternative or complementary medicine still commands a large following. The World Health Organization has estimated that over 80% of the world's inhabitants rely mainly on traditional medicine for their primary health care needs (1). Even in developed countries, the increasing public acceptance of alternative medicine has resulted in medical schools offering courses in this specialty and insurance companies and managed care organizations giving benefits to those who seek treatment from alternative medicine practitioners.

The most widely practiced alternative therapy is herbal medicine. Recognizing the vast potential of plants as natural resources for drugs, many international and local government agencies are encouraging basic science research and clinical studies on medicinal plants. A convenient starting point for researchers is the investigation of biological activities of herbal preparations that have been used by traditional practitioners. Unfortunately, scientific investigations on these preparations are complicated by the fact that herbs are frequently used in combination, their active principles are often unknown, the composition of herbal mixtures is not always disclosed and is often altered to suit the constitution of the consumer.

EW1 is a powder prepared from a mixture of herbs that have been used by traditional healers in China for the treatment of carbuncles. The composition of the mixture is a well-kept trade secret. It is usually applied as a paste onto the skin, and has been reported by the users to be effective in reducing swelling, redness, purulent discharge and hastening re-epithelialization. As carbuncles are usually caused by staphylococci, an investigation was carried out to test the *in vitro* activity of this herbal mixture on control and clinical strains of staphylococci.

Materials and method

Bacterial strains

The 18 bacterial strains tested consisted of a control *Staphylococcus aureus* (ATCC strain 29213), three clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), 4 isolates of methicillin-sensitive *Staphylococcus aureus* (MSSA) and 10 isolates of coagulase-negative staphylococci (CNS), of which 5 were methicillin-resistant and 5 methicillin-sensitive. The clinical isolates were from wound swabs of in-patients of the University of Malaya Medical Centre, Kuala Lumpur.

Preparation of stock solution

The EW1 powder was transported from Shanghai, China, at room temperature and kept at 4°C for several weeks until used for testing. Different conventional solvents were used to dissolve the powder including chloroform, dimethylformamide, dimethylsulfoxide, glacial acetic acid, absolute methanol, absolute ethanol, saturated sodium bicarbonate, 7% sodium bicarbonate, 2N NaOH, water, 0.1N HCl and a combination of glacial acetic acid and 7% sodium bicarbonate. Of these 12 solvents, only HCl was able to bring the powder into solution without heating. Hence, a stock solution of 10mg/ml was made in 0.1N HCl by dissolving 10 mg in 900 µl of sterile millipore-filtered water and 100 µl

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of 1N HCl, filter-sterilized using a 0.22 μm membrane filter and stored at –20°C.

Determination of Minimal Inhibitory Concentrations (M.I.C.) by agar dilution

The EW1 stock solution was diluted in sterile distilled water to give 3.125 to 800 mg/l. Two ml of each dilution was added to 18 ml of molten Mueller-Hinton agar to make a series of agar plates containing 0.3 to 80 mg/l of EW1. The 18 staphylococcal strains to be tested were incubated in Nutrient Broth (NB, Oxoid) overnight and then diluted 1:100 to approximately 10^5 organisms/ml. Using a micropipettor, 100 μl of each diluted culture was delivered onto each of the EW1-containing agar plates. The plates were then incubated overnight at 37°C and read for growth of bacteria after 18 hours. The M.I.C. was the lowest concentration of EW1 that totally inhibited the growth of bacteria.

Direct application of EW1 onto a bacterial lawn

As EW1 is usually applied as a thick patch directly onto the skin, the concentration of the active principle at the site of application may be higher than those used in the M.I.C. determination. To investigate the inhibitory effect of EW1 at higher concentrations, overnight NB cultures of the 18 staphylococcal strains were inoculated onto Mueller-Hinton agar plates with sterile cotton wool swabs and allowed to dry on the bench. The stock EW1 solution was diluted to 1000 mg/l and 20 μl volumes of the diluted solution were pipetted onto the inoculated agar plates. In addition, one gram of EW1 powder was made into a paste with a few drops of water and 0.1N HCL and directly applied onto the surface of the inoculated agar plates in circular patches of about 7 mm diameter. All plates were incubated overnight at 37 °C and then read for growth inhibition zones.

