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Editorial

Telemetric EEG and the Rat: A Guide For Neuroscientists

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Abstract

Telemetric EEG in the rat's brain has been used for experiments which tests the effects of an antiepileptic compound on it's antiseizures activity. A simple classification correlating epileptiform discharge and Racine's behavioral activity is discussed.

Keywords: *electroencephalogram, neuroscience, rat, telemetry*

Animal models for seizures and epilepsy have played a fundamental role in advancing our understanding of basic mechanisms underlying ictogenesis and epileptogenesis and have been instrumental in the discovery and preclinical development of novel antiepileptic drugs. Despite the successful development of various new antiepileptic drugs in recent decades, the search for new therapies with better efficacy and tolerability remains an important goal (1). The discovery and development of a new antiepileptic drug relies heavily on the preclinical use of animal models to establish efficacy and safety prior to first trials in humans (2).

Racine's scale is one of the most frequently used tools to determine the intensity of a seizure in rodent models of experiment epilepsy. Racine developed the scale by investigating the relationship between electroencephalogram (EEG)-changes and the development of motor seizures in the amygdala-kindling model, characterized by partial seizures with secondary generalization The motor symptoms include "mouth and facial movements" (intensity stage 1); "head nodding" (stage 2); "forelimb clonus" (stage 3); seizures characterized by rearing, (stage 4) and seizures characterized by rearing and falling (stage 5) (3).

Racine's scale is frequently applied to many other models for seizures and epilepsy. It is however questionable, whether the use of Racine's scale for the assessment of seizure intensities in other epilepsy or seizure models is justifiable, given the well known relation between activated brain part and corresponding expressed behavior of Sprague Dawley rats (160–350 g) which are suitable animals to induce status epilepticus models and to record continuous EEG spikes and wave discharges (4–6).

We used a total of 10 male Sprague Dawley and 6 Genetic Absence Epilepsy Rats from Strasbourg (GAERS) rats (7), four to six months of age and weighing 187–325 g. The parent GAERS rats were gifts from the Kyoto University, Japan. The animals were born and raised under environmentally controlled conditions (12 hours light/dark cycles, 20–22 °C in the animal facility house of the Universiti Sains Malaysia Health Campus, with food and *water ad libitum*. All animals were treated according to the guidelines approved by the Animal Ethics Committee of Universiti Sains Malaysia.

Prior to surgery (5–15 min), the animals were anaesthetized with ketamine and xylazine (80 mg/kg and 7.5 mg/kg, respectively, i.p.) and additional ketamine (5 mg/kg, i.p.) were given during surgery when a sensorial pain stimulus by squeezing the footpad, elicited motor reflexes (8). After proper anaesthesia, the fur on the head and back were clipped rostral to the medial canthus of the eyes to immediately cranial to the last cervical vertebra in a strip approximately 3 cm wide. The

animals were placed on a heating pad and secured in a stereotaxic apparatus (Stoelting Model 516 00; Illinois, U.S.A.). The surgical site and surrounding area were swabbed with 70% ethyl alcohol and scrubbed with a 4% chlorohexidine solution. A 3–4 cm mid-sagittal incision was made on the scalp and the skin reflected with haemostats to expose the entire dorsal portion of the skull. The periosteum was removed and haemostasis achieved with sterile cotton-tip applicators. Bregma was marked and two holes bored through the skull with drilling (# 105 drill bit). Stainless steel electrodes (DSI Model F40-EET; St. Paul, MN, U.S.A.) insulated except at the tip were implanted bilaterally into the brain over the parietal cortex. The other two electrodes were placed in the neck muscle of EMG recording to compare with the EEG spikes. The EEG electrodes were fixed to the skull of rat with dental acrylic. The radio telemetry unit was placed subcutaneously into the pocket over and caudal to the scapula. Using blunt-ended scissors, a subcutaneous pocket was made caudally from the incision by pushing aside connective tissue and then skin was sutured (Figure 1A-1D). The method of telemetry implantation was followed from White *et al.* (2006; 2010). The surgical procedures of our experiment were considered as minimum to mild pain scale according to the pain assessment, and it was managed by local anaesthesia (9).

Kainic acid was administered intraperitoneally on Sprague Dawley rats (n = 6) to induce an episode of status epilepticus. One to 2 weeks after telemetry implantation, Sprague Dawley rats were injected with kainic acid (5 mg/kg; IP: Sigma, St. Louis, MO) diluted in sterile 0.9% saline at 2.5 mg/ml. Rats were continuously monitored for electroencephalogram and motor seizures. Racine scale was used to characterize motor seizure severity (3). Kainic acid injections were repeated at 1 hours intervals until class III, IV, or V seizures were evoked for at least 3 hrs (i.e., 10 convulsive seizures per hour). If animals were nearing its endpoint, half-doses (2.5 mg/kg) were given to avoid excessive toxicity and mortality. Kainate administration was terminated for animals displaying electrographic seizures with few or no motor seizures after receiving four full doses. Control animals (n = 4) were treated with normal saline with same volume and number of injections. In GAERS rats (n = 6) only DSI telemetry are implanted without injecting kainic acid. All rats were given 5 ml lactated Ringer's (subcutaneously) and apple slices following treatment.

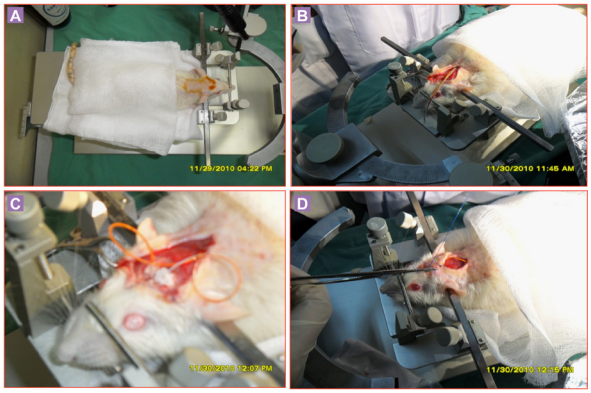

Figure 1A-1D: Implantation of DSI telemetry system into Sragues Dawley rat.

The EEG activity was acquired by Dataquest DSI telemetry, USA software and analysed off-line using Neuro-Score software, DSI, USA, configured for automatic detection and saving of spikes and wave discharges, and seizures. Setting for seizure detection were the following: amplitude threshold, 3; seizures duration, > 2s; detection threshold, 0; minimum frequency, 3 Hz; short burst detection was turned off; length of EEG kept before and after each seizure was 0.5 min. The duration of the acquisition time was 24 hours. Analysis of EEG was performed by a 'blinded' unbiased investigator. All seizure EEGs were revised manually. The spike bursts lasting less than 3 s are not counted as seizures.

The EEG of GAERS rat was used for positive control in this study. In GAERS rats, the fully developed spike and wave discharges typically have the following features: a fundamental frequency in the range of 7–12 Hz; an amplitude varying from 300 to 1000 μV, and a duration in the range of 1–65 s (Figure 2) that was fully comparable with the previous study (10).

We detected a total five different types of electroencephalographic spike and wave epileptic discharges and coupled with the Racine's behavioural scale (Table 1) throughout the recording periods that were different from the baseline recordings of EEG in Sprague Dawley rats.

The first epileptic EEG was characterized by sharp-spike discharges with a frequency of 2–4 Hz, a mean duration of 10 s (ranging from 3 s to 1–5 min) (Figure 3A), and a downward spikes amplitude of around 600 μV (baseline up to 200 μV) (Figure 3B shows a close-up view). During this EEG discharges pattern linked to the stage 1 and stage 2 behavioral categories of Racine's scale. The rats were behaviorally arrested for a while, stiffness of the neck muscles, facial jerking and neck jerks.

The second epileptic EEG discharge (Figure 3C shows a close-up view) consisted of clonic epileptic discharges with a frequency of 2 to 3 Hz and poly spikes and waves amplitude was around 600 μV (baseline up to 200 μV). These discharges had irregular spikes, sometimes consisted of poly spikes, and the spikes were less sharp than those of the first type of discharge pattern. Forelimb clonus symptoms were observed in this stage.

The third epileptiform EEG discharge pattern was long lasting characterized by a frequency of 6 to 10 Hz sharp irregular spikes, sometimes with poly-spikes, and a spike amplitude of around 800 μV. A close-up look of this EEG deviation is found in Figure 3D. The tonic behavioral symptoms, rearing were observed in kainic acid induced epileptic rats.

Another distinct tonic-clonic epileptic EEG abnormality consisted of very characteristic high

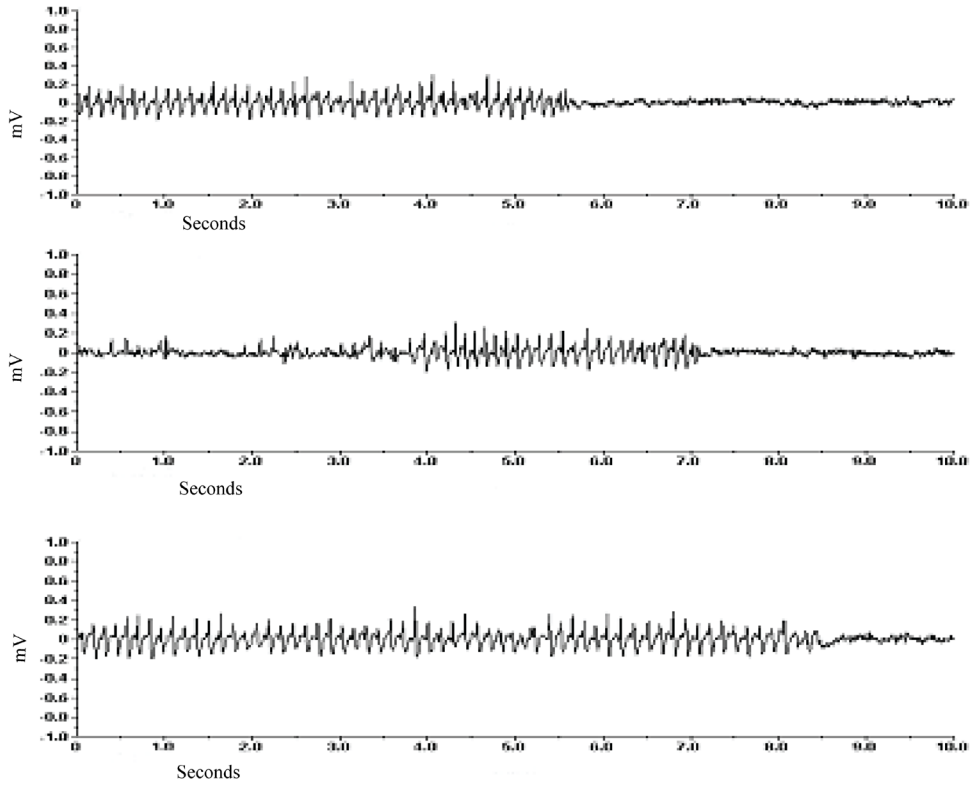

Figure 2: Spike and wave discharges (SWDs) of EEG in Genetic Absence Epilepsy Rats from Strasbourg (GAERs) (n = 6). X axis is showing amplitude in mV and Y axis is showing frequency (Hz) in seconds.

Table 1: Different types of electroencephalographic epileptic discharges in kainic acid-induced Sprague Dawley rats that are coupled with Racine's scale (n = 6)

Class	Epileptiform discharge	Racine's Scale Behavioral Activity
1 and 2	Sharp wave epileptic discharge (sharp/ spikes pattern)	Mouth and facial movements (class 1), and Head nodding (wet dog shakes) (class 2)
3	clonic epileptic discharge (rhythmic pattern/ spike and wave)	Forelimb clonus
4	Tonic epileptic discharge (rapid sharp pattern/poly spikes)	Rearing
5	Tonic-clonic (irregular pattern/ poly spikes and wave)	Rearing and falling

amplitude poly spikes (up to 900 μV), which were sometimes embedded in some irregular spikes of smaller amplitude (Figure 3E).

A last irregular pattern of epileptic EEG discharge found consisted mostly of long lasting (1 to 5 min), high amplitude single spiking with short amplitude poly-spikes, and sometimes it followed by high frequency oscillations. These high frequency oscillations ranged from 10 to 20 Hz. A close-up look at this type of EEG deviation can be found in Figure 3F.

This classification will be able to guide us

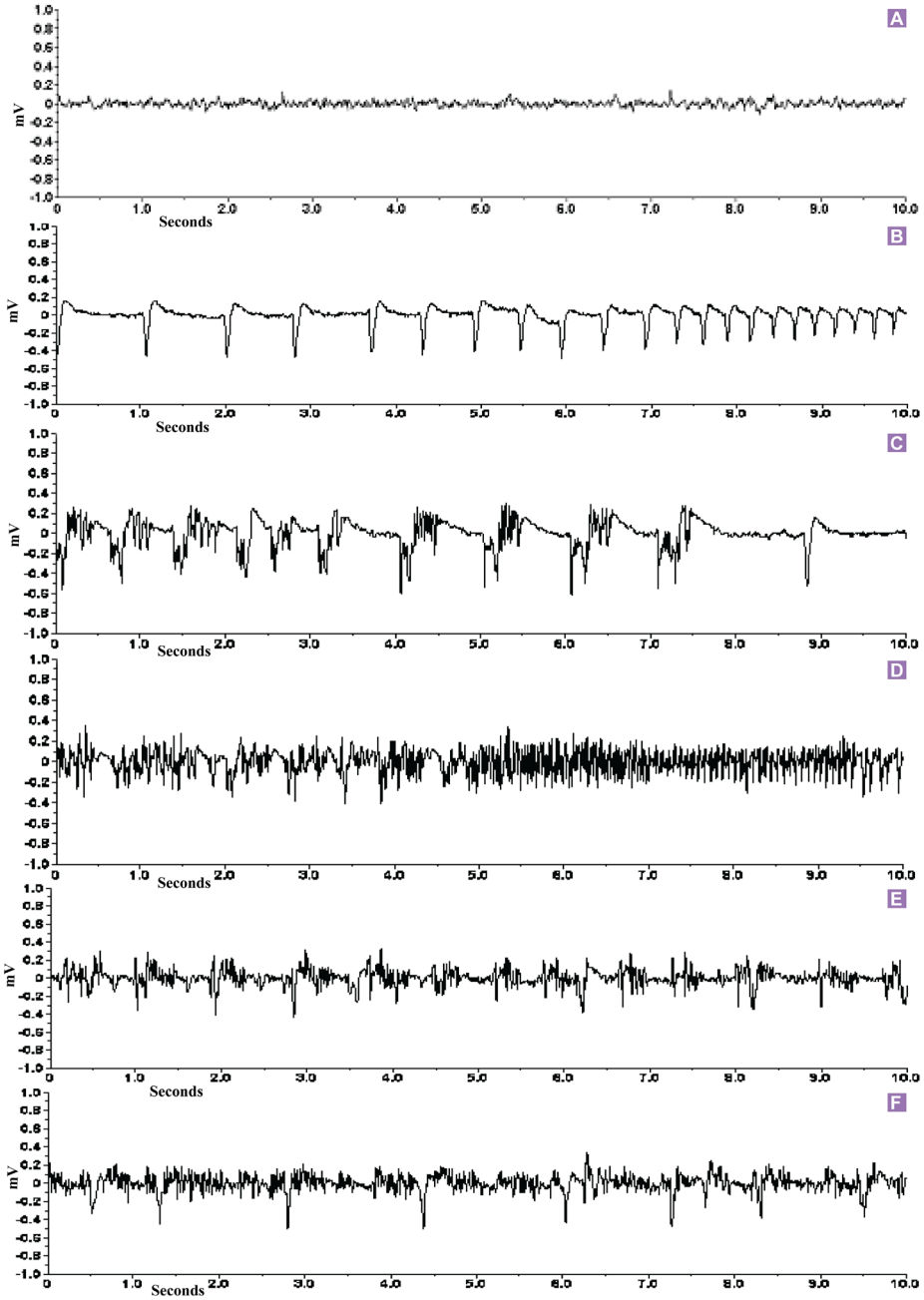

Figure 3A-3F: EGG records in Sprague Dawley rats (n = 6). (A) Baseline EEG recording in Sprague Dawley rats before kainic acid injection. (B) Downward sharp epileptic EEG discharges in kainite-induced rats. (C) Clonic epileptic EEG discharges. (D) Tonic epileptic EEG discharges. (E) Tonic-clonic epileptic EEG discharges. (F) Irregular epileptic EEG recording of kainite-induced Sprague Dawley rats. X axis is showing amplitude in mV and Y axis is showing frequency (Hz) in seconds.

when one observes the EEG changes when the effects of the proposed antiseizure compound is being monitored.

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Review Article

Homo Heuristicus: Less-is-More Effects in Adaptive Cognition

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Abstract

Heuristics are efficient cognitive processes that ignore information. In contrast to the widely held view that less processing reduces accuracy, the study of heuristics shows that less information, computation, and time can in fact improve accuracy. We discuss some of the major progress made so far, focusing on the discovery of less-is-more effects and the study of the ecological rationality of heuristics which examines in which environments a given strategy succeeds or fails, and why. Homo heuristicus has a biased mind and ignores part of the available information, yet a biased mind can handle uncertainty more efficiently and robustly than an unbiased mind relying on more resource-intensive and general-purpose processing strategies.

Keywords: *cognition, heuristics, uncertainty*

Introduction

As far as we know, animals have always relied on heuristics to solve adaptive problems, and so have humans. To measure the area of a candidate nest cavity, a narrow crack in a rock, an ant has no yardstick but a rule of thumb: Run around on an irregular path for a fixed period while laying down a pheromone trail, and then leave. Return, move around on a different irregular path, and estimate the size of the cavity by the frequency of encountering the old trail. This heuristic is remarkably precise: Nests half the area of others yielded re-encounter frequencies 1.96 times greater (1). Many evolved rules of thumb are amazingly simple and efficient (2).

The Old Testament says that God created humans in his image and let them dominate all animals, from whom they fundamentally differ (Genesis 1:26). It might not be entirely accidental that in cognitive science some form of omniscience (knowledge of all relevant probabilities and utilities, for instance) and omnipotence (the ability to compute complex functions in a split second) has shaped models of human cognition. Yet humans and animals have common ancestors, related sensory and motor processes, and even share common cognitive heuristics. Consider how a baseball outfielder catches a ball. The view of cognition favoring omniscience and omnipotence suggests that complex problems are solved with complex mental algorithms. Richard Dawkins, for example, argues that "He behaves as if he had solved a set of differential equations in predicting the trajectory of the ball. At some subconscious level, something functionally equivalent to the mathematical calculations is going on" (3, p 96). Dawkins carefully inserts "as if" to indicate that he is not quite sure whether brains actually perform these computations.

And there is indeed no evidence that brains do. Instead, experiments have shown that players rely on several heuristics. The gaze heuristic is the simplest one and works if the ball is already high up in the air: Fix your gaze on the ball, start running, and adjust your running speed so that the angle of gaze remains constant (4). A player who relies on the gaze heuristic can ignore all causal variables necessary to compute the trajectory of the ball–the initial distance, velocity, angle, air resistance, speed and direction of wind, and spin, among others. By paying attention to only one variable, the player will end up where the ball comes down without computing the exact spot. The same heuristic is also used by animal species for catching prey and for intercepting potential mates. In pursuit and predation, bats, birds, and dragonflies maintain a constant optical angle between themselves and their prey, as do dogs when catching a Frisbee (5).

In the 1950s, Herbert Simon proposed that people satisfice rather than maximize (6,7). Maximization means optimization, the process of finding the best solution for a problem, whereas satisficing (a Northumbrian word for "satisfying") means finding a good-enough solution. For

Simon, humans rely on heuristics not simply because their cognitive limitations prevent them from optimizing, but also because of the task environment. For instance, chess has an optimal solution, but no computer or mind, be it Deep Blue or Kasparov, can find this optimal sequence of moves because the sequence is computationally intractable to discover and verify. In the 1970s, the term heuristic acquired a different connotation, undergoing a shift from being regarded as a method that makes computers smart to one that explains why people are not smart.

Daniel Kahneman, Amos Tversky, and their collaborators published a series of experiments in which people's reasoning was interpreted as exhibiting fallacies. "Heuristics and biases" became one phrase. It was repeatedly emphasized that heuristics are sometimes good and sometimes bad, but virtually every experiment was designed to show that people violate a law of logic, probability, or some other standard of rationality. By the end of the 20th century, the use of heuristics became associated with shoddy mental software, generating three widespread misconceptions:

1. Heuristics are always second-best.
2. We use heuristics only because of our cognitive limitations.
3. More time, more information, and more computation would always be better.

These three beliefs are based on the so-called accuracy-effort trade-off, which is considered a general law of cognition: If you invest less effort, the cost is lower accuracy. Effort refers to searching for more information, performing more computation, or taking more time. In fact, these typically go together. Heuristics, on the other hand, allow for fast and frugal decisions; thus it is commonly assumed that they are second-best approximations of more complex "optimal" computations and serve the purpose of trading off accuracy for effort. Contrary to the belief in a general accuracy-effort trade-off, less information and computation can actually lead to higher accuracy, and in these situations the mind does not need to make trade-offs. Here, a less-is-more effect holds. That simple heuristics can be more accurate than complex procedures is one of the major discoveries of the last decades. Heuristics achieve this accuracy by successfully exploiting evolved mental abilities and environmental structures. Since this initial finding a systematic science of heuristics has emerged.

The discovery of less-is-more

Many theories of cognition–from exemplar models to prospect theory to Bayesian models of cognition–assume that all pieces of information should be combined in the final judgment. The classical critique of these models is that in the real world, search for information costs time or money, so there is a point where the costs of further search are no longer justified. This has led to optimization-under-constraints theories in which search in the world (9) or in memory (10) is terminated when the expected costs exceed the benefits. Note that in this "rational analysis of cognition," more information is still considered better, apart from its costs. Similarly, the seminal analysis of the adaptive decision maker (11) rests on the assumption that the rationale for heuristics is a trade-off between accuracy and effort, where effort is a function of the amount of information and computation consumed:

> *Accuracy-effort trade-off:* Information and computation costs time and effort; therefore minds rely on simple heuristics that are less accurate than strategies that use more information and computation.

Here is the first important discovery: Heuristics can lead to more accurate inferences than strategies that use more information and computation (see below). Thus, the accuracy-effort trade-off does not generally hold; there are situations where one attains higher accuracy with less effort. Even when information and computation is entirely free, there is typically a point where less is more:

> *Less-is-more effects:* More information or computation can decrease accuracy; therefore, minds rely on simple heuristics in order to be more accurate than strategies that use more information and time.

To justify the use of heuristics by accuracy-effort trade-offs means that it is not worth the effort to rely on more complex estimations and computations. A less-is-more effect, however, means that minds would not gain anything from relying on complex strategies, even if direct costs and opportunity costs were zero. Accuracy-effort trade-offs are the conventional justification for why the cognitive system relies on heuristics (12,13), which refrains from any normative implications. Less-is-more effects are a second justification with normative consequences: They

challenge the classical definition of rational decision-making as the process of weighting and adding all information. Note that the term less-is-more does not mean that the less information one uses, the better the performance. Rather, it refers to the existence of a point at which more information or computation becomes detrimental, independent of costs.

Ignoring information can lead to more accurate predictions

In the 1970s, researchers discovered that equal (or random) weights can predict almost as accurately as, and sometimes better than, multiple linear regression (14–17). Weighting equally is also termed *tallying*, reminiscent of the tally sticks for counting, which can be traced back some 30 000 years in human history. These results came as a surprise to the scientific community. When Robin Dawes presented the results at professional conferences, distinguished attendees told him that they were "impossible," his paper with Corrigan was first rejected and deemed "pre-mature". A sample of recent textbooks in econometrics revealed that none referred to the findings of Dawes and Corrigan (18). In these original demonstrations, there was a slight imbalance: Multiple regression was tested by cross-validation (that is, the model was fitted to one half of the data and tested on the other half) but tallying was not. Czerlinski, Gigerenzer, and Goldstein conducted 20 studies in which both tallying and multiple regression were tested by cross-validation, correcting for this imbalance (19). All tasks were paired comparisons. For instance, estimating which of two Chicago high schools will have a higher drop-out rate, based on cues such as writing score and proportion of Hispanic students. Ten of the 20 data sets were taken from a textbook on applied multiple regression (20). Averaged across all data sets, tallying achieved a higher predictive accuracy than multiple regression (Figure 1). Regression tended to overfit the data, as can be seen by the cross-over of lines: It had a higher fit than tallying, but a lower predictive accuracy.

The point here is not that tallying leads to more accurate predictions than multiple regression. The real and new question is in which environments simple tallying is more accurate than multiple regression, and in which environments it is not. This is the question of the *ecological rationality of tallying*. Tallying avoids precise computation of cue weights. Next, we consider less-is-more effects which arise by ignoring cues. The take-the-best heuristic is a model of how people infer which of two objects has a higher value on a criterion, based on binary cue values retrieved from memory. For convenience, the cue value that signals a higher criterion value is 1, and the other cue value is 0. Take-the-best consists of 3 building blocks:

1. Search rule: Search through cues in order of their validity.
2. Stopping rule: Stop on finding the first cue that discriminates between the objects (i.e., cue values are 1 and 0).
3. Decision rule: Infer that the object with the positive cue value (1) has the higher criterion value.

Take-the-best is a member of the one good-reason family of heuristics because of its stopping rule: Search is stopped after finding the first cue that enables an inference to be made. Take-the-best simplifies decision making by both stopping after the first cue and by ordering cues unconditionally by validity, which for it cue is given by:

v_i = number of correct inferences using cue i / number of possible inferences using cue i.

Both these simplifications have been observed in the behavior of humans and other animals, but routinely interpreted as signs of irrationality rather than adaptive behavior. In the late 1990s, our research group tested how accurately this simple heuristic predicts which of two cities has the larger population, using real-world cities and binary cues, such as whether the city has a soccer team in the major league (21,22). The unexpected result was that inferences relying on one good reason were more accurate than both multiple regression and tallying. We obtained the same result, on average, for 20 studies (Figure 1). This result came as a surprise to both us and the rest of the scientific community. But there were more surprises to come. Chater et al., (23) used the city population problem and tested take-the-best against heavy-weight non linear strategies: A three-layer feedforward connectionist network, trained using the backpropagation algorithm (24), 2 exemplar-based models the nearest-neighbor classifier (25), and Nosofsky's generalised context model (26), and the decision tree induction algorithm C4.5 (27). The predictive accuracy of the four complex strategies was rather similar, but the performance of take-the-best differed considerably. When the percentage of training

examples (the sample size) was small or moderate (up to 40% of all objects), take-the-best out performed or matched all the competitors, but when the sample size was larger, more information and computation seemed to be better. This was the first time that relying on one good reason was shown to be as accurate as nonlinear methods, such as a neural network. Yet, as Brighton (28) showed in a re-analysis, Chater et al.'s method of fitting the models on the learning sample and then testing these models on the entire sample

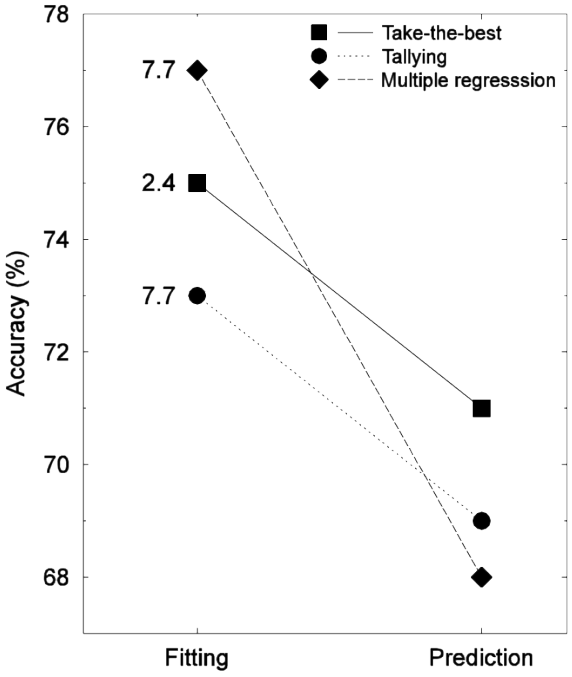

Figure 1: Less-is-more effects. Both tallying and take-the-best predict more accurately than multiple regression, despite using less information and computation. Note that multiple regression excels in data fitting ("hindsight"), that is fitting its parameters to data that is already known, but performs relatively poorly in prediction ("foresight," as in cross-validation). Take-the-best is the most frugal, that is, it looks up, on average, only 2.4 cues when making inferences. In contrast, both multiple regression and tallying look up 7.7 cues on average. The results shown are averaged across 20 studies, including psychological, biological, sociological, and economic inference tasks (19).

(including the learning sample), favored those models that overfit the data, especially at high sample sizes. When cross-validation was used, there was a new surprise: The predictive accuracy of take-the-best exceeded that of all rival models over the entire range of sample sizes (Figure 2). Cross-validation provides a far more reliable model selection criterion and is standard practice for assessing the relative performance of models of inductive inference (29,30).

Once again, another less-if-more effect was discovered, and a new question emerged: In which environments does relying on one good reason result in better performance than when relying on a neural network or on other linear and non-linear inference strategies? The success of take-the-best seems to be due to the fact that it ignores dependencies between cues in what turns out to be an adaptive processing policy when observations are sparse. Whereas all the competitors in Figure 2 attempt to estimate the dependencies between cues in order to make better inferences, take-the-best ignores them by ordering the cues by validity. In fact, when one alters the search rule of take-the-best by carrying out the more resource-intensive process of ordering cues by conditional validity, performance drops to the level of the more resource-intensive algorithms (Figure 2a). Conditional validity takes into account the fact that when one cue appears before another in the cue order, this first cue is likely to affect the validity of the second cue and all subsequent ones.

These two results are instances of a broader class of less-is-more effects found in the last decades, both analytically and experimentally. We use *less-is-more* here as a generic term for the class of phenomena in which the accuracy-effort trade-off does not hold, although the individual phenomena differ in their nature and explanation. Findings that show how less can be more have often been regarded as curiosities rather than as opportunities to re-think how the mind works. We turn now to the second step of progress made: the development of an understanding of *why* and *when* heuristics are more accurate than strategies that use more information and computation. The answer is not in the heuristic alone, but in the match between a heuristic and its environment. The rationality of heuristics is therefore ecological, not logical.

Ecological rationality

All inductive processes, including heuristics, make bets. This is why a heuristic is not inherently good or bad, or accurate or inaccurate, as is sometimes believed. Its accuracy is always

relative to the structure of the environment. The study of the ecological rationality asks the following question: In which environments will a given heuristic succeed, and in which will it fail? Understanding when a heuristic succeeds is often made easier by first asking why it succeeds. As we have shown, when analysing the success of heuristics, we often find that they avoid overfitting the observations. The statistical concept of overfitting is part of the explanation for why heuristics succeed, but to gain a clearer understanding of how and when heuristics exploit the structure of the environment, this issue can be examined more closely.

Heuristics and bias

The study of heuristics is often associated with the term bias. The heuristics and biases program

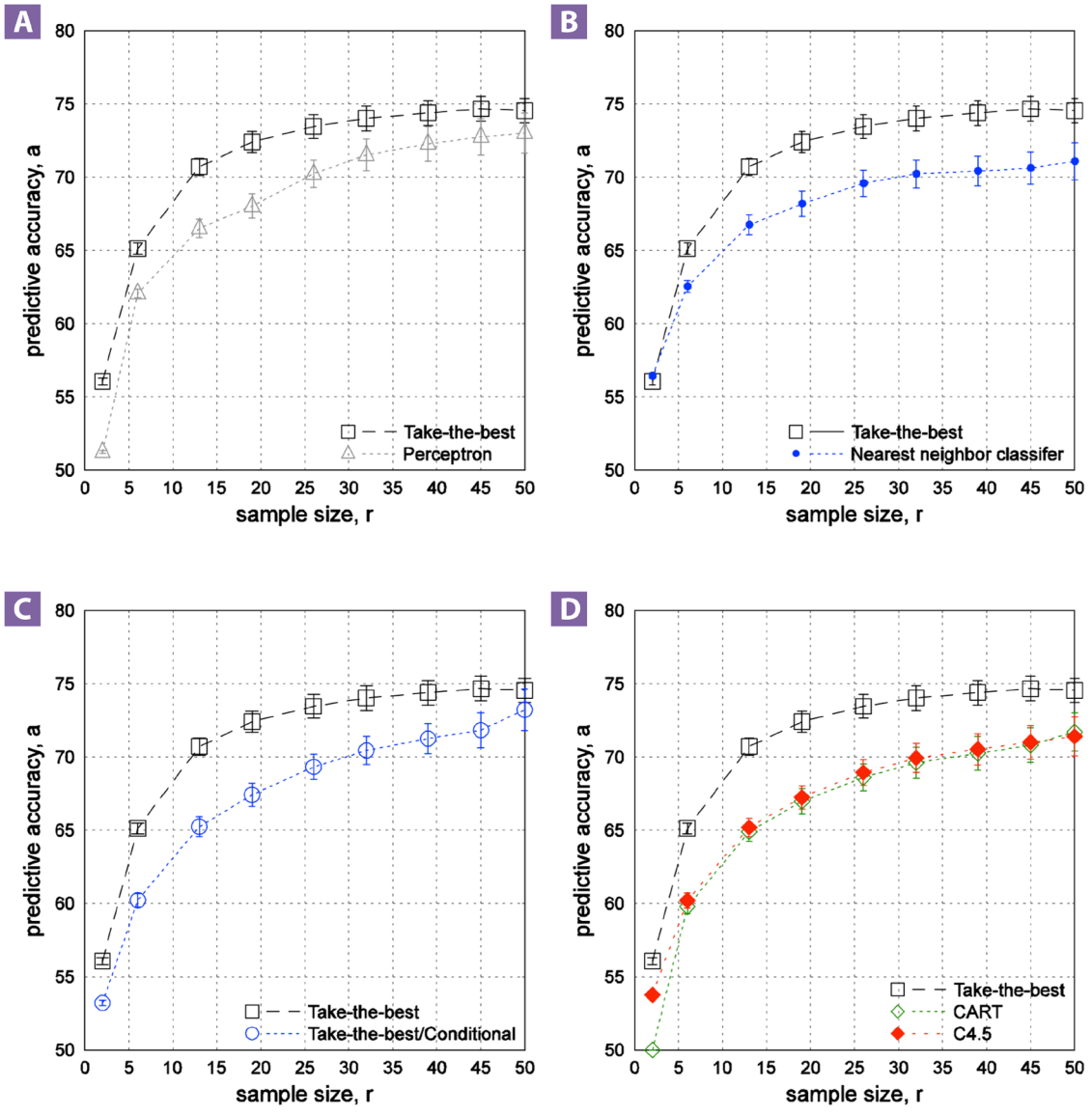

Figure 2: For the city population task, the performance of take-the-best compared to five alternative models. Each panel plots the predictive accuracy of take-the-best and a rival model as a function of the number of objects used to train the model. Take-the-best outperforms; (A) A linear perceptron (essentially logistic regression), (B) the nearest neighbor classifier, (C) a variant of take-the-best that uses a more resource-intensive search rule that orders cues by conditional validity, and (D) two tree induction algorithms, C4.5, and CART (classification and regression trees).

of Kahneman and Tversky used the term with a negative connotation: Reasoning errors reveal human biases that, if overcome, would result in better decisions. In this view, a bias is defined as the difference between human judgment and a "rational" norm, often taken as a law of logic or probability, such as statistical independence as in the gamblers' fallacy. In contrast to this negative use of bias, simple heuristics are perhaps best understood from the perspective of pattern recognition and machine learning, where there are many examples of how a biased induction algorithm can predict more accurately than an unbiased one (29). Findings such as these can be explained by decomposing prediction error into the sum of three components, only one of which is bias:

$$\text{Total error} = (\text{bias})^2 + \text{variance} + \text{noise}.$$

The derivation of this expression can be found in many machine learning and statistical inference textbooks (30–33) (29), but is perhaps most thoroughly set out and discussed in a landmark article by Geman et al., (34). The concepts of bias and variance can be understood by first imagining an underlying (true) function that some induction algorithm is attempting to learn. The algorithm attempts to learn the function from only a (potentially noisy) data sample generated by this function. Averaged across all possible data samples of a given size, the bias of the algorithm is defined as the difference between the underlying function and the mean function induced by the algorithm from these data samples. Thus, zero bias is achieved if this mean function is precisely the underlying function. Variance captures how sensitive the induction algorithm is to the contents of these individual samples and is defined as the sum squared difference between the mean function, mentioned above, and the individual functions induced from each of the samples.