Time-kill studies

Two tests were carried out to compare the growth of *S. aureus* ATCC 29213 in NB with that in EW1 and in the solvent HCl. In the first test, the ATCC strain was incubated in NB overnight. Ten μl of this broth culture was used to inoculate each of three tubes containing a) 1 ml NB with 100 mg/l of EW1, b) 1 ml of NB with HCl (final concentration of 0.001N), and c) 1ml of NB. The inoculated tubes were then incubated in a 37°C water-bath. At 0, 2, 4, 5 and 6 hr after incubation, 20 μl was removed from each tube and diluted from 10^{-1} to 10^{-8}. Twenty μl of each dilution was then spotted on to a Nutrient Agar plate and incubated overnight. The number of colonies grown on the agar plate was counted to obtain the number of cfu/ml. In the second test, the whole procedure was repeated without the HCl control tube.

Results

In the agar dilution test, the ATCC control did not grow on agar containing ≥ 10 mg/l of EW1 but there was no demonstrable growth inhibition for any of the clinical strains even up to 80 mg/l (Table 1).

Table 1. Minimal Inhibitory Concentration of EW1 for staphylococci

Bacteria	No.of strains	MIC (mg/l)
S. aureus ATCC 29213	1	10
Methicillin-sensitive *S. aureus*	3	>80
Methicillin-resistant *S. aureus*	4	>80
Methicillin-sensitive CNS	5	>80
Methicillin-resistant CNS	5	>80

CNS, coagulase-negative staphylococci

When EW1 was applied as a paste, there was a clear zone of growth inhibition of 10 mm diameter around the ATCC control but not around any of the clinical strains. When applied as a 1000 mg/l solution, 3 clinical strains besides the ATCC control were partially inhibited as shown by the presence of tiny colonies within the zone of inhibition formed in the area where EW1 was dropped onto the staphylococcal lawn.

In the first time-kill study, the colony count of ATCC 29213 in NB grew from $1.5x10^3$ at 0 hour to $3.5x10^6$ at 6 hours of incubation. The corresponding counts in HCl and EW1 at 6 hours were $8.5x10^6$ and $2.0x10^5$ respectively. These counts indicated that, at the final concentration of 0.001N, the HCl solvent did not interfere with the growth of staphylococci while EW1 reduced the colony count by more than 1 log. This reduction in viable count was again seen in the second study where the count of ATCC 29213 rose from $5x10^2$ at 0 hour to $1x10^7$ in NB and $1.5x10^5$ in EW1 at 6 hours. Pooling together the results of both studies, the mean colony count in the absence of EW1 was $7.3x10^6$ cfu/ml while that in the presence of EW1 was $1.75x10^5$ cfu/ml, making a mean reduction of 1.5 log (Fig.1).

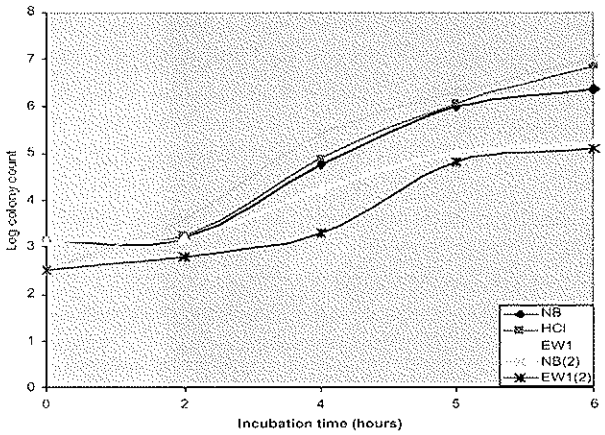

Figure 1. The effect of EW1 on the growth of *S. aureus* ATCC 29213

Discussion

Herbal medicine has been used for thousands of years by many different cultures. Its curative ability is established by the collective experience from large numbers of users. Scientific documentation of efficacy is scarce. The evaluation of herbal remedies may require strategies that are different from those used to evaluate drugs used in modern medicine. The biological activity of herbs may vary according to factors such as the age of the plants, the time of year in which the plants are collected and the geographical area from which the plants are collected. In addition, herbs are often used in combination with other plants, minerals or animal parts, all of which may vary in biological activity according to how and where they are obtained. Hence the therapeutic activity of herbal preparations may be less predictable or reproducible than that of synthesized drugs.