Notice that an unbiased algorithm may suffer from high variance, because the mean function may be precisely the underlying function but the individual functions may suffer from excess variance and hence high error. An algorithm's susceptibility to bias and variance will always depend on the underlying function and how many observations of this function are available. Our cognitive systems are confronted with the bias-variance dilemma whenever they attempt to make inferences about the world. What can this tell us about the cognitive processes used to make these inferences? First of all, cognitive science is increasingly stressing the senses in which the cognitive system performs remarkably well when generalizing from few observations, so much so that human performance is often characterized as optimal (35,36). These findings place considerable constraints on the range of potential processing models capable of explaining human performance. From the perspective of the bias-variance dilemma, the ability of the cognitive system to make accurate predictions despite sparse exposure to the environment strongly indicates that the variance component of error is successfully being kept within acceptable limits. Although variance is likely to be the dominant source of error when observations are sparse, it is nevertheless controllable. This analysis has important implications for the possibility of general-purpose models. To control variance, one must abandon the ideal of general-purpose inductive inference and instead, consider to one degree or another, specialisation (34). Put simply, the bias-variance dilemma shows formally why a mind can be better off with an adaptive toolbox of biased, specialised heuristics. A single, general-purpose tool with many adjustable parameters is likely to be unstable and incur greater prediction error as a result of high variance.

Biased minds for making better predictions

The relationship between mind and environment is often viewed from the perspective of bias, following the "mirror view" of adaptive cognition (37). In this view, a good mental model or processing strategy is assumed to be one that mirrors the properties of the world as closely as possible, preferably with no systematic bias, just as a linear model is assumed to be appropriate if the world is also linear. A cognitive system with a systematic bias, in contrast, is seen as a source of error and the cause of cognitive illusions. If this were true, how can cognitive heuristics that rely only on one good reason and ignore the rest make more accurate inferences than strategies that use more information and computation do, as illustrated in Figure 2? We have identified three reasons:

1. The advantage of simplicity is not because the world is similarly simple, as suggested by the mirror view. This is illustrated by the apparent paradox that although natural environments exhibit dependencies between cues (such as the environment considered in Figure 2, where correlations between cues range between -0.25 and 0.54), take-the-best can make accurate predictions by ignoring

those dependencies, so much so that it can outperform strategies that explicitly set out to model these dependencies. Superior performance is achieved by betting on lower variance, not lower bias.

2. As a consequence, if observations are sparse, simple heuristics like take-the-best are likely to outperform more general, flexible strategies. It is under these conditions that variance will be the most dominant component of error.
3. Similarly, the more noise in the observations, the more likely a simple heuristic like take-the-best will outperform more flexible strategies. The greater the degree of noise, the more dominant the variance component of error is likely to be.

This argument is supported by a diverse set of related findings. First, consider how a retail marketing executive might distinguish between active and nonactive customers. Experienced managers tend to rely on a simple hiatus heuristic: Customers who have not made a purchase for 9 months are considered inactive. Yet there are more sophisticated methods, such as the Pareto/Negative Binomial Distribution (NBD) model, which considers more information and relies on more complex computations. But when tested, these methods turned out to be less accurate in predicting inactive customers than the hiatus rule (38). Second, consider the problem of searching literature databases, where the task is to order a large number of articles so that the most relevant ones appear at the top of the list. In this task, a "one-reason" heuristic (inspired by take-the-best) using limited search outperformed both a "rational" Bayesian model that considered all of the available information and PsychINFO (39). Third, consider the problem of investing money into N funds. Harry Markowitz received the Noble Prize in economics for finding the optimal solution, the mean-variance portfolio. When he made his own retirement investments, however, he did not use his optimizing strategy, but instead relied on a simple heuristic: 1/*N*, that is, allocate your money equally to each of N alternatives (see Table 1 below). Was his intuition correct? Taking 7 investment problems, a study compared the 1/*N* rule with 14 optimizing models, including the mean-variance portfolio and Bayesian and non-Bayesian models (40). The optimizing strategies had 10 years of stock data to estimate their parameters and on that basis had to predict the next month's performance. Next, the 10-year window was moved 1 month ahead, and the next month had to be predicted and so on until the data ran out. 1/*N*, in contrast, does not need any past information. In spite (or because) of this, 1/*N* ranked first (out of 15) on certainty equivalent returns, second on turnover, and fifth on the Sharpe ratio, respectively.

Unpacking the adaptive toolbox

The adaptive toolbox is a metaphor used to conceptualize the stock of strategies available to the organism. Research on the adaptive toolbox attempts to formulate a deeper understanding of the heuristics that humans and other animals use, the building blocks of heuristics that can be used to generate new ones, and the evolved capacities that these building blocks exploit (41). Table 1 shows ten heuristics in the adaptive toolbox of humans. But how does the mind select a heuristic that is reasonable for the task at hand? Although far from a complete understanding of this mostly unconscious process, we know there are at least three selection principles. The first is that memory constrains the choice set of heuristics and thereby creates specific cognitive niches for different heuristics (42). Consider the choice between the first three heuristics in Table 1: (1) the recognition heuristic, (2) the fluency heuristic, and (3) take-the-best. Assume it is 2003, and a visitor has been invited to the third round of the Wimbledon Gentlemen's tennis tournament and encouraged to place a bet on who will win. The two players are Andy Roddick and Tommy Robredo. First, assume that the visitor is fairly ignorant about tennis and has heard of Roddick but not of Robredo. This state of memory restricts the choice set to the recognition heuristic:

> If you have heard of one player but not the other, predict that the recognized player will win the game.

As it happened, Roddick won the match. In fact, this correct inference is not an exception. This simple heuristic predicted the matches of Wimbledon 2003 and 2005 with equal or higher accuracy than the ATP rankings and the seeding of the Wimbledon experts did (56,57). Now assume that the visitor has heard of both players, but recalls nothing else about them. That state of memory limits the choice set to the fluency heuristic:

> If you have heard of both players, but the name of one came faster to your mind than the other, predict that this player will win the game.

Table 1: Ten well-studied heuristics for which there is evidence that they are in the adaptive toolbox of humans. Each heuristic can be used to solve problems in social and nonsocial environments. See the references given for more information regarding their ecological rationality, and the surprising predictions they entail

Heuristic	Definition[1]	Ecologically rational, if	Surprising findings (examples)
Recognition heuristic (43,44)	If one of two alternatives is recognized, infer that it has the higher value on the criterion	Recognition validity > 0.5	Less-is-more effect if $\alpha > \beta$; systematic forgetting can be beneficial (45)
Fluency heuristic (46)	If both alternatives are recognized but one is recognized faster, infer that it has the higher value on the criterion	Fluency validity > 0.5	Less-is-more effect; systematic forgetting can be beneficial (45)
Take-the-best (21)	To infer which of two alternatives has the higher value: (1) search through cues in order of validity (2) stop search as soon as a cue discriminates (3) choose the alternative this cue favors	See Table 1	Often predicts more accurately than multiple regression (19,28)
Tallying (15)	To estimate a criterion, do not estimate weights but simply count the number of positive cues	Cue validities vary little, low redundancy (47,48)	Often predict equally or more accurately than multiple regression (19)
Satisficing (6,49)	Search through alternatives and choose the first one that exceeds your aspiration level	Number of alternatives decreases rapidly over time, such as in seasonal mating pools (50)	Aspiration levels can lead to significantly better choices than chance, even if they are arbitrary (51,52)
1/N; equality heuristic (40)	Allocate resources equally to each of N alternatives	High unpredictability, small learning sample, and large N	Can outperform optimal asset allocation portfolios
Default heuristic (53)	If there is a default, do nothing	Values of those who set defaults match those of the decision maker, when the consequences of a choice are hard to foresee	Explains why mass mailing has little effect on organ donor registration; predicts behavior when trait and preference theories fail
Tit-for-tat (54)	Cooperate first and then imitate your partner's last behavior	The other players also play tit-for-tat; the rules of the game allow for defection or cooperation but not divorce	Can lead to a higher payoff than optimization (backward induction)
Imitate the majority (55)	Consider the majority of people in your peer group and imitate their behavior	Environment is stable or only changes slowly; info search is costly or time-consuming	A driving force in bonding, group identification, and moral behavior
Imitate the successful (55)	Consider the most successful person and imitate his or her behavior	Individual learning is slow; information search is costly or time-consuming	A driving force in cultural evolution

[1] For formal definitions, see references.

Finally, assume that the visitor is more knowledgeable and can recall various facts about both players. That again eliminates the recognition heuristic and leaves a choice between the fluency heuristic and take-the-best. According to the experimental evidence, the majority of subjects switch to knowledge-based heuristics such as take-the-best when the values of both alternatives on relevant cues can be recalled (8), consistent with an analysis of the relative ecological rationality of the two heuristics in this situation. The general point is that memory "selects" heuristics in a way that makes it easier and faster to apply a heuristic when it is likely to yield accurate decisions (42). In the extreme case where the visitor has not heard of any of the players, none of the heuristics can be used. In this event, the visitor can resort to social heuristics, such as imitate the majority: Bet on the player on whom most others bet.

The second known selection principle, after memory, is feedback. Strategy selection theory (58) provides a quantitative model that can be understood as a reinforcement theory where the unit of reinforcement is not a behavior, but a heuristic. This model allows predictions about the probability that a person selects one strategy within a defined set of strategies. The third selection principle relies on the structure of the environment, as analyzed in the study of ecological rationality. For instance, the recognition heuristic is likely to lead to fast and accurate judgments if the recognition validity is high, that is, a strong correlation between recognition and the criterion exists, as is the case for tennis and other sports tournaments. There is experimental evidence that people tend to rely on this heuristic if the recognition validity is high but less so if the recognition validity α is low or at chance level ($\alpha = 0.5$). For instance, name recognition of Swiss cities is a valid predictor for their population ($\alpha = 0.86$), but not for their distance from the center of Switzerland, the city of Interlaken ($\alpha = 0.51$). Pohl (59) reported that 89% of participants relied on the recognition heuristic in judgments of population, but only 54% in judgments of distance to Interlaken. Thus, the use of the recognition heuristic involves two processes: first, recognition in order to see whether the heuristic can be applied, and second, evaluation in order to judge whether it should be applied.

Homo heuristicus

In this article, we summarized a vision of human nature based on an adaptive toolbox of heuristics rather than on traits, attitudes, preferences, and similar internal explanations. We discussed the progress made in developing a science of heuristics, beginning with the discovery of less-is-more effects that contradict the prevailing explanation in terms of accuracy-effort trade-offs. Instead, we argue that the answer to the question "Why heuristics?" lies in their ecological rationality, that is, in the environmental structures to which a given heuristic is adapted. Appealing to the bias-variance dilemma, we proposed how the ecological rationality of heuristics can be formally studied, focusing on uncertain criteria and small samples that constitute environmental structures which fast and frugal heuristics can exploit. Homo heuristicus can rely on heuristics because they are accurate, not because they require less effort at the cost of some accuracy (60). We hope to have raised our readers curiosity about the emerging science of heuristics, and also hope that some might be inspired to solve some of the open questions, such as whether there is a system of building blocks of heuristics, similar to the elements in chemistry, and how a vocabulary for describing relevant environmental structures can be found.

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Original Article

The Effectiveness of Methadone Maintenance Therapy Among Opiate-Dependants Registered with Hospital Raja Perempuan Zainab II Kota Bharu, Kelantan

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Abstract

***Background*: The objective of this study was to determine the effectiveness of MMT program among injecting drug users (IDUs) in Kota Bharu, Kelantan.**

***Methods:* The study was a retrospective study based on the records of injecting drug users (IDUs) involved in the MMT program from November 2005 to 31st Jan 2008, registered at the Psychiatric Clinic of Hospital Raja Perempuan Zainab II. Opiate Treatment Index (OTI) was used as the research instrument. Repeated measures ANCOVA was used to compare the mean scores during the entry period and after completing twelve months of MMT program after adjusted for age, marital status, and level of education.**

***Results:* A total of 117 file records were reviewed. There was significant reduction in the mean scores after 12 months of heroin Q score, HIV Risk-taking Behavior Scale and health scale after adjusted for age, marital status, and level of education. For Heroin Q score, mean difference was 2.01 (95% CI: 1.45, 2.56), for HIV Risk-taking Behavior Scale, mean difference was 7.64 (95% CI: 6.03, 9.26), and for health scale, mean difference was 5.35(95% CI: 3.90, 6.79).**

***Conclusion:* This study supports the evidence that MMT program is effective in treating heroin and opiate dependence.**

Keywords: *methadone, intravenous drug users, opiate treatment index, Kelantan*

Introduction

According to the World Health Report 2002, 8.9% of total burden of disease worldwide is due to psychoactive substance abuse, which includes tobacco, alcohol and illicit drugs (1). The World Drug Report 2006 revealed that almost 16 million or 0.4% of the world population age 15 to 64 years old is abusers of opiates. Opiates continue to be the main problem drug worldwide, accounting for almost two thirds of all treatment demand in Asia and also 60% of treatment demand in Europe (2).

The harm associated with high-risk injected opiate use and the threat of HIV epidemic among injecting drug users has become a worldwide problem (3). The number of injecting drug users (IDUs) worldwide was estimated at approximately 13.2 million. Over 10 million (78%) live in developing and transitional countries. HIV prevalence among IDUs of over 20% was reported for at least one site in 25 countries and territories (4).

Twenty years ago, due to a rapid increase in mortality rates among injecting drug users and the upcoming threat of HIV, the first harm-reduction program was implemented in the Western world (3). Harm reduction can be defined as any effort that attempts to minimize the negative consequences associated with substance use (either to the individual, their families, their communities, or society as a whole) without requiring the cessation of such use. It provides an alternative to the moralistic and medical models of drug and alcohol treatment, acknowledging that some individual may be unable or unwilling to refrain from its use (5). Methadone maintenance treatment is an important component of harm-reduction approach because it is the largest drug

treatment modality for heroin addiction that has been proven effective in reducing injection drug use. The general objective of Methadone Maintenance Therapy (MMT) is to improve the quality of life of persons with opiate dependence by reducing relapse, improving their physical, and mental condition, reducing the spread of infection among IDUs and those sharing needles as well as improving their psychosocial functioning (6).

Methadone is a potent synthetic opiate agonist. It is administered orally in daily doses and quickly achieves steady-state plasma levels after repeated administration (7). Its effects are qualitatively similar to morphine and other opiates (6). Prescribing methadone at adequate therapeutic doses would reduce craving. It prevents the onset of withdrawal. It is not intoxicating or sedating, and its use does not interfere with normal activities of daily living (8,9). In addition, MMT significantly lowers illicit opioid drug use, reduces crime, and enhances social productivity (10).

In Malaysia, drug addiction has been declared as the nation's number one enemy since 1983. Geographically close to the Golden triangle (Myanmar, Laos, Thailand), rapid progress and urbanization contributed to substantial rise in the number of new and relapsing drug users although there have been draconian punishments (11). Currently it has been estimated that about 400 000 to 800 000 drug users in Malaysia. However, the issue of HIV and a failure of recent modalities such as regimental detention in rehabilitation centers were the reasons why the government and public realize the seriousness of the situation. The HIV epidemic has been affecting IDUs in Malaysia for many years (11) as a result of high risk behaviors such as needle sharing and unsafe sex (12). It was estimated that 75% and 80% of new HIV cases were IDUs in Malaysia and Kelantan respectively (13,14).

In Malaysia, Harm Reduction Working group (HRWG) was established in January 2004 to advocate the implementation of Harm reduction initiatives, consisting of Needle Syringe Exchange Program (NSEP), provision of condom and methadone maintenance therapy (11).

The methadone maintenance therapy (MMT) program was launched in October 2005. There were 1241 clients registered with the MMT program clinics in December 2006. The program was implemented at hospitals, government health clinics and private clinics all over the country (14). Several criteria were set by the Ministry of Health for a person to be involved in the program. The criteria include the following: a patient must volunteer into the treatment program; dependency or addiction must be established; chronic cases of opiate addiction; a patient must abide by the program regulation and procedures and previous unsuccessful methadone or buphrenophine treatment should not exclude a patient from further methadone treatment. The exclusion criteria include the following: opiate addiction less than 2 years; age less than 18 years old; polysubstance dependence; abnormal liver function test; hypersensitivity to methadone and acute medical or psychiatric disorders (6).

Few studies conducted in other countries revealed that MMT program was effective in reducing drug use, HIV risk and crimes as well as improving societal and family functions (15–18) and decreasing mortality (19). A similar study assessing the improvement in the quality of life using WHOQOL-Bref [(a shorter/ brief 26 item version of WHOQOL-100 (WHO quality of life)] as a research tool was conducted previously in Malaysia. However, it involved only a small number of patients (20). The present study was conducted with a much bigger sample size, using the Opiate Treatment Index (OTI) as a research instrument. The OTI was reported to have facilitated the evaluation of treatment for opiate users (21). The present study supports the demand and need for MMT services particularly in Kelantan.

The objective of the present study was to determine the effectiveness of Methadone Maintenance Therapy (MMT) among IDUs registered with Hospital Raja Perempuan Zainab II Kota Bharu, Kelantan in improving their quality of life by reducing relapse, improving their health condition and psychosocial function as well as reducing the HIV infection .

Materials And Methods

This study was a retrospective cohort record review on Injecting Drug Users involved in the Methadone Maintenance Treatment program at the Psychiatric Clinic of Hospital Raja Perempuan Zainab II, Kota Bharu, Kelantan. It was conducted from Nov 2005 to 31st Jan 2008. A total of 216 cases were registered within this period of time. However, those who had completed twelve months of treatment were included. A sample size calculation was done using PS Software, version 1.0.13 (22). Taking a significance level of 0.05, a study power of 80%, Standard deviation for crime domain of 0.14 (23), a detectable difference of 0.05 (the smallest but meaningful mean difference in the criminal score before and after

twelve months of being in MMT program), the minimum sample size required was 64 subjects.

A validated research instrument, known as Opiate treatment Index (OTI) was used to assess the effectiveness of MMT. The OTI was validated in United Kingdom, New Zealand and Australia. It provides a valid and reliable instrument for assessing opiate treatment outcome across a broad range of drug related problems (20). The assessment was performed by medical doctors in charged of the psychiatric clinic at the time when patients entered the MMT program and again twelve months after they had participated in the program. The OTI consists of several domains including drug use score, HIV Risk-taking Behavior Scale (HRBS), Social Functioning Scale, Criminality Scale and health scale.

The drug use domain examines the reported recent behavior of the client, assessed based on the information collected on the last three days of drug use for heroin and opiate drug category. For each drug class, they were asked on the three most recent days that they used drug, and how much drug was used on the last two occasions (6). The HIV Risk-taking Behavior Scale (HRBS) measures the behavior of injecting drug users that puts them at risk of either contracting or passing on HIV and other blood borne viruses to other people. The information obtained includes the drug use and their sexual activities (6). The Social Functioning Scale addresses major aspects of social integration which include involvement of the individual in the drug sub-culture such as whether the person is living with or befriending current drug users, their employment status, conflict with relatives, friends or partners and friend's support. The score was calculated by simply adding up the individual scores for each of the twelve questions asked (6). The Criminality Scale attempts to assess the respondents' frequency of recent involvement in four crime areas including property crime, dealing, fraud and crimes involving violence. The total score was calculated by adding up the score for each of the four crime areas (6). The health scale is a symptom check-list that has been designed to give an indication of the subject's current state of health, especially injection-related health problems. It addresses symptoms and signs in each of the major organ systems. The score for the Health Scale was derived by adding up the total scores for each sub-section. For all domains, the lower the scores, the better the conditions are (6).

Socio demographics of all subjects were tabulated for descriptive statistics. Paired t test was used to compare the mean scores of all six OTI domains during entry period and after completing twelve months of MMT program. Repeated measures ANCOVA within group analysis was used to compare the mean scores of all six OTI domains during entry period and after completing twelve months of MMT program after adjusted for age, marital status and level of education. Significant level for comparing the mean difference was set at 5% level. Results were analyzed using SPSS version 12.

Ethical approval was obtained from the Research and Ethics Committee, University Sains Malaysia. Approval was also obtained from the director of Hospital Raja Perempuan Zainab II, Kelantan State Health Director and Medical Research Ethics Committee (MREC), Ministry of Health.

Results

A total of 117 records were reviewed. Table 1 shows the sociodemographic characteristics of the subjects. The majority were males (99.1%), Malays (98.3%) and unmarried (74.4%). Their mean age was 31.4 years old. The age range from 20 to 51 years old. Most of them (98.3%) had education level up to secondary schools.

Table 2 shows significant reduction in the mean scores after 12 months of all domains

Table 1: Sociodemographic characteristics of Injecting Drug Users involved in the MMT program at the Psychiatric clinic, Hospital Raja Perempuan Zainab II, Kota Bharu, Kelantan[a] mean (SD)

Variables	*n* (%)
Age	31.4 (5.35)[a]
Gender	
Female	1 (0.90)
Male	116 (99.1)
Race	
Malay	115 (98.3)
Non-Malay	2 (1.70)
Marital status	
Married	30 (25.6)
Single	87 (74.4)
Education level	
Primary school	2 (1.70)
Lower secondary school	35 (29.9)
Upper secondary school	80 (68.3)

except for the social functioning domain. For Heroin Q score, mean difference was 2.18 (95% CI: 1.72, 2.64), for Opiate Q score, mean difference was 1.02 (95% CI: 0.33, 1.72), for HIV Risk-taking Behavior Scale, mean difference was 8.25 (95% CI: 6.90, 9.60); for Criminality Scale, mean difference was 0.20 (95% CI: 0.03, 0.28); and for health scale, mean difference was 5.30 (95% CI: 4.31, 6.67).

Table 3 shows significant reduction in the mean scores after 12 months of heroin Q score, HIV Risk-taking Behavior Scale and health scale after adjusted for age, marital status and level of education. For Heroin Q score, mean difference was 2.01 (95% CI: 1.45, 2.56), for HIV Risk-taking Behavior Scale, mean difference was 7.64 (95% CI: 6.03, 9.26); and for health scale, mean difference was 5.35 (95% CI: 3.90, 6.79).

Discussion

Although MMT programs operate differently in different countries, they generally have four similar goals including reduction in drug use and addiction related to HIV risk behavior, thereby reducing HIV transmission. It is also aimed at reducing addiction related crimes and helping patients to resume their societal and familial functions (15,18). In the present study, there was an improvement in the clients' quality of life as shown by the reduction in the scores of all tested domains after undergoing 12 months of the MMT program although the significant reductions after adjusted for age, marital status and level of education were observed in a few domains such as heroin use, HIV risk-taking behavior and health scale. The improvement is consistent with the

Table 2: Mean Opiate Treatment Index (OTI) at baseline and after twelve months in the program among Injecting Drug Users involved in the MMT program at the Psychiatric Clinic in Hospital Raja Perempuan Zainab II, Kota Bharu (n = 117)

Domains	Score mean (SD)		Mean score	t-stat	*P*-value[a]
	Intake	12 months	difference (95%CI)[b]	(df)	
Drug use					
Heroin Q score	2.17 (2.51)	0.00 (0.00)	2.18 (1.72, 2.64)	9.38 (116)	< 0.001
Opiate Q score	1.18 (3.71)	0.16 (0.77)	1.02 (0.33, 1.72)	2.91 (116)	0.004
HRBS[c]	10.22 (5.61)	1.96 (4.14)	8.25 (6.90, 9.60)	12.10 (113)	< 0.001
Social function	13.72 (5.88)	13.14 (5.32)	0.51 (−8.47, 1.87)	0.75 (110)	0.456
Crime	0.21 (0.63)	0.01 (0.10)	0.20 (0.03, 0.28)	2.41 (105)	0.018
Health	7.22 (5.97)	1.92 (2.98)	5.30 (4.31, 6.67)	9.20 (105)	< 0.001

[a] paired t test.
[b] 95% Confidence Interval.
[c] HIV Risk-taking Behavior Scale.

Table 3: Mean Opiate Treatment Index (OTI) at baseline and after twelve months in the program among Injecting Drug Users involved in the MMT program at the Psychiatric Clinic in Hospital Raja Perempuan Zainab II, Kota Bharu after adjusted for age, marital status, and level of education (n = 117)

Domains	Mean score difference	95% Confidence Interval	*P* value[a]
Drug use			
Heroin Q score	2.01	1.45, 2.56	< 0.001
Opiate Q score	8.74	−0.030, 1.78	0.059
HRBS[c]	7.64	6.03, 9.26	< 0.001
Social function	0.16	−1.48, 1.81	0.843
Crime	0.09	−0.06, 0.24	0.242
Health	5.35	3.90, 6.79	< 0.001

[a] Repeated measures ANCOVA within group analysis was applied. Potential covariate (age) and categorical confounders (marital status and level of education) were controlled by repeated measures ANCOVA.
[b] HIV Risk-taking Behavior Scale.

results from other studies as well (15–17,20,24). However, among the studies (15–17,20,24) only one study (23) used the OTI as their research tool while the others used WHOQOL-Bref. The WHOQOL-Bref consists of four domains which assess the physical, psychological, social relationships and the environment (19). To our knowledge, our study is the first published study in Malaysia that assessed the improvement in the quality of life of the injecting drug users using OTI. Indeed it is proven that the tool is sufficiently comprehensive to evaluate the outcomes of treatment for opiate users in which drug use constitutes the major outcome domain followed by a measurement of current HIV risk-taking behavior (21).

The upcoming threat of HIV among injecting drug users has been the main reason for the implementation of MMT program. The reduction in heroin and opiate use revealed by this study may be taken as an indication that the MMT program helps to reduce drug use thus reducing injecting behavior. These results are in keeping with some other studies (15,17,25). The significant reduction in HIV risk behavior supports other study as well (26). Further, long term follow up study needs to be conducted to evaluate the effectiveness of the program in reducing the incidence of HIV.

Although statistically insignificant, our study also revealed that MMT reduces crime as shown in the case of Australia. When given in adequate therapeutic doses, methadone blocks the euphoric effects of injected heroin, thereby providing an opportunity for the individual to improve his or her social functioning (27).

This study shows no significant improvement in the social function domain. Similar to other studies (17,20) the finding could be due to the social stigma faced by the clients thus preventing them from improving their social life including their family relationship. Indeed, family relationship has an impact on the quality of life of patients involved in the MMT program (28). In contrast with our finding, however, another study (18) reported that there was an improvement in family relationship of the patients involved.

We acknowledge some limitations in this study despite the apparent improvement in the mean scores of some OTI domains. The scoring system in the records is based on self reports and this may lead to either an overestimation or underestimation of the benefits. For instance, the reduction in drug use may have been overestimated, while the extent of criminal activities may have been underestimated, thus exaggerating the benefits of the program.

Interviewer bias may pose a problem as the clients were interviewed by different medical officers. The quality of the information obtained depends on the rapport established between the interviewer and the clients. To some extent, the findings may not portray the true effectiveness of the program as there was no controls (IDUs who were not in the MMT program) involved for comparison.

Generally, this study finding supports the evidence from other studies that MMT program is an effective treatment for injecting drug users especially in reducing HIV risk behavior which may help to reduce the spread of HIV. However, the finding might not be able to be generalized to all clients of MMT because only those who completed twelve months of treatment were included in the study. It is important to revise and relook at the present program as other services such as support and counseling including family counseling (29) should be added in order to improve the quality of life of opiate dependants as well as to ensure the improvement is sustained.

Authors' Contribution

Conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, and collection and assembly of data: PDJ
Conception and design, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, and statistical expertise: AAR
Critical revision of the article for important intellectual content and statistical expertise: WZWM
Provision of study materials or patients and administrative, technical, or logistic support: MAMN
Administrative, technical, or logistic support: NHA

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Original Article

Expression Trend of Selected Ribosomal Protein Genes in Nasopharyngeal Carcinoma

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Abstract

Background: **Ribosomal proteins are traditionally associated with protein biosynthesis until recent studies that implicated their extraribosomal functions in human diseases and cancers. Our previous studies using GeneFishing™ DEG method and microarray revealed underexpression of three ribosomal protein genes, *RPS26*, *RPS27*, and *RPL32* in cancer of the nasopharynx. Herein, we investigated the expression pattern and nucleotide sequence integrity of these genes in nasopharyngeal carcinoma to further delineate their involvement in tumourigenesis. The relationship of expression level with clinicopathologic factors was also statistically studied.**

Methods: **Quantitative Polymerase Chain Reaction was performed on nasopharyngeal carcinoma and their paired normal tissues. Expression and sequence of these three genes were analysed.**

Results: **All three ribosomal protein genes showed no significant difference in transcript expressions and no association could be established with clinicopathologic factors studied. No nucleotide aberrancy was detected in the coding regions of these genes.**

Conclusion: **There is no early evidence to substantiate possible involvement of *RPS26*, *RPS27*, and *RPL32* genes in NPC tumourigenesis.**

Keywords: *NPC, RP, RPS27, RPS26, RPL32, transcript expression*

Introduction

Nasopharyngeal carcinoma (NPC) is a distinct type of head and neck cancer that refers to the malignancy of the nasopharynx tissue. NPC has its highest incidence in Southern China and South East Asia, and is more prevalent in the population of Cantonese-Chinese heritage (1). Interestingly, in the East Malaysian state of Sarawak, the native Bidayuh population was found to exhibit highest age-standardized rates of NPC occurrence in the world (2). Although many molecular studies have been carried out, NPC remains one of the most commonly misdiagnosed diseases due to the nature of the disease itself (3). Thus, the development of a suitable biomarker is important and essential in the early diagnosis of the disease to better control the prognosis of the cancer.

Traditionally, ribosomal proteins (RP) are thought to play an important role mainly in catalysing protein translation. However, in 1996 extraribosomal functions of RPs was discovered (4). In a more recent review (5) a list of RPs associated with many extraribosomal functions that are independent of their own involvement in the protein biosynthesis was summarizes. Ribosomal proteins have been implicated in many human diseases and disorders. Gazda's group (6) reported association of *RPS19* with Diamond-

Blackfan Anemia, in which mutations of *RPS19* together with downregulation of other RP genes, alter transcription, translation, apoptosis and promote oncogenic pathways in the disease. In colorectal carcinoma, the differential expression of RP genes has been found (7,8). Studies by Amesterdam's group (9) using Zebrafish as model suggested RP genes to be candidate cancer causing genes. Developmental defects were also reported in RP knockdown Zebrafish (10). A recent study by Maclnnes et al. (11) reported loss of *p53* synthesis in Zebrafish carrying heterozygous mutations for 17 different RP genes hence possibly predisposing Zebrafish to malignant peripheral nerve sheath tumours.

In our previous studies, *RPS26* and *RPS27* genes encoding proteins for small ribosomal subunit were identified to be downregulated in nasopharyngeal carcinoma (12). A subset of RP genes for the large ribosomal subunit was also found to be differentially expressed among cell lines derived from the human nasopharyngeal epithelium (13). Microarray screening on an NPC case also revealed differential expression of a few RPs that includes *RPL32* (unpublished data). This study was aimed at delineating possible involvement of *RPS26*, *RPS27*, and *RPL32* in NPC tumourigenesis, as well as determining the relationship of the expression levels of these RP genes with NPC associated clinicopathologic factors.

Materials and Methods

Tissue biopsies and total RNA extraction

Ethical approval for this study was provided by the Medical Research Ethics Committee (Ministry of Health Malaysia, Ref: (H) dlm. KKM/NIHSEC/08/0804/MRG-IMR). Biopsies of tumouric growths and their adjacent normal tissues were obtained via forceps-biopsy method from NPC suspects admitted to Sarawak General Hospital and Hospital Serian. These biopsy specimens were immediately kept in RNA later RNA stabilizing solution (Qiagen, USA) prior to total RNA extraction using Trizol method (Invitrogen, USA). Assessment of RNA quality and quantity was later performed via spectrophotometric analysis and gel electrophoresis. Only patients diagnosed as NPC cases were subjected to subsequent expression study. For each NPC subjects, the RNA extracted from the adjacent normal tissues would serve as controls. Both the NPC and normal tissues had been confirmed histopathologically by pathologists. The details of the NPC subjects in this study are as listed in Table 1.

Quantitative polymerase chain reaction (qPCR) analysis

Real-time quantitative PCR was carried out on eleven sample pairs. The extracted RNA was first DNase treated with RQ1 RNase-Free DNase

Table 1: Clinicopathologic details of NPC subjects in this study

Patient ID	Age	Gender	Ethnicity	TNM Staging	WHO Classification
GH20	36	M	Iban	II	III
GH22	44	M	Chinese	II	III
GH24	54	M	Iban	IV	III
GH27	58	M	Malay	IV	II
GH39	69	M	Malay	IV	III
GH41	68	M	Bidayuh	I	III
GH48	49	M	Malay	IV	II
GH54	61	M	Bidayuh	III	III
GH55	37	F	Lun Dayak	II	II
GH67	49	M	Bidayuh	III	III
HS96	56	M	Bidayuh	II	III

GH: Subjects admitted to Sarawak General Hospital, HS: Subjects admitted to Hospital Serian; M: Male, F: Female. Iban, Bidayuh and Lun Dayak are natives of Sarawak. TNM Staging: A cancer staging system that describes the extent of cancer in a patient's body based on size of tumour (T), whether regional lymph nodes are involved or not (N) and whether metastasis has occurred (M). Small, low-grade cancers with no metastasis and no spread to regional lymph nodes are classified as Stage I or II. High, large-grade cancers with spread to regional lymph nodes or organs are classified as Stage III, whereas Stage IV refers to cancers that have metastasized. WHO Classification: Classification by World Health Organization based on histopathological types. Type I - keratinizing carcinoma, Type II - non-keratinizing carcinoma, Type III - undifferentiated carcinoma.

(Promega, USA) and heat inactivated according to manufacturer's protocol. First strand cDNA synthesis was synthesized from 2 µg of total RNA using oligo-dT primers, catalysed by MML-V reverse transcriptase (Promega, USA) in a reaction volume of 25 µl. Then, 0.5 µl of the first strand cDNA was used as template for subsequent PCR amplification. A total of 4 ng of cDNA was added to a final reaction volume of 25 µl containing 1X Rotor-Gene™ SYBR Green PCR Master Mix (Qiagen, USA) and 1 µM of each forward and reverse primer. All qPCR primers used in this study are listed in Table 2. The *β-actin* (14) and *RPS27* (15) qPCR primers are established primers. Singleplex amplification was then carried out in Rotor-Gene™ 6000 Rotary Analyzer (Qiagen, USA) with initial denaturation for 5 min at 95 °C followed by 40 cycles of denaturation at 95 °C for 5 sec and annealing or extension at 60 °C for 10 sec. Changes in fluorescence of SYBR Green dye in every cycle were monitored with the aid of Rotor-Gene™ 6000 software version 1.7 (Qiagen, USA). The threshold cycle (CT) value for amplification of each gene was determined by auto threshold function of the software. Prior to the amplification, PCR efficiency and primers compatibility of gene of interest and reference gene were validated via standard curve method (16). Melting curve analysis with temperature rampling from 55 °C–99 °C was also carried out in each run to confirm specificity of PCR amplification. *β-actin* which served as reference gene was used for normalization of the cDNA input. In each NPC cases studied, its respective normal controls served as calibrator. The ΔC_T value and relative quantitative value $2^{-\Delta\Delta CT}$ were calculated (16) and statistical significance was later tested using $\Delta\Delta$CT value (17). The experiment was carried out in duplicate.

Sequence analysis

RPS27 amplification was carried out in a mixture volume of 25 µl with final reaction concentrations of 1X GoTaq™ Reaction Buffer, 0.2 mM dNTPs, 3mM MgCl2, 1 µM of each *RPS27* specific primers (forward: 5'-ACGACCTACGCACACGAGA-3'; reverse: 5'-CACTCATCTTGACTCAGAGTGCT-3') and 1.25 U GoTaq® Flexi DNA Polymerase (Promega, USA). PCR amplifications were performed using PTC-2000 Peltier Thermal Cycler (MJ Research) converter. The mixture was first incubated at 96 °C for 1 min, followed by denaturation at 94 °C for 30 s and annealing at 64 °C for 30 s and then extension at 72 °C for 30 s. The complete amplification procedure was carried out for 30 cycles followed by further incubation at 72 °C for 5 min. On the other hand, amplifications of *RPS26* was carried out using 1 µM of each *RPS26* specific primers (18) at annealing temperature 54 °C. For the case of *RPL32* amplification, *RPL32* specific primers (forward: 5'-GTGGCAGCCATCTCCTTCT-3'; reverse: 5'-GAAAACGTGCACATGAGCTG-3') were used. Aliquots of PCR products were later size-fractionated by agarose gel electrophoresis. The amplicons ranged in size from 243-455 bp.