The results of the preliminary studies on EW1 described in this report show that this herbal preparation does have some anti-staphylococcal activity as indicated by the M.I.C. of 10 mg/l against ATCC 29213. However, this M.I.C. is considerably less than that of commonly used anti-staphylococcal antibiotics such as cloxacillin and vancomycin, both of which have a M.I.C. of 0.5-2.0 mg/l for ATCC 29213. This weak inhibitory activity was also seen in the time-kill studies where the mean reduction in colony count after 6 hours of incubation was less than 100-fold. With clinical isolates, the results were even less impressive. None of the 17 clinical strains tested were inhibited when EW1 was applied as a paste to simulate clinical usage, and only 3 strains of methicillin-sensitive *S aureus* were partially inhibited at a very high concentration of 1000 mg/l. These results suggest that the reported therapeutic effect of EW1 on carbuncles may not be solely due to its anti-staphylococcal activity.

For acceptance as an evidence-based therapeutic agent, further studies on EW1 are required to establish its *in vitro* and *in vivo* efficacy.

Acknowledgement

The authors are grateful to Dr J de Silva for the supply of EW1 powder and background information on this traditional herbal remedy.

Reference

1. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. WHO Bulletin 1985;63:965-81.

RUPTURE OF THE CAECUM DUE TO INVASIVE AMOEBIASIS

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ABSTRACT: This paper describes a case of invasive amoebiasis in a 72 year old woman. About 1 week prior to admission she had right iliac fossa pain and physical examination suggested perforated appendix or carcinoma of caecum. Laparotomy revealed perforation of caecum. Histopathology of tissue removed showed abundant trophozoites of *Entamoeba histolytica*. After surgery treatment was instituted and patient had an uneventful recovery. *(JUMMEC 2000; 1: 51-52)*

KEYWORDS: Rupture of caecum, amoebiasis

Introduction

Amoebiasis is more commonly seen in tropical and subtropical regions and is endemic in Malaysia. Amoebiasis is due to invasion of the colonic mucosa by the enteric protozoan *Entamoeba histolytica*. *Entamoeba histolytica* usually lives as a harmless commensal in the lumen of the human large bowel. Occasionally *E. histolytica* becomes a pathogen. Amoebae invade the tissues by secreting lytic enzymes (6) and through the activation of the complement system. This lytic and pseudopodial activities of the amoebae is the major cause of its penetration and necrotising activity in the tissues. In rare instances, they invade the muscle layers and further outward invasion of tissues results in perforation of the gut. Immune suppression due to any cause, and other bowel infections favour this invasion. Precipitating causes for perforation eg. parturition, external trauma and surgery have been mentioned (6). Perforation of the colon is a rare but frequently fatal complication of amoebiasis (2). Owing to varying presentations, the condition is often not diagnosed preoperatively (2).

Case report

The patient, a 72 year old Chinese woman complained of right iliac fossa pain of one week duration. One month previously she had experienced a few episodes of bloody diarrhea. Examination revealed a febrile patient with a temperature of 38 °C. Abdomen was tender at right iliac fossa with a mass. A presumptive diagnosis was made as perforated appendix or carcinoma of caecum. A laparotomy was done with right paramedial incision. Omentum was found to have a flimsy adhesion on separation.

There was faecal discharge coming from a 3 cm perforation at anterior surface of caecum. Examination of appendix, liver and other organs appeared normal. Right hemicolectomy was done. The specimen consisting of caecum, appendix and ileum was sent to the Pathology laboratory for gross and microscopic examinations. For microscopic examination sections of tissues removed were submitted in blocks. Sections of 5 – 6 μm were made and stained with hematoxylin and eosin. Patient's blood was taken and sent to the Department of Parasitology for serological test for amebiasis. ELISA was performed on the patient's serum.

Direct ELISA

Antigen : *Entamoeba histolytica* in house antigen was used.

Test procedure: Plate was coated with antigen, 50 μl/well. A flex plate bottom was used. Plate was incubated in wet chamber overnight at 4 °C. It was then washed off 3 times with PBS-tween, 5 minutes in between each wash. It was then blocked with 0.5% BSA, 200 μl/well. Plate was covered with aluminium foil and incubated at Room temperature for 2 hours. It was then washed off 3 times with PBS-tween. Serial dilutions of patient's sera were added to the antigen wells, 50 μl / well. Dilutions were made both for IgM and IgG. Antigen and serum dilutions were incubated for 1 hour at room temperature. Excess seen were washed off 3 times with PBS-tween. After being dried the antigen wells were covered with antihuman sera, 50 μl/well and were incubated for 1 hour at Room temperature. After excess antihuman sera had been washed 3 times with PBS – tween, substrate [(PNPP (P–Nitrophenyl Phosphate Tablets) dissolved in 5 ml of DEA (Diethyl amine)] was added to the wells, 50

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µl/ well and incubated for 15 minutes at room temperature. Then the plate was read using the ELISA machine using a 405 filter. A titre of 1:64 was considered positive.