PCR products of the correct size were purified using Gel Extraction System extraction kit (Viogene, USA) according to manufacturer's

Table 2: qPCR primers used in real-time PCR expression study

Gene	Primer Sequence (5'-3')	PCR efficiency	r^2 of calibration curve
β-actin	F: GCCAACCGCGAGAAGATGA R: CATCACGATGCCAGTGGTA (14)	0.99	0.99944
RPS26	F: GCCGCAGCAGTCAGGGACAT R: GGCAGCACCCGCAGGTC AA	1.05	0.99265
RPS27	F: GTGAAATGCCCAGGATGCTATA R: TGTAGGCTGGCAGAGGACAG (15)	0.98	0.98496
RPL32	F: GAAGTTCCTGGTCCACAACG R: GCGATCTCGGCACAGTAAG	1.08	0.99521
p53	F: TCAACAAGATGTTTTGCCAACTG R: ATGTGCTGTGACTGCTTGTAGATG	1.03	0.99384
Paxillin	F: GAGGCTCGCGGCGGAAAAGT R: AGGGCGTCGAGGTCGTCCAT	0.96	0.98714

F: forward primer, R: reverse primer. Calibration curve: 5x dilution of cDNA input ranging from 40 ng to 0.064 ng except for *RPS27* which starts from 20 ng and ends at 0.032 ng.

instructions and then sent to a commercial sequencing service provider (1st Base Laboratory Sdn Bhd, Malaysia). All purified DNA samples were sequenced in both forward and reverse directions. All sequences obtained were verified by comparative analysis with the sequences in GenBank database (*RPS26* [GenBank: NM001029]; *RPS27* [GenBank: NM001030]; *RPL32* [GenBank: NM000994]). The sequence corresponding to the amplified gene or region of interest was searched using the Blastn program (http://www.ncbi.nlm.nih.gov) with the nucleotide sequence obtained as a query sequence. Verifications of forward and reverse sequences were performed using blast2q alignment tool available at the NCBI website.

Statistical analysis

Paired Student's *t*-test and Wilcoxon Signed Ranks test were used to test the significance of the difference in expression of gene of interest between the NPC cases and controls. Results were expressed as mean ± SD. Multiple Linear Regression (MLR) test was used to assess the association between demographic/clinicopathologic factors and expression of the RP gene(s). The correlation between *p53* or *Paxillin* expression and RP gene expression was calculated either with Pearson or Spearman correlation test. All statistical analyses were performed using SPSS® software version 17.0 (SPSS Inc., USA). Statistical significance was set as $P < 0.05$.

Results

Expressions of RPS26, RPS27, and RPL32 genes and their association with clinicopathologic factors

Real time qPCR analysis on 11 NPC cases namely GH20, GH22, GH24, GH27, GH39, GH41, GH48, GH54, GH55, GH67, and HS96 revealed that there was no significant difference in expressions of all 3 RP genes when comparing NPC cases to controls ($0.093 \leq P \leq 0.929$) (Table 3). Although statistically they are not significant, *RPS27* and *RPL32* did display a pattern of underexpression in 7 out of 11 cases (64%) and 8 out of 11 cases (73%), respectively. An association study using MLR analysis was performed to further investigate any possible hidden relationship between these RP genes and clinicopathogic factors. MLR analysis on NPC associated factors revealed no relationship between *RPS26*, *RPS27,* and *RPL32* expressions with age, ethnic group, TNM staging and WHO classification. There was also no linear relationship of each RP genes with each of the factors when we examined them at univariate level. The result was as shown in Table 4. The gender factor was not analysed because the ratio of cases was biased, M:F = 10:1.

Nucleotide surveillance of coding regions of RPS26, RPS27, and RPL32

In order to detect presence of any genetic alterations that may be associated with NPC, full

Table 3: Expression of genes of interest in NPC cases and their paired normal controls ($n = 11$)

Gene	Expression Mean (SD)	*t* statistic (df)	*P* value
RPS26	N : −2.125 (3.7100) T : −1.250 (2.9800)	−0.089 #	0.929
RPS27	N : −5.516 (6.5733) T : −4.079 (3.8269)	−0.606 (10)	0.558
RPL32	N : −6.012 (3.6969) T : −3.841 (1.5102)	−1.856 (10)	0.093
p53	N : 5.751 (3.0480) T : 4.846 (1.5555)	−1.129 (10)	0.285
PXN	N : 11.726 (2.9667) T : 10.470 (2.8300)	−1.036 (10)	0.324

SD = standard deviation, df = degree of freedom, N = normal controls, T = NPC cases, # Z statistic. Expression was calculated as ΔC_T in which C_T of *β-actin* was subtracted from C_T of gene of interest. The C_T (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold. Thus, no unit is required. The smaller the value of ΔC_T is, the higher the expression of a gene is. Statistical significance was determined using Paired Student's *t* test except for *RPS26*. In the case of *RPS26*, the distribution of data is skewed to the left. Thus, expression is reported as median and interquartile range (IQR) and analysed using Wilcoxon Signed Ranks Test.

length cDNAs encompassing the entire coding regions of *RPS26*, *RPS27,* and *RPL32* were amplified and sequenced. Not all samples used in the expression study were subjected to sequence analysis due to sample limitation. For *RPS26* and *RPL32*, sequence analysis was carried out on GH20, GH22, GH24, GH27, GH39, GH41, GH48, and GH55 whereas sequence analysis of *RPS27* was performed on all eleven samples. Off all the samples screened, no nucleotide aberrancy could be detected in the entire coding regions of the 3 RP genes studied.

Comparison of expression pattern: p53 and Paxillin Vs RP genes

The qPCR analysis for *p53* and *Paxillin* genes were carried out on sample GH20, GH22, GH24, GH27, GH39, GH41, GH48, GH54, GH55, GH67, and HS96. No significant difference was observed for both genes (Table 3). The relationship of these two genes with each RP genes was determined using Pearson's correlation test. For data spread that was not linear or that did not show bivariate normal distribution, Spearman's correlation test was used. For *p53*, correlations between *p53* and each RP genes were tested using Spearman's correlation test. No significant correlation was found ($0.298 \leq P \leq 0.979$). For *Paxillin*, Pearson correlation test was used except for the case of RS26. The analysis revealed that there was no significant correlation between *Paxillin* and each of *RPS27* and *RPL32* ($P = 0.977$ & 0.295, respectively). Spearman's correlation test on *RPS26* also showed that there was no correlation between the gene and *Paxillin* ($P = 0.689$).

Table 4: Simple linear regression analysis on NPC associated clinicopathologic variables with RP gene expression

Independent Variable	*P* value		
	RPS26	*RPS27*	*RPL32*
Age	0.919	0.893	0.735
Ethnic group	0.245	0.186	0.365
TNM staging	0.505	0.587	0.917
WHO classification	0.313	0.093	0.403

Expression was calculated as $\Delta\Delta C_T$ in which ΔC_T of normal controls was subtracted from ΔC_T of NPC cases. Ethnicity is categorised as (i) high risk group that includes Bidayuh (2) and Chinese (1) (ii) others that include Malay, Iban and Lun Dayak. TNM staging refers to cancer staging of the subjects eg. Stage I, II, III, and IV whereas WHO classification categorises the NPC tumours into Type I, II, and III based on the degree of differentiation.

Discussion

RPS26 had previously been implicated in type 1 diabetes (19) and Diamond-Blackfan Anemia (DBA) (19,20). Studies had shown that DBA is associated with predisposition to cancer in particularly acute myeloid leukemia and osteogenic sarcoma (21,22) and mutation of *RPS26* in DBA is common (19). In this study, however, we did not detect any nucleotide aberrancy in the entire coding region of *RPS26*. The mutation of *RPS26* therefore might not be a common event in some cancer types. Expression study and MLR analysis on qPCR data further confirmed that *RPS26* is unlikely to be associated with NPC tumourigenesis.

Overexpression of RPS27 or MPS-1 was reported previously in many different types of tumours and evidence had shown that it might involve in progression towards malignancy (23–28). Stack's group (29,30) using patients' serum protein in their studies, proposed that it could be a potential biomarker in the early detection and diagnosis of head and neck squamous cell carcinoma (HNSCC). In our study, we examined the expression of RPS27 at the transcript level using qPCR method and found that the expression is not significantly different in NPC cases relative to their paired normal controls. Since this current study incorporated more samples, our findings also invalidates our previous suspicion of the underexpression of RPS26 and RPS27 in NPC (12). Our findings also did not agree with findings by Stack's which reported RPS27 overexpression in HNSCC (26,30). In their study (30), they compared RPS27 level in the serum of 125 subjects and 89 controls. However, of the 125 subjects examined, only 4 were tumours of the nasopharynx and they were all from stage IV. Our study, which consists of eleven paired normal and NPC samples (that are difficult to come by), and incorporates tumours of all four stages, should therefore be more representative of the expression pattern of RPS27 in NPC progression. We could not establish any linear relationship between RPS27 and age and gender. Others had reported similar findings in breast and gastric cancers (24,27) although their data was based on protein level analysis and thus could not be compared directly with our findings from transcript level. Although studies in many cancers including HNSCC had shown that RPS27 had positive correlation with tumour grades and stages at the protein level (26–28), we did not observe such pattern in our study at the transcript level. A recent study demonstrated

that RPS27 was capable of reducing Paxillin mRNA and protein levels (31) in head and neck cancer cell line but the role of Paxillin remains unclear in HNSCC progression. Our expression analysis on Paxillin transcript did not reveal such trend in NPC cases studied. We are well aware that findings from protein level would have added meaningful insights to this study, but given the fact that the biopsy samples obtained were often less than 5mm3 in size, we did not have enough of the remains to perform any protein extraction or analysis after isolating the RNA. The absence of mutation in the coding region of the RPS27 gene further indicated that this RP gene might not be associated with tumourigenesis of NPC.

Differential expression of *RPL32* had been reported in prostate cancer cells and colorectal cancer (7,32). A recent study in *Schizosaccharomyces pombe* also suggested that *RPL32* might be a potential transcriptional regulator (33). In our study however, we could not find any evidence that support possible involvement of *RPL32* in NPC tumourigenesis. Expression and sequence analysis of the transcripts showed no significant differential expression and absence of nucleotide variation, respectively. No relationship could be established between clinicopathologic factors and *RPL32*.

Recently, some ribosomal proteins, such as *RPS3*, *RPS7*, *RPL11* and also our gene of interest *RPS27*, were reported to be able to regulate *p53* activity by binding to MDM2 (34–37). The *p53-MDM2* pathway is an important regulatory mechanism in the cells that could cause cell-cycle arrest or apoptosis. In order to investigate if there's any relation between the expressions of *RPS26*, *RPS27* and *RPL32* with *p53*, a correlation analysis was performed. No correlation was found between expressions of *RPS26* and *RPL32* with *p53*. We speculate that perhaps these 2 RP genes were not involved in the *MDM2-p53* pathway, or at least not in the way similar to *RPS3*, *RPS7,* or *RPL11*. As for *RPS27*, although Sun's group (37) reported transcriptional repression of *RPS27* by *p53* gene and that ectopic expression of *RPS27* protein could increase *p53* protein level, we could not detect any correlation between transcript expressions of these 2 genes in our study ($P = 0.977$, $n = 11$). However, it's worth mentioning that in an earlier, preliminary study by our group using reverse transcription PCR and with a larger sample size ($n = 19$), statistical analysis did show significant underexpression of this gene in NPC cases ($P = 0.034$, data not shown). Of these 19 samples, 9 were used in this current study: GH20, GH22, GH24, GH27, GH39, GH41, GH48, GH54, and GH55 but due to insufficient amount of RNA left, we could not perform qPCR analysis on all 19 NPC cases.

On overall, from our study, there is no evidence to show that *RPS26*, *RPS27,* and *RPL32* are involved in the tumourigenesis of NPC. It should be noted however that this study is based on a relatively small sample size ($n < 20$) and is carried out at the transcript level only. We focused on transcript expression because RP mRNA abundance is kept within a fairly narrow range in normal healthy cells and any variation in RP mRNA expression therefore may reflect possible involvements in extraribosomal functions (38). For the case of *RPS27*, although differential expression at protein level had been reported previously (26,30), we reported no differential expression of the transcripts. Such inconsistencies in findings arise perhaps as a result of the complexity of regulatory process of the ribosome biogenesis and also due to the limitation of our current knowledge on extraribosomal functions of RPs. It could also be for the reason that NPC is a distinct form of HNSCC cancer and thus the molecular pathogenesis pathway varies.

Conclusion

Expression study on *RPS26*, *RPS27,* and *RPL32* showed no differential expression in NPC cases. No relationship could be established between these RP genes with age, gender, ethnicity, cancer staging, and WHO classification. There was no correlation found between *p53* and all three RP genes studied. Absence of nucleotide aberrancy in the coding regions indicated that mutation of any of these RP genes might not be a common event in NPC tumourigenesis. Based on the sample size used, there is no empirical evidence that could suggest possible involvement of these three RP genes in the tumourigenesis of NPC.

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Competing interests

The author(s) declare that they have no competing interests.

Authors' Contribution

Conception and design, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, and statistical expertise: EUS, ASK, XMA, TYL, SKS, TST

Critical revision of the article for important intellectual content and statistical expertise: EUS, ASK

Provision of study materials or patients and administrative, technical, or logistic support: ASK, TST, SKS

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Original Article

Validity and Reliability of the Iranian Version of the Insomnia Severity Index

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Abstract

Background: **The Insomnia Severity Index (ISI) is a short subjective questionnaire which helps physicians in making decisions about patients suffering from insomnia. The present study was an attempt to test the reliability and validity of the Iranian version of the ISI and to measure the correlation between ISI items and polysomnography results in chronic insomnia patients.**

Methods: **Two groups responded to the Persian translation of four questionnaires; ISI, Pittsburg Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), and General Health Questionnaire (GHQ). The first group consisted of 135 patients diagnosed with chronic insomnia, and the second group was comprised of 55 normal people. After completing the questionnaires, the insomniac patients underwent standard overnight polysomnography.**

Results: **The internal consistency demonstrated by Cronbach's alpha coefficient was above 0.8 for both groups. The Intra-class correlation coefficient was above 0.7 after two weeks for both groups. The correlations between ISI, PSQI, ESS, and GHQ were high. In addition, close correlations were found between scores obtained from the ISI questionnaire items in insomniac patients with corresponding polysomnographic variables.**

Conclusion: **The Iranian version of the ISI is a reliable and valid instrument. It is a valuable short and first-line questionnaire for insomnia research and clinical work.**

Keywords: *insomnia, Insomnia Severity Index, Iran, reliability, validity*

Introduction

Insomnia is a common sleep disorder in the world (2,3), affecting at least 10–35% of the adult population (4). Insomnia is characterized by the perception of inadequate quantity or quality of sleep with associated prominent negative impact on health and daily functioning. Insomniac patients suffer from high levels of absenteeism from the workplace and increased use of health care services. They are also prone to psychiatric morbidities (5). Epidemiologic studies show that insomnia has been associated with affective disorders, substance abuse, lower life expectancy, and other adverse health outcomes (6).

The four most common complaints about insomnia are difficulty initiating sleep, frequent awakenings from sleep, difficulty falling back to sleep after nocturnal awakenings, and spontaneous early morning awakening. Therefore, definition of insomnia relies on the patient's perspective and decision of the physician (7,8). There is no diagnostic test to define insomnia, and taking a history is sufficient to establish the condition. With this in mind, designing a valid and reliable self-reporting questionnaire would help physicians to rapidly and inexpensively gather information from patients about their sleep complaints (9).

There are relatively few self-reporting questionnaires for assessment and clinical diagnosis of insomnia. The Insomnia Severity Index (ISI) is a short subjective instrument for measuring insomnia symptoms and consequences. The ISI is composed of seven items assessing sleep onset, sleep maintenance, early morning awakening, interference with daily functioning, perceived prominence of impairment attributed to the sleep problem, concerns about sleep problems, and satisfaction with sleep patterns (1). Perceived severity of each item is rated on a 0–4 scale. A total score ranging from 0 to 28 is obtained from summing the seven ratings.

Since its introduction in 1993, the ISI has been widely used for research and clinical purposes (1). Studies show that the ISI is a useful questionnaire with acceptable validity and

reliability for evaluating and screening in the context of primary insomnia (10,11).

The purpose of the present study was to assess the validity and reliability of the Iranian version of the ISI questionnaire. We assessed the internal consistency and concurrent validity (with polysomnography) at a sleep clinic in a sample of chronic insomniac patients. Furthermore, we assessed the internal consistency and concurrent validity (with three other questionnaires) in a normal group.

Subjects and Methods

We first had a bilingual expert panel of sleep physicians, psychiatrics, and occupational medicine specialists translate the ISI questionnaire into Persian. Each item was discussed until agreement was reached on an appropriate translation. We made every effort to keep the sentences of the questionnaire conceptually understandable and simple. Then, we had two external translators translate it back into English. Lastly, we had a few fluent users of English compare the original ISI with the back-translated edition. This revealed only one mistake in the translation. As it was not possible to show emphasis using capitalization in the Persian edition, we used bold words.

The purpose of the study was explained to all participants, and their informed consent was obtained. The experimental group consisted of 135 patients who had been diagnosed with chronic insomnia by a single sleep physician according to ICD-10 criteria. These patients attended the sleep clinic between November 2008 and March 2009. In addition, they underwent medical examinations to rule out other medical disorders. The control group consisted of 55 cases without insomnia in the same age range as the members of the experimental group. They were recruited from the staff of a university not occupied with shift work. They had normal sleep habits and did not report insomnia, loud snoring, or excessive daytime sleepiness.

Subjects with underlying chronic illnesses or history of taking medication or substances affecting the sleep-wake cycle such as hypnotics or stimulants were excluded from the study. Twenty people from each group were randomly selected for participation in the test-retest process. The interval between the test and the retest was two weeks.

Four sleep questionnaires were used: ISI, Pittsburg Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), and General Health Questionnaire (GHQ-28) (1,12–14). The Persian versions of these questionnaires had been used in previous studies (15–17). After filling the questionnaires, the patients with chronic insomnia were subjected to overnight polysomnography in a sleep laboratory.

SPSS 11.5 was used for statistical analysis. Summary statistics for descriptive data were obtained for means and standard deviations. We used Cronbach's alpha to test the internal consistency reliabilities and the intra-class correlation coefficients (ICC) to compute test-retest reliability of the ISI scores. The correlation between ISI items and polysomnography results was computed using two-tailed the Spearman correlation coefficient. For all data, a $p < 0.05$ was considered statistically significant.

Results

A total of 135 insomniac patients (56 men and 79 women) and 55 normal people (26 men and 29 women) took part in this study. Table 1 shows the demographic variables in both groups.

According to PSG results, 12 patients were diagnosed with mild obstructive sleep apnea with an apnea hypopnea index (AHI) > = 5, and two patients had moderate sleep apnea (AHI > = 10). There were no cases with severe sleep apnea, narcolepsy, and PLMD.

The results from the ISI questionnaire were interpreted according to Morin study (1). Of all the participants in the experimental group, 26.6% (n = 36) exhibited clinically insignificant insomnia (0–7), 32.6% (n = 44) sub-threshold insomnia (8–14), 36.3% (n = 49) clinical insomnia of moderate severity (15–21), and 4.4% (n = 6) severe clinical insomnia (22–28). The mean and standard deviation turned out to be 12.1 and 6.7, respectively.

The results of Cronbach's alpha coefficients revealed a high internal consistency in both insomniac and normal groups (0.82 and 0.87, respectively). The ICC was 0.84 (95% CI, 0.78–0.89). In 20 insomniac patients and 20 control subjects, the ICC was 0.87 (95% CI, 0.74–0.91) and 0.79 (95% CI, 0.68-0.85), respectively. The performances of the patients on each of the items on the questionnaire and their total score on the questionnaire were significantly correlated. In the insomniac group, the item-to-total correlation coefficients were in the range of 0.56 to 0.91, with a higher correlation found between sleep latency and the number of awakenings during the night. Also, the item-to-total correlation coefficients were in the range of

0.61 to 0.85 in normal respondents (Table 2).

Table 3 shows the correlation between ISI items and corresponding polysomnographic variables. There was a significant correlation between subjective complaint about difficulty in sleep initiation and sleep onset latency in PSG (r = 0.56, p < 0.001). Furthermore, there was a significant correlation between subjective complaint about difficulty in sleep maintenance and the number of awakenings in PSG (r = 0.19, p = 0.025). A low patient satisfaction from sleep was associated with lower total sleep time in PSG (r = −0.3, p = 0.02). No significant correlation was found between patient satisfaction from sleep and sleep efficiency in PSG (r = −0.34, p = 0.26). Also, there was no significant correlation between polysomnographic variables and the last three questions of the ISI questionnaire (i.e., interference, noticeability, and worried) (p > 0.05).

As tables 4 and 5 show, there were significant correlations between the ISI, PSQI, ESS, and GHQ. The correlation between the ISI questionnaire and the PSQI was stronger in insomniac patients than in healthy patients.

Discussion

Despite recent advances in insomnia treatment and introducing new drugs, diagnosis remains a challenge. On the other hand, insomnia may develop with different symptoms which make it more difficult to diagnose. Several studies show that sleep deprivation causes many harmful effects on the quality of life and daily functioning (15,18).

Table 1: Clinical and demographic variables in insomniac and normal people

Categories	Insomniac patients n = 135 Mean (SD)		Normal respondent n = 55 Mean (SD)		*P* value
Age (years)	42.3	(5.8)	38.7	(8.3)	0.110
BMI (Kg/m²)	24.3	(5.7)	25.7	(7.1)	0.090
Education	10.4	(2.6)	11.6	(4.1)	0.071
ISI scores	12.1	(6.7)	5.1	(1.7)	0.003
Total sleep time (min)	339.6	(78.5)	363.5	(80.3)	0.005
Sleep latency (min)	22.2	(27.6)	13.2	(18.2)	0.030
Number of awakening	25.8	(13.8)	16.5	(12.8)	0.001
Apnea-hypopnea index	12.3	(4.8)	13.1	(7.5)	0.090
Total limb movement	38.6	(11.7)	24.9	(9.3)	0.080

Table 2: Insomnia Severity Index items-to-total correlations in patient and control groups

Question	Item-to-total correlations (*P* value)		Cronbach's alpha	
	Insomniac patients	Normal respondents	Insomniac patients	Normal respondents
Difficulties of falling asleep	0.91 (0.001)	0.81 (0.040)	0.78	0.75
Nocturnal awakenings	0.83 (0.045)	0.78 (0.009)	0.71	0.87
Early morning awakenings	0.67 (0.007)	0.61 (0.002)	0.64	0.79
Dissatisfaction	0.79 (0.008)	0.85 (0.010)	0.88	0.73
Interference	0.72 (0.011)	0.72 (0.006)	0.79	0.83
Noticeability	0.56 (0.031)	0.79 (0.004)	0.85	0.78
Distress	0.61 (0.040)	0.69 (0.008)	0.81	0.88
Total			0.82	0.87

In this study, we translated the ISI questionnaire into Persian and then back-translated it into English to assess the validity and reliability of its Persian version. We took care to make the translation free from any error.

A significant correlation was found between the results obtained from the ISI questionnaire and those from PSQI and GHQ questionnaires.

Also, the correlations between items on the ISI questionnaire and corresponding polysomnographic variables were found to be appropriate. The correlations were more significant in the first three questions of the questionnaire. This demonstrates that the ISI is more useful in quantifying insomnia severity at a sleep clinic than in detecting dissatisfaction from sleep. We can also conclude that this questionnaire can differentiate between different types of insomnia.

Furthermore, there was a significant correlation between the ISI questionnaire and the ESS. Previous studies disagree on this.

Table 3: Correlations between Insomnia Severity Index and polysomnographic results

Insomnia Severity Index	Polysomnography measures			
	SOL [a]	NA [b]	SE [c]	TST [d]
	r (P)	*r (P)*	*r (P)*	*r (P)*
Difficulties of falling asleep	0.56 (< 0.001)			−0.31 (< 0.001)
Nocturnal awakenings		0.19 (0.025)		−0.37 (< 0.001)
Early morning awakenings				−0.21 (0.002)
Dissatisfaction			−0.34 (0.260)	−0.3 (0.020)
Total score				−0.29 (0.001)

[a] SOL, sleep onset latency.
[b] NA, number of awakenings.
[c] SE, sleep efficiency.
[d] TST, total sleep time.

Table 4: Descriptive statistics and Pearson correlation between the ISI questionnaire with PSQI, ESS, and GHQ28 in insomniac patients

Categories	*r* (*P* value)							
	1		2		3		4	
Insomnia Severity Index								
Pittsburg Sleep Quality Index	0.58	(0.046)						
Epworth sleepiness scale	0.42	(0.041)	0.37	(0.001)				
GHQ 28	0.67	(0.043)	0.69	(0.023)	0.41	(0.009)		
Mean (SD)	12.1	(6.7)	8.6	(2.1)	8.4	(1.8)	5.2	(0.9)

Table 5: Descriptive statistics and Pearson correlation between the ISI questionnaire with PSQI, ESS, and GHQ28 in normal group

Categories	*r* (*P* value)							
	1		2		3		4	
Insomnia Severity Index								
Pittsburg Sleep Quality Index	0.16	(0.043)						
Epworth sleepiness scale	0.59	(0.007)	0.28	(0.037)				
GHQ 28	0.62	(0.024)	0.64	(0.008)	0.38	(0.009)		
Mean (SD)	5.1	(1.7)	6.1	(1.8)	6.1	(1.5)	4.6	(1.2)

Some studies have found a stronger correlation between the ISI and fatigue than between the ISI and sleepiness. This means that, in spite of less nocturnal sleep, insomniac patients do not have excessive daytime sleepiness. That is to say, insomnia is a condition of 24 hours hyperarousal (19,20).

Another study has found a strong correlation between the ISI questionnaire and the ESS (5). Daytime sleepiness in insomniac patients might be a consequence of nocturnal sleep disturbance or other disorders such as narcolepsy and obstructive sleep apnea.

Also, our findings suggest that the ISI is a homogenous scale. For all the items, component scores were significantly correlated with the global score. This is more obvious for components related to sleep quantity (sleep latency, and the number of awakenings). These findings are consistent with previous studies about the ISI (5,20,21).

Given the high prevalence of insomnia and its harmful consequences on health, it seems that there should be a reliable and valid questionnaire for rapid screening of insomnia.

The results of our study showed that the Persian edition of the ISI is a reliable and valid tool to screen insomnia in the Iranian population. It is also a useful tool to measure insomnia in both chronic insomniac patients and normal people.

This questionnaire was developed by Morin and colleagues for the screening of insomnia and measuring the effects of treatment on the severity of the condition (1). The ISI is now widely employed in English- and non-English-speaking countries, and its reliability and validity have been measured in various languages (5,10,20–22).

Our results about the reliability and validity of the Persian version of the ISI were close to the studies performed in other languages including English, Chinese, Spanish, Turkish, and Arabic (5,10,20,22,24).

Also, we found a stronger correlation between the ISI questionnaire and the PSQI in insomniac patients than in healthy patients. This indicates that validity of the ISI is appropriate in insomniac patients. This finding agrees with previous studies (10,20).

Conclusion

Insomnia is a serious health problem and can exacerbate other problems like epilepsy (3). Therefore, early diagnosis and treatment is very important. We can claim that the Persian version of the ISI questionnaire is a valuable tool for clinical and research work. Furthermore, it is a useful short and first-line questionnaire to evaluate insomnia symptoms.

Our study had several limitations. We did not examine results from the post-treatment ISI. This was because the treated patients did not return to the clinic to complete the questionnaire. Second, the PSG was not done on the healthy control group as we considered it acceptable and not different from previously published studies (5,10,19,21). Third, only one PSG recording was conducted for insomniac participants, indicating that the information gathered could be subject to a first-night effect. This means that in the sleep laboratory setting, disturbed sleep is experienced on the first night even in adults with normal sleep (25). Due to the high cost of overnight polysomnography, it was impossible to perform two night PSG exams for each participant. Future researchers can evaluate results from the Persian version of the ISI questionnaire before and after treatment in patients suffering from insomnia. In addition, the reliability of this questionnaire can be studied in other populations with sleep disorders such as shift workers.

Authors' Contribution

Conception and design, analysis and interpretation of the data, drafting of the article, and critical revision of the article for important intellectual content: ZY
Conception and design and analysis and interpretation of the data: KSH
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Original Article

Nutrition Quality of Life among Female-Majority Malay Undergraduate Students of Health Sciences

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Abstract

Background: **University students generally tend to engage in problematic eating behaviours, including unhealthy dieting, skipping meals, and high intake of fast food, although they are aware of the negative consequences. Eating behaviours have been shown to be interestingly related to quality of life (QoL). Our study aimed to 1) assess general nutrition quality of life (NQoL) status and 2) compare NQoL status based on gender, financial resources, study courses, year of study, and body mass index (BMI) profiles.**

Methods: **This study was conducted among undergraduates of health sciences in a local public university in Terengganu. Students completed the Malay version of NQoL (6 domains; 50 items; Likert-type responses 1–5). Data analysis was carried out by using SPSS 16.0, utilising descriptive and parametric statistics.**

Results: **A total of 241 students were enrolled [age = 19.7 (0.1) years; female (83.0%); Malay (96.7%)]. *Social/Interpersonal Factors* [3.84 (0.43)] emerged as the best component, while *Food Impact* [3.10 (0.40)] was the worst. Across all variables, only gender and study courses showed significantly different NQoL. Females scored better than males in *Self-Efficacy* (confidence in food selection ability) ($P < 0.05$). Nursing students also experienced significantly greater NQoL (mean = 3.58, 95% CI = 3.47, 3.68) than radiography students in *Self-Efficacy* ($P < 0.05$). Medical laboratory technology students had a significantly more favourable NQoL rating (mean = 3.62, 95% CI = 3.47, 3.76) than nursing students in *Self-Image* ($p < 0.05$). Study courses significantly influenced the NQoL status of students with *Good NQoL*, while those with *Poor NQoL* were mostly influenced by gender and financial resources ($P < 0.05$).**

Conclusion: **These outcomes indicate that specific demographic characteristics seemed to make a difference in the NQoL of undergraduate students.**

Keywords: *nutrition, quality of life, undergraduates, health sciences*

Introduction

Food intake and eating behaviour play an important role in a person's physical, mental, and emotional well-being (1). It is expected that what we eat will affect our quality of life (QoL), as our identification of self, social interactions, and psychological well-being are usually influenced by our food intake (2).

Many methods have been developed to measure population-specific nutrition quality of life (NQoL), such as among patients with irritable bowel syndrome (3), cardiovascular metabolic disease (4), cancer (5), and malnutrition due to HIV infection (6). However, these measures cannot be generalized to the normal population, and the more well-established, generic ones, such as the short-form health survey with 36 questions (SF-36), fail to include items important to respondents dealing with nutritional problems (7). A detailed, nutrition-related QoL measure is needed to obtain accurate information presenting food-related behavioural problems within the normal population. Thus, NQoL version 1.4, developed by Barr & Schumacher (2), was suitable for individuals with a fifth grade or higher reading ability and should take no longer than ten minutes to complete. This tool consists of 6 categories, which cover the physical, social, psychological, food impact, self-image, and self-efficacy components.

Most of the time, eating habits become worse during college years, due to several factors, such as financial problems, meal-skipping, inadequate variety of food intake, snacking, and physical inactivity (8,9). Other factors, such as time

constraint, convenience, availability, cost, taste, lack of knowledge, and peer influence also strongly influence university students' food choices (10). University students tend to engage in problematic eating behaviours, including unhealthy dieting, high intake of fast food, low intake of fruits and vegetables, and minimal consumption of dairy products, although they are generally aware of the negative consequences of those habits (11,12). These findings are mirrored in several reported studies conducted in Malaysia among university students (13–15). In addition, these young adult populations are in a transformation phase of their lives, moving away from parental guidance to being independent young adults, and therefore, are more exposed to unfavourable changes in food intake when starting university life (16–18).

Healthy dietary habits and QoL among students of health sciences are vital, as they themselves will become healthcare professionals, and students who ignore adopting a healthy lifestyle are more likely to fail to convince or promote health for their patients or clients (19,20). Essentially, QoL of the student population is influenced by a variety of factors, such as depression, poor social interaction, low self-esteem, poor academic performance, and bad behaviour (21,22). Therefore, our study intended to evaluate general NQoL status and to compare NQoL status and risk factors, including gender, financial resources, course of study, year of study, and body mass index (BMI) profiles among health science undergraduates in a Malaysian public university.

Subjects and Methods

Study procedure and Ethical Approval

Permission to conduct the study was obtained from the Institute for Health Behavioural Research (IHBR), Clinical Research Centre (CRC) and Ministry of Health Research and Ethics Committee (MREC), Malaysia. Institutional approval was also sought prior to the data collection process.

Study design and sample selection

This study was based on a preliminary, cross-sectional design. Participants were a convenience sample of undergraduates with health sciences backgrounds, between the ages 18 to 24 years, who were studying in a local public university in Terengganu. Data were collected between November 2010 and March 2011. Sample size was determined using Naing's formula (23):

$$n = \left[\frac{z}{\Delta}\right]^2 p\,(1-p)$$

where n = sample size; z = z-value at confidence interval of 95%, which is 1.96; P = percentage of the obesity population that is expected in this study, which is 14.0% (24); and Δ = detectable difference, which was set at 7%. The minimum sample size calculated for this study was 104 respondents (after considering a 10% dropout rate). However, a higher number of respondents was chosen to ensure sufficient data were obtained for more accurate and precise results. Hence, at the end of this study, 241 health science undergraduates participated in this study.

Instruments

All students initially completed a personal information form, which was comprised of demographic questions recording their gender, religion, race, living arrangement, field of study, educational level, year of study, financial resources, and BMI. The BMI (kg/m^2) was calculated using the individual's height and weight, and classified according to the Asian population categorisation (25).

Nutrition Quality of Life (NQoL) instrument

Nutrition related to QoL was measured via the Malay version of the Nutrition Quality of Life, NQoL (*Kaji selidik Kualiti Nutrisi Kehidupan*) instrument, which consists of 50 items. This Malay translated version was produced based on the English NQoL Survey version 1.4 (2,7), which was developed as a tool to monitor the impact of medical nutrition therapy (MNT), a nutrition-based treatment that involves selecting the appropriate food. The original NQoL questionnaire was initially forward translated into Malay and later back translated into English. Finally, cross-cultural adaptations were conducted to polish the items. This cross-cultural adaptation was conducted and reviewed by an expert committee, comprised of methodologists and health professionals, who ensured that the items were translated correctly and were relevant in the new setting when used in a different language (26–28). This instrument assesses six domains: *Food Impact (9 items), Self-Image (6 items), Psychological Factors (10 items), Social/Interpersonal Factors (7 items), Physical Functioning (9 items), and Self-Efficacy (9 items). Example of the items for each domain are: I ate enough food to be satisfied (Food Impact); Liked the way I look (Self-Image); Rewarded myself*

with food (Psychological Factors); My family/ friends have nagged me about food I ate (Social/ Interpersonal Factors); Walking at a moderate pace for 30 minutes (Physical Functioning); and Knew what type of food I should have been eating for my healthy lifestyle (Self-Efficacy). Several NQoL items are described in Appendix 1. For each item, the responses were based on a five-point Likert-type scale: 5 = all of the time, 4 = most of the time, 3 = some of the time, 2 = a little of the time, and 1 = none of the time. The *Overall Score* was derived from the total mean of all 6 domains. The frame of reference for all questions was the preceding two weeks. Scores for 28 of the 50 items were transposed so that higher scores indicated better NQoL. Only for one domain, *Self-Efficacy,* were the scores not transposed, as all the questions were already positively worded. In addition, another instrument, the *Breast Cancer* and *Chemotherapy Questionnaire (BCCQ)* (29,30) was administered in parallel for the purpose of examining the concurrent validity of the Malay version of NQoL.