Results

Gross appearance of specimen is as seen in Figure 1 which shows caecum together with attached terminal part of ileum and appendix. There was an area of friable necrotic perforation of the caecal wall. The site of perforation was covered over the serosa surface by adherent omental fat. The caecal wall including the mucosa around it was oedematous and congested in appearance.

Microscopic examination of the caecum is as depicted in Figure 2. The caecal specimen shows a cluster of trophozoites of *E. histolytica* densely infiltrated by acute inflammatory cells and fibrinous debris. The trophozoites have a tiny, eccentrically located nucleus. Some trophozoites contain erythrocytes.

Serological test

The serum was positive for both IgM and IgG. IgG is positive with a titre of 1:2048.

Discussion

Clinical presentation of amoebic colitis is vague and hence the condition is likely to be mistaken for other common abdominal ailments. If the diagnosis is missed, the condition may lead to high mortality rates. Treated in time, it is a curable disease. In the case presented, diagnosis of amoebiasis was missed. The diagnosis could have been made preoperatively in this patient who presented with an acute abdomen and with a history of bloody diarrhoea but it had not been considered. The involvement was primarily caecal and the symptoms mimic ruptured appendicitis or ruptured carcinoma of caecum. Early diagnosis by modern serological tests and prompt treatment with effective anti-amoebic drugs, can prevent complications(6). Therefore it is pertinent for physicians to carry out serodiagnosis for amoebiasis to establish or exclude the diagnosis in patients who present with bloody diarrhoea. For this patient, after the operation she was put on treatment with metronidazole and she had an uneventful recovery.

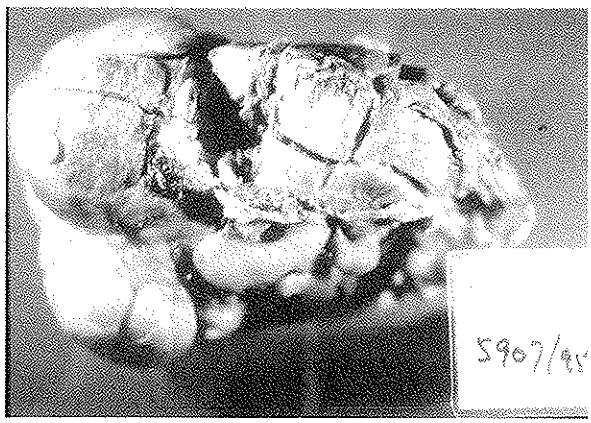

Figure 1. Caecum: necrotic with perforation.

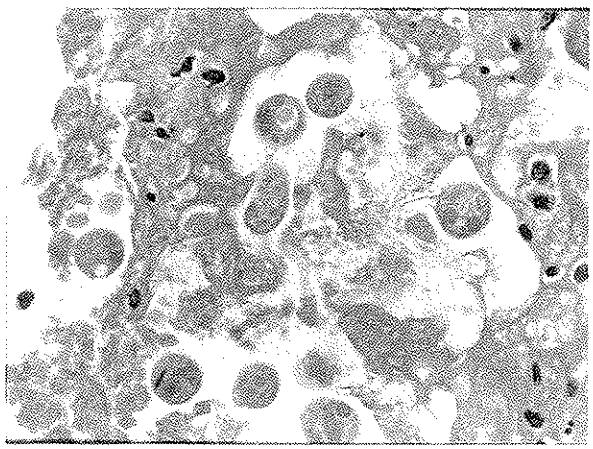

Figure 2. Microscopic appearance showing clusters of trophozitee of *E. histolytica.*

References

1. Adams EB and MacLeod IN. Invasive amebiasis. I.Amebic dysentery and its complications. Medicine, 1977; 56:315 – 23.
2. Eggleston FC, Verghese M and Handa AK. Amoebic perforation of the bowel : experience with 26 cases. Brit, J. Surg. 1978; 65: 748 – 51.
3. Harries J. Amoebiasis : a review. Journal of the Royal Society of Medicine 1982; 75 :190–7.
4. Judy KL. Amebiasis presenting as an acute abdomen Am Jof Surg, 1974; 127: 275–9.
5. Manukaran MN, Ahmad H and Abdullah I. Amoebiasis with multiple colonic perforations and ruptured liver abscess Med. J. Malaysia 1983; 38 : 71–3.
6. Thuse MG. Fatal intestinal amoebiasis. Postgrad. Med. J. 1979; 55:548-52.

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