Appendix 1: Examples of items of the NQoL version 1.4 instrument

Food Impact

1. I had plenty of choice in the food I ate
2. I could afford to buy the food that was best for me

Self-Image

3. Liked the way my clothes fit
4. Was pleased with the way I managed what I ate

Psychological Factors

5. Was happy with the food I ate
6. Was frustrated about limiting the food I ate

Social/ Interpersonal Factors

7. I have cut down the amount of time I spend on work or other activities because of my food-related condition
8. I had someone I could talk to who understood the struggles I have had with food

Physical Functioning

9. Walking slowly for 10 minutes
10. Walking up a flight of stairs

Self-Efficacy

11. Knew what type of food I should have been eating for my healthy lifestyle
12. Made healthy food choices

Reliability and validity of the Malay version of NQoL instrument

Cronbach's alpha coefficient (for internal consistency reliability) and Spearman's correlation coefficient (for divergent validity) were employed for the purpose of reliability and validity tests of the Malay version of the NQoL instrument. Overall, Cronbach's alpha for all NQoL domains ranged from 0.217 to 0.908, in which *Physical Functioning, Self-Efficacy,* and *Overall Score* demonstrated acceptable values of greater than 0.700 (31). On the other hand, NQoL domains correlated weakly and insignificantly with the chemotherapy (r_s = −0.058, 0.178) and breast cancer subscales of the *BCCQ* (r_s = −0.002, 0.216), indicating its divergent validity. In addition, evidence of convergent validity was shown by the correlation coefficients among all NQoL domains and *Total NQoL* as being high and significant (r_s = 0.442, 0.643; $P < 0.05$). For the purpose of concurrent study, the outcomes were considered acceptable and adequate. These preliminary findings provide some early evidence of the reliability and validity of the Malay NQoL, but further validation exercises in larger samples are essential.

Statistical analysis

The primary analysis involved descriptive statistics for all ten demographic characteristics, which were presented as frequencies and percentages. An initial normality test was carried out,utilising age and mean scores of NQoL domains as dependent variables. Outcomes complied with the normality requirements, in which the score distribution was normally distributed as indicated in Kolmogorov–Smirnov statistics by $P > 0.05$. Subsequently, parametric univariate analyses were employed for each independent variable for 1) all respondents and 2) respondents stratified according to *Total NQoL Score* (*Poor NQoL* = score < mean; and *Good NQoL* = score ≥ mean) (32). Thus, an independent t-test was used to compare score differences between groups, and a one-way ANOVA was used to compare more than two groups. Multiple logistic regression analyses were conducted to determine whether gender, course of study, year of study, and BMI profiles (as confounders) were associated with good NQoL (as reference group) or poor NQoL. The results were presented in the form of mean, standard

deviation (SD), confidence interval (CI), and *p* value. A value of $P < 0.05$ was considered statistically significant.

Results

Demographic characteristics

The basic demographic characteristics of students are presented in Table 1a and 1b. A total of 241 students, with 100% response rate, participated in the study (nursing = 120, radiography = 64, and medical laboratory technology [MLT] = 57). The majority of the students were female (83.0%), Malay (96.7%), Muslim (97.9%), and not taking supplements (83.8%). The mean (SD) age of all students was 19.7 (0.9) years, ranging from 18 to 24 years. Their mean height was 1.6 (0.1) meters, and the overall weight was 52.8 (1.3) kg. Most (52.3%) were within the desirable BMI range (18.5–22.9). Approximately 77.6% of the students were living with friends, with the majority receiving an education loan scheme (either by the Perbadanan Tabung Pendidikan Tinggi Nasional/ PTPTN or Majlis Amanah Rakyat/ MARA) (76.3%).

General Nutrition Quality of Life (NQoL) status

The median, range, standard deviation (SD), and mean scale scores of NQoL subscales are depicted in Table 2. Overall, *Social/Interpersonal Factors* [3.84 (0.43)] emerged as the highest-scored subscale, while the lowest rating was obtained for *Food Impact* [3.10 (0.40)].

Comparison of NQoL status by socio-demographic characteristics

Gender

There was a significant difference in terms of *Self-Efficacy* between genders, with females scoring higher than males ($P = 0.043$, 95% CI = -0.59, -0.01). Among the NQoL domains, male students showed the highest mean score in the *Physical Functioning* [3.88 (1.03)] component, while female students reported the highest score in *Social/Interpersonal Factors* [3.85 (0.43)]. The lowest mean score in *Food Impact* component was generated by both genders [male = 3.08 (0.46); female = 3.11 (0.38)]. Overall, there was a general tendency for NQoL scores to be higher among female respondents (Table 2).

Financial resources

No significant differences were found between students receiving an education loan scheme and students not receiving an education loan scheme. However, both groups of students experienced higher NQoL in the *Social/ Interpersonal* aspect. They also reported lower NQoL with regard to the *Food Impact* subscale [receiving loan = 3.09 (0.36); not receiving loan = 3.12 (0.50)]. In the majority of NQoL domains, undergraduates who were not receiving a PTPTN/ MARA loan, interestingly, reported a trend of better NQoL status than those receiving the loan (data not shown).

Course of study

NQoL comparisons by the three different study courses—nursing, radiography, and MLT—are reported in Table 3. Nursing students experienced significantly greater NQoL ratings (mean = 3.58, 95% CI = 3.47, 3.68) than radiography students in *Self-Efficacy* ($P < 0.05$). On the other hand, MLT students had a significantly more favourable score (mean = 3.62, 95% CI = 3.47, 3.76) than nursing students in *Self-Image* ($P < 0.05$). However, in all other scales, there were no significant differences, although generally, ratings for all NQoL domains were higher among MLT students compared to nursing and radiography students.

Year of study

NQoL comparisons by year of study are also reported in Table 3. The results showed a total absence of any significant differences across the year of study (first year; $n = 80$, second year; $n = 101$; and third year, $n = 60$). Nonetheless,

Table 1a: Demographic characteristics of students ($n = 241$)

Variable	Mean	Standard deviation	Median	Minimum, maximum
Age (years)	19.7	0.9	20.0	18.0, 24.0
Weight (kg)	52.8	1.3	50.0	37.0, 120.0
Height (m)	1.6	0.1	1.6	1.4, 1.9
BMI (kg/m^2)	21.1	4.0	20.3	13.0, 37.9

Table 1b: Categories of students (n = 241)

Categories	Frequency (n)	Percentage (%)
Gender		
a) Radiography	64	26.6
Male	20	31.2
Female	44	68.8
b) Medical Laboratory Technology (MLT)	57	23.7
Male	9	15.8
Female	47	82.5
c) Nursing	120	49.8
Male	10	8.3
Female	109	90.8
Religion		
Muslim	236	97.9
Buddhist	1	0.4
Hinduism	2	0.8
Christian	1	0.4
Race		
Malay	233	96.7
Chinese	2	0.8
Indian	3	1.2
Others	3	1.2
Living arrangement		
Alone	6	2.5
With partners/ family	48	19.9
With friends	187	77.6
Course of study		
a) Radiography		
First year	25	39.1
Second year	23	35.9
Third year	16	25.0
b) MLT		
First year	15	26.3
Second year	26	45.6
Third year	16	28.1
c) Nursing		
First year	40	33.3
Second year	52	43.3
Third year	28	23.3
Financial resources		
Education loan scheme	184	76.3
Scholarship	21	8.7
Family	27	11.2
Own saving	3	1.2
Others	6	2.5
Body Mass Index (BMI) (kg/m^2)		
Underweight (< 18.5)	56	23.2
Normal weight (18.5–22.9)	126	52.3
Overweight (≥ 23.0)	49	20.7

Table 2: General level of NQoL status and the differences of NQoL domains based on gender and BMI profiles (n = 241)

Domains	General			Gender, mean (SD)		95% CI
	Median	Mean (SD)	Min, max	Male (n = 39)	Female (n = 200)	
FI	3.11	3.10 (0.40)	2.00, 4.33	3.08 (0.46)	3.11 (0.38)	−0.17, 0.10
SI	3.40	3.47 (0.53)	2.20, 5.00	3.54 (0.59)	3.46 (0.52)	−0.10, 0.27
PF	3.50	3.46 (0.41)	2.40, 4.30	3.37 (0.49)	3.48 (0.40)	−0.27, 0.06
Soc	3.86	3.84 (0.43)	2.43, 4.71	3.80 (0.43)	3.85 (0.43)	−0.19, 0.10
Phy	3.89	3.77 (1.01)	1.00, 5.00	3.88 (1.03)	3.76 (0.99)	−0.22, 0.47
SE	3.56	3.47 (0.67)	1.00, 5.00	3.18 (0.83)	3.52 (0.62)	−0.59, −0.01
Total NQoL	3.53	3.52 (0.30)	2.78, 4.12	3.48 (0.34)	3.53 (0.29)	−0.18, 0.03

Domain	t-statistic (df)	*P*-value*	BMI profiles, mean (SD)			F-statistic (df)	*P*-value[a]
			UW (n = 56)	NW (n = 126)	OW (n = 49)		
FI	−0.48 (237)	0.683	3.03 (0.39)	3.14 (0.40)	3.10 (0.40)	1.41 (2, 230)	0.247
SI	0.93 (237)	0.351	3.44 (0.52)	3.48 (0.55)	3.48 (0.51)	0.13 (2, 230)	0.876
PF	−1.27 (48.19)	0.134	3.56 (0.37)	3.42 (0.44)	3.43 (0.37)	2.32 (2, 230)	0.100
Soc	−0.60 (237)	0.286	3.82 (0.43)	3.86 (0.43)	3.80 (0.43)	0.42 (2, 230)	0.656
Phy	0.71 (237)	0.887	3.65 (1.03)	3.86 (0.99)	3.65 (1.03)	1.25 (2, 230)	0.290
SE	−2.40 (45.38)	0.043	3.38 (0.66)	3.49 (0.66)	3.42 (0.68)	0.59 (2, 228)	0.556
Total NQoL	−1.00 (237)	0.159	3.48 (0.32)	3.54 (0.30)	3.48 (0.28)	1.26 (2, 230)	0.286

*Independent t–test; aOne-Way ANOVA test; FI = Food Impact; SI = Self-Image; PF = Psychological Factors; Soc = Social/ Interpersonal Factors; Phy = Physical Functioning; SE = Self-Efficacy; BMI = Body Mass Index; UW = Underweight; NW = Normal-weight; OW = Overweight; Min = Minimum; Max = Maximum; SD = Standard Deviation.

all groups showed higher NQoL in *Social/ Interpersonal* aspects, with third-year students [3.86 (0.41)] having the highest rating compared to first-year [3.83 (0.44)] and second-year students [3.83 (0.43)]. In contrast, the *Food Impact* subscale was rated lowest by all respondents, with first-year students [3.05 (0.39)] showing the poorest rating compared to second-year [3.09 (0.40)] and third-year respondents [3.21 (0.37)]. Overall, students in the third year reported the best NQoL in almost all dimensions compared to students of other years.

Different BMI profiles

Table 2 shows that there were no significant differences among participants with various BMI profiles in all NQoL components. Nonetheless, students with normal weight had better NQoL in almost all dimensions compared to students who were either underweight or overweight. Intriguingly, better NQoL, particularly in *Social/ Interpersonal Factors* aspect, was observed among underweight [3.82 (0.43)] and overweight students [3.80 (0.43)]. Otherwise, lower NQoL was notably present in *Food Impact* relative to other subscales in all groups [underweight = 3.03 (0.39); normal weight = 3.14 (0.40); overweight = 3.10 (0.40)].

Stratified respondents

The mean score for Total NQoL was 3.52, and this was used as the cut-off score for stratifying respondents according to Poor NQoL (n = 118) and Good NQoL (n = 123). In Table 4, an overall report for stratified respondents is presented (Table 4).

a) Poor NQoL

No significant differences were detected in NQoL domains among all independent variables, except for gender and financial resources. Females reported significantly higher levels of *Self-Efficacy* compared to males (P = 0.005). Significantly better *Food Impact* was shown among those who received an education loan scheme compared to those who did not receive an education loan scheme (P = 0.041) (data not shown).

Table 3: Differences of NQoL domains based on different courses and years of study (n = 241)

Domain	Mean (SD)			F-statistic (*df*)	*P*-value [a]
	A: Nursing (n = 120)	B: Radiography (n = 65)	C: MLT (n = 57)		
Food Impact	3.11 (0.37)	3.03 (0.46)	3.13 (0.38)	0.35 (2240)	0.703
Self-Image	3.41 (0.46)	3.46 (0.61)	3.62 (0.54)	3.12 (2240)	0.046 (A vs. C)
Psychological Factors	3.44 (0.37)	3.45 (0.47)	3.48 (0.43)	0.29 (2240)	0.751
Social/ Interpersonal	3.87 (0.38)	3.79 (0.42)	3.89 (0.49)	2.59 (2240)	0.077
Physical Functioning	3.89 (0.93)	3.57 (1.04)	3.68 (1.13)	1.65 (2240)	0.194
Self-Efficacy	3.58 (0.57)	3.26 (0.69)	3.41 (0.81)	4.02 (2238)	0.019 (A vs. B)
Total NQoL	3.53 (0.29)	3.41 (0.29)	3.51 (0.30)	2.52 (2240)	0.083
Domain	**Mean (SD)**			**F-statistic (*df*)**	***P*-value [a]**
	First year (n = 80)	**Second year (n = 101)**	**Third year (n = 60)**		
Food Impact	3.05 (0.39)	3.09 (0.40)	3.21 (0.37)	2.97 (2240)	0.053
Self-Image	3.49 (0.55)	3.47 (0.49)	3.46 (0.57)	0.39 (2240)	0.962
Psychological Factors	3.40 (0.43)	3.48 (0.41)	3.50 (0.40)	1.12 (2240)	0.327
Social/ Interpersonal	3.83 (0.44)	3.83 (0.43)	3.86 (0.41)	0.13 (2240)	0.875
Physical Functioning	3.74 (0.97)	3.79 (1.01)	3.77 (1.08)	0.06 (2240)	0.947
Self-Efficacy	3.46 (0.66)	3.38 (0.64)	3.61 (0.71)	2.22 (2238)	0.111
Total NQoL	3.50 (0.30)	3.51 (0.30)	3.57 (0.30)	1.12 (2240)	0.329

[a] One-Way ANOVA test; SD = Standard Deviation; MLT = Medical Laboratory Technology.

b) Good NQoL

Across all independent variables, course of study was the only variable that showed a significant difference in the *Self-Image* aspect, with MLT students experiencing a significantly higher level compared to nursing students (P = 0.008). However, nursing students reported significantly more favourable NQoL than radiography students in the *Self-Efficacy* aspect (P = 0.019). MLT students possessed significantly better NQoL in the *Social/ Interpersonal* aspect (P = 0.031) and Total NQoL (P = 0.024) compared to other students (data not shown).

For multiple logistic regression outcomes, normal-weight students had 2.66 times the odds of having good NQoL (95% CI = 1.30, 5.43; P < 0.05) compared to overweight students when adjusted for gender, year of study, and study courses (Table 5).

Discussion

This study evaluated the general NQoL status among undergraduate students of health sciences from three different courses at a local public university in Terengganu. The results provided several important insights for understanding general NQoL status based on gender, financial resources, study course, year of study, and BMI profile.

With regard to general NQoL status, *Social/ Interpersonal Factors* emerged as the highest-ranking subscale. This result might be due to

Table 4: Score description across all respondents [presented as mean (SD)]

Domains	Respondent Categorisation		95% CI	t-statistic (df)	*P*-value [a]
	Poor NQoL (*n* = 118)	Good NQoL (*n* = 123)			
Food Impact	2.92 (0.36)	3.28 (0.34)	–0.46, –0.28	–8.11 (239)	< 0.001
Self-Image	3.31 (0.50)	3.63 (0.51)	–0.45, –0.19	–4.90 (239)	< 0.001
Psychological Factors	3.33 (0.40)	3.59 (0.39)	–0.36, –0.16	–5.06 (239)	< 0.001
Social/ Interpersonal Factors	3.68 (0.43)	3.99 (0.37)	–0.41, –0.20	–5.86 (230)	< 0.001
Physical Functioning	3.24 (0.98)	4.28 (0.75)	–1.26, –0.81	–9.21 (219)	< 0.001
Self-Efficacy	3.21 (0.63)	3.71 (0.60)	–0.66, –0.34	–6.25 (237)	< 0.001
Total NQoL	3.28 (0.20)	3.75 (0.18)	–0.51, –0.42	–18.80 (239)	< 0.001

* Independent *t*-test.

Table 5: Multiple logistic regression analysis of NQoL status

Variable	Crude OR	*P*-value	Adjusted OR (95% CI)	*P*-value
Gender				
Male	0.53 (0.26, 1.08)	0.079	0.73 (0.34, 1.56)	0.409
Female	1 (reference)		1 (reference)	
Year of study				
First year	1.57 (0.80, 3.08)	0.188	1.70 (0.84, 3.42)	0.139
Second year	1.25 (0.66, 2.37)	0.500	1.30 (0.65, 2.57)	0.459
Third year	1 (reference)		1 (reference)	
BMI profiles				
Underweight	1.92 (0.87, 4.25)	0.107	1.88 (0.83, 4.26)	0.132
Normal-weight	2.84 (1.42, 5.69)	0.003	2.66 (1.30, 5.43)	0.007
Overweight	1 (reference)			
Course of study				
MLT*	1 (reference)		1 (reference)	
Radiography	0.93 (0.45, 1.90)	0.837	1.04 (0.47, 2.29)	0.925
Nursing	1.61 (0.86, 3.04)	0.140	1.69 (0.85, 3.38)	0.135

* MLT = medical laboratory technology.

the majority of the students living with friends (77.6%), an arrangement that would inevitably influence their dietary choices (33). Moreover, most items in this domain asked about their dietary habits when accompanied by friends; e.g. *"I had someone I could talk to who understood the struggles I have had with food"* (2). Therefore, they would have experienced desirable social communication, being able to share their problems or joys with their friends, particularly while having meals together. Meanwhile, *Food Impact* appeared as the lowest-ranking domain, which emphasizes the impact of food or nutrition on a person's physical, mental (mind), and social well-being (e.g. "*I could afford to buy the food that was best for me*") (2). This situation could be due

to the new environment, which is associated with the changes in lifestyle induced by the transition of moving away from their family homes and assuming responsibility for their own diets (34). In order to improve their NQoL status, they should slowly adapt to the new university surroundings and learn how to practice proper dietary habits, information which is readily obtained through the Internet, magazines, books, brochures, and leaflets.

In our study, female students had slightly higher NQoL scores compared to male students in most of the six dimensions of the Malay version of the NQoL instrument, except for the *Physical Functioning* and *Self-Image* components. This trend somehow indicates that female students adopted a more sedentary lifestyle, with minimal regular physical activity compared to male students. Lack of energy, time, and self-confidence have always been reported among females (35,36). The rising rates of inactivity are often linked to obesity, diabetes, heart disease, and certain cancers, commonly referred to as the "diseases of inactivity" (37). Therefore, it is essential to promote regular physical activity as a part of the strategy to improve NQoL in students. Furthermore, the *Self-Image* aspect appeared to be scored lower by female students, possibly because of the substantial percentage of underweight and overweight students among them (42.6%). Nonetheless, this finding was only our observation in this population, and such a condition may have contributed to body image dissatisfaction among university students, exposing them to the development of eating disorders, such as anorexia nervosa and bulimia nervosa (38,39). Even so, female students reported significantly higher *Self-Efficacy*, or more confidence in their ability to choose healthy foods, such as those high in calcium and low in fat, compared to male students.

Based on the sample of students surveyed in this study, 76.3% of students are receiving an education loan scheme (from PTPTN or MARA). This group of students experienced poorer NQoL in almost all domains, except *Self-Efficacy*. The main reason could be due to their restricted financial resources, as well as the tendency to be thriftier with their spending. Consequently, the likelihood of changing their eating habits was greater when starting university life. This situation could have indirectly decreased their weekly consumption of fresh fruits, vegetables, oily fish, and seafood, and at the same time "encouraged" their fast food intake and meal-skipping practices (40). Despite the fact that the majority of the students are unemployed and their main source of income comes from educational loans (PTPTN = RM 3000 per semester), they also might be receiving money from their parents (41). Thus, this condition might be seen as quite lucrative, even though they are just beginning a major transition in their lives.

The findings of this study seem to indicate further that nursing students possessed a significantly more positive perception of their *Self-Efficacy* towards their eating habits compared to MLT and radiography students. It could be that nursing students are more exposed to a wealth of information and different academic experiences during their university years. They might also have a rather positive perception of health, and therefore, value health status differently compared to the general population (20). This is encouraging, because only when nursing students have confidence in their own abilities are they able to focus on the needs of their patients (42). However, the *Self-Image* of nursing students was lower compared to MLT students; it is probable that the high percentages of underweight (22.5%) and overweight (18.3%) students in the nursing group, factors that are commonly associated with lower body image perception, contributed to this phenomenon (43). On the other hand, undergraduates in MLT courses appeared to have higher QoL in the majority of NQoL subscales, especially in the aspects of *Food Impact, Self-Image, Psychological Factors,* and *Social/ Interpersonal Factors,* compared to their counterparts. The possible explanation could be that these students enjoy better personal relationships and stronger social lives than the other students (19), although the actual reason could not be elucidated at this point. Possibly due to the lack of a nutrition component in their syllabus, radiography students showed lower NQoL in all subscales. Nonetheless, as future healthcare providers, they too should be expected to possess minimal knowledge about nutrition in the process of providing general health advice.

In addition, the analysis of relationships among NQoL dimensions and different years of study showed that students in the third year had the highest scores in all domains, except for *Physical Functioning* and *Self-Image*. This finding could be because the senior students (third year) had already adapted to the environment of university life and were probably more "aware" in selecting healthier food compared to their juniors (first and second year). Limited knowledge and information related to healthy eating also might have been another possible factor, as the junior

students were likely to be more ignorant of the importance of healthy eating, as a consequence of the process of adaptation to a new study and living environment. A previous study by King et al., (10) found that most junior students live in dormitories and rely on hostel food or fast food served there as their main source of nutrition. Based on these results, fresh recruits should be exposed to healthy eating guidelines and its impact on nutritional habits during their orientation period.

The relatively lower NQoL among overweight students than students with normal weight might be due to skipping breakfast, a habit that normally contributes to obesity (44), as well as their sedentary lifestyles, coupled with minimal physical activity (8). The positive influence of being normal-weight was confirmed in the subsequent multiple regression analysis for those with good NQoL. Generally, BMI profiles have been strongly associated with QoL, whereby QoL impairment has been shown to worsen with increasing obesity and the probability of developing chronic conditions, such as type 2 diabetes and cardiovascular diseases (45–47). In addition, underweight students have been shown to exhibit poorer NQoL, suggesting that some aspects of QoL could be attributed to being underweight (48). Unfortunately, studies have been less concerned with identifying trends in QoL among underweight populations, although many reasons for low body weight might exist; for example, someone classified as "underweight" may be so because of dysfunctional eating behaviours (e.g., food restriction and excessive exercise), suffering from distinct medical conditions (e.g., long-term chronic health conditions), or some factors unrelated to health status, such as naturally low BMI (49). With these findings, it is clear that efforts are needed to explore the relationship between BMI and QoL in longitudinal studies by using larger samples with Asian-version BMI profiles.

Lastly, according to the stratified respondents of Poor NQoL and Good NQoL, it was apparent that those with Good NQoL possessed better scores in all domains than those with Poor NQoL. However, the huge differences in the score of *Physical Functioning* could be due to these young adults with Good NQoL being more active compared to those with Poor NQoL. A similar observation was reported in a systematic review showing that higher physical activity level was consistently associated with better QoL among the general adult population (50). A study by Huang (51) also reported that over 70% of college students were found to perform active and moderately active physical activities daily. Such physical activities, coupled with regular exercise, are especially beneficial to university students for increasing their overall body fitness and well-being, as well as reducing their risk of various diseases (35). Nevertheless, due to the lack of direct physical activity assessment in our current study, concrete evidence to support this finding requires further investigation.

Although our study relied on the inclusion of a large, convenient sample, a major limitation still exists. Essentially, the sample was rather imbalanced between genders, due to the recruitment of more females than males as respondents; hence, the results were heavily biased towards females. This is due to a higher proportion of female students in their faculty, which represents a common trend in the universities in our country, Malaysia (52). Nevertheless, our data generally included all the representatives of the three different courses in this health sciences population. In addition, our sample from public university was highly dominated by a Malay population (96.7%) practicing Islam, which prohibits the consumption of alcoholic beverages. Other than that, the instrument used was considered reliable for this current population, whereby several NQoL domains showed Cronbach's alpha values of greater than 0.700. In addition, the instrument used was considered valid when the NQoL domains correlated weakly and insignificantly with BCCQ, which conceptually measured completely unrelated issues. Moreover, convergent validity was confirmed via strong relationships between *Total NQoL* and the respective domains. Nonetheless, further psychometric analysis for this instrument should be encouraged in future. It also would have been more appropriate to ask the respondents to identify whether or not an item was completely understood (26). Because of the cross-sectional nature of this study, no definite conclusion regarding the factors affecting NQoL can also be drawn at this moment. Future longitudinal studies are needed to determine factors influencing NQoL specifically in this population. In addition, the actual eating habits and food intake of university students in the current cohort should be explored, as this study was rather focused on the general perceptions and beliefs towards eating habits.

Conclusion

The current evidence suggests that gender and course of study seemed to make a difference in NQoL status among undergraduates. However,

NQoL status did not differ by financial resources, course of study, or BMI profiles. Additional strategies to increase healthy nutritional habits in university students are clearly warranted, even though some may not be directly involved in this specialized area. As health science students will become future healthcare professionals, it is vital for them to have appropriate NQoL themselves before educating their patients. A simple healthy eating guideline would be a useful mechanism for promoting such changes, especially within the period of their studies in university.

Conflict Of Interest Statement

The authors have no conflict of interest or relevant financial relationships in this study.

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Authors' Contribution

Conception and design, critical revision of the article for important intellectual content, final approval of the article, and statistical expertise: LPL
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Original Article

Suicide and the Publicly Exposed Pedophile

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Abstract

Background: **Current clinical wisdom is that the vast majority of those who complete suicide suffer from a mental disorder. Uncritical adherence to this belief may limit our understanding and restrict the full range of prevention activities. We aimed to examine the public record for accounts of suicide by men who had been, or were about to be, investigated or apprehended for "sex only" child sex offences, with a view to presenting a collection of case histories, and identifying examples of suicide in the apparent absence of mental disorder other than pedophilia.**

Method: **The public record (hard and electronic copy) was examined.**

Results: **Twenty case histories were identified of men with no apparent mental disorder (other than pedophilia) who completed suicide shortly after exposure or threatened public exposure and/or early or potential legal punishment.**

Conclusion: **This evidence strongly suggests that exposure or threatened public exposure of pedophilia and/or early or potential legal punishment creates a predicament, which may lead to completed suicide.**

Keywords: *communications media, mental disorders, suicide, pedophilia, prevention*

Introduction

For the last half century, the prevailing medical theory has been that all or almost all of those who complete suicide are suffering a mental disorder (1,2). While people with mental disorder are at greater risk of suicide than those without, exclusive application of the "mental disorder is necessary" model of suicide may diminish the importance of life context and the preventive potential of considered social actions.

Recent studies from China reporting mental illness in less than 50% of suicide completers have raised important questions (3). Reports from India (4,5) have found mental disorder in less than 40% of completers, and a report from the West (Faroe Islands) has found evidence of psychiatric or drug disorders in only 61% of those who completed suicide (6). In a recent psychological autopsy study across several countries (7), up to two-thirds of suicide cases remained without psychiatric diagnosis in those studies that only examined Axis I disorders.

Adverse life events, independent of mental disorder, can contribute to suicide (8). A recent "sociological autopsy" found that relationship breakdown was the main trigger in 34% of cases (9). The seminal work of Emil Durkheim (10) is also relevant. Durkheim did not deny that mental illness was a cause of suicide. However, he regarded social factors as the major cause, stating, "The individual yields to the slightest shock of circumstances because the state of society has made him a ready prey to suicide".

We have argued that while mental disorder may be sufficient for suicide, it is not necessary, and suicide may be a consequence of 'predicaments' such as slow, painful and undignified death, reputation damage, shame and financial loss (11–14).

The suicide rate (after apprehension) of male child sex offenders who engaged exclusively in sex (as distinct from those who performed multiple types of crimes and those who were violent to children), is reported to be 183 times higher than that of members of the general population (15).

This high suicide rate has been noted in the blog, "Sex Offender Suicides and Other Deaths", where the deaths have been considered "unintentional burdens of punishment" (16).

We were interested to examine the public record of suicide by men who had recently been investigated or apprehended for "sex only" (15) child sex offences, in search of evidence that suicide can occur as a consequence of a 'predicament'. Such results may be

complementary to, and take our knowledge beyond, epidemiological understanding.

Accordingly, the aim of this study was to examine the public record for accounts of suicide by men who had recently been investigated or apprehended for "sex only" child sex offences, with a view to presenting a collection of case histories of men, with no apparent mental disorder other than pedophilia, who apparently completed suicide in response to their predicament. [We note here that the question arises as to whether pedophilia should be regarded as a mental disorder. From the DSM-IV-TR perspective, pedophilia is a clinical condition, but that "does not imply that the condition meets legal or other non-medical criteria for what constitutes a mental disorder". Our background is clinical, thus we accept pedophilia as a mental disorder and refer to the "absence of mental disorder other than pedophilia".]

Methods

The public record (hard and electronic copy) was searched, cases were documented, and a summary made. We have an extensive collection of newspaper reports of suicide covering the last 32 years, and employed electronic search methods using the words "suicide", "pedophilia" and "suicide child sex offence". The summary took the form of a table and simple calculations.

Results

Case histories

Lewis Harcourt, 1st Viscount Harcourt (59 years; 1922) has been described as a sexual predator who attempted to rape both males and females. His behavior was tolerated and kept private. However, after he attempted to rape a 12 year old boy, the boy's mother made the matter public. Harcourt died by overdose in his study at his London home. He was born to a well connected family and educated at Eaton. His father, Sir William, had been Home Secretary. Harcourt married at 33 years and fathered four children. He had been a liberal MP (1904–1916), and had held various ministerial positions, including Secretary of State to the colonies (17).

Neil Hocquart (40 years; 1991) was a photographer associated with Nichols Rabet (see later) and a large group of other pedophiles at a privately funded children's activity center, "The Stables". He died of a drug overdose in London, after police raided his house and confiscated 300 pornographic videos and 100 pornographic photographs. Hocquart, born Foster, took the name from a Guernsey sailor. Captain Hocquart willed considerable wealth to Foster, and soon after, died of an apparent heart attack. At this time, Rabet fled to Thailand (18).

William Lucan-Roberts (61 years; 2000), was facing 67 child sex charges, and was dreading his picture appearing in the newspaper. He left a suicide note, "...I felt it was better I go..." and drowned himself in a private swimming pool in Sydney, Australia. Lucan-Roberts was a classics teacher and cricket coach at a prestigious Sydney secondary school (19).

William Brown (52 years; 2004) was charged with child sex offences in Indonesia and jailed for 13 years; within days, he hanged himself at Police Headquarters, Karangasem, Bali. He had once worked in the Australian Embassy in Jakarta. However, "his overt homosexuality had led to his dismissal as a diplomat" in 1984. He then worked as an English teacher in a tourism school in Bali (20).

Bruce Crosby (59 years; 2006) was an educated US citizen living in Panama. His computer was confiscated by the Panamanian police. Four days later, while charges were being prepared, he asphyxiated himself with butane cooking gas in his bathroom, on the island of Bastimentos. He was believed to have been an active pedophile, using local boys, and made a living by selling pornography and arranging pedophile sex tours of Panama to clients from around the world (21).

Louis Conradt (56 years; 2006) had been Texas district attorney for more than 20 years and at the time of his death he was Rockwall County chief felony assistant district attorney. He shot himself in the head when a SWAT team entered his home. Conradt had been investigated by an anti-pedophile group, Perverted-Justice, which arranged for actors pretending to be under-aged children to make email and telephone contact with him. Perverted-Justice was associated with the television show, To Catch a Predator. The police were informed of various findings; they obtained a warrant, broke down Conradt's front door and entered. After Conradt died, pornographic material was found on his computer. There was subsequent criticism of aspects of police and media staff behavior. Conradt graduated in law from the University of Texas, and lived alone (22).

Wolfgang Priklopil (44 years; 2006) abducted 10-year-old school girl, Natasha Kampusch, near Vienna, in 1998. He kept her prisoner in the basement of his house for 8 years. When she escaped and the police were alerted, Priklopil

suicided by jumping in front of a train. Details of actual sexual offences have not been reported, and Natasha Kampusch has remained silent on the subject. Priklopil was a communication technician with a good work history. He owned his own house (which he inherited from his grandfather) and a luxury car (23).

Nichols Rabet (57 years; 2006) handcuffed his hands to his ankles, taped a plastic bag over his head and died of suffocation, in Pattaya, Thailand, days before he was to appear in court on multiple pedophile offences, including sex acts with children as young as six years. He had been on bail for 10 months. Rabet was a British citizen. He had been the deputy head of a council-run children's home in London but left after being accused of child molestation. His name was placed on a register of people deemed unsuitable to work with children. He then financed and operated a children's activity center in Sussex, called 'The Stables' (his money was left to him by an elderly woman he had befriended-events similar to those by which his colleague, Neil Hocquart, came into money). Rabet fled to Thailand in the 1980s, when accused of molestation of children. His suicide note read: "Sorry I have to leave like this but it is the only way to escape the stress of my life" (24).

J D "Roy" Atchison, (53 years; 2007) was an assistant United States prosecutor living in Florida. He was married to a school teacher and had three children. Atchison was president of the Gulf Breeze Sports Association for soccer, t-ball and other activities. He was snared in a police operation and charged with traveling from Florida to Michigan to have sex with a 5-year-old girl. He pleaded guilty. He was moved from one Michigan gaol to another because of suicide concerns and was placed on suicide watch. Authorities found he "demonstrated no evidence of despondency", but before sentence was passed, he hanged himself (25).

Helmet Nehls (64 years; 2007) was being held in gaol in Udon Thani (Thailand). He had been arrested and charged with having abducted and raped a 12 year old girl, a crime punishable by life in prison. He stuffed socks in his mouth, cut his wrists and died in custody. Nehls was a computer technician, a German national, from Zeppernick, East Germany. He had frequently visited Thailand over a period of 15 years and was fluent in the language. Police claimed he was in the habit of paying families to have the use of pre-pubescent children for weekends, and produced pornographic material which they found at his home (26).

Bob Collins (61 years; 2007) died of an overdose of medication and alcohol in Northern Territory, Australia, following a battle with bowel cancer, and three days before he was to face a committal hearing on 21 charges of sex with children dating back 30 years. Other charges were in preparation, including possession of child pornography images. Collins lived a high profile political life. At 35 years of age, he became a member of the Northern Territory Legislative Assembly. At 41 years, he was elected to the Australian Senate and was soon elevated to the Cabinet. His party lost power in 1996, and Collins retired from parliament two years later, at 52 years of age. He then held senior government positions, until he was charged with child-sex offences. He had received a civil decoration for his community work (27).

Elliott Lash (26 years; 2009) was a pediatric nurse who jumped to his death from the 4th floor of a building in Nashville, Tennessee. His wife had discovered pictures on his computer, of him molesting a male child, and informed the police. A police officer contacted Lash by telephone and asked him to come to the police station and to bring his lawyer. Later that day Lash died; he had the name and telephone number of the detective who spoke to him written on his hand. The police confiscated three of his computers and a large quantity of videos and still photographs. Lash was married (but recently separated) and had four children. He was a Navy veteran and had spent a short period at Austin Peay State University (28).

David Dewees (32 years; 2009) had been teaching English and Latin at Jarvis Collegiate Institute (Toronto, Canada) for six years. He was charged with sex offences involving two children in Ontario. He was released on bail, and lay on a railway track (in clear view of witnesses) and was killed by a train. "He was an excellent teacher, he was very popular with the kids," said a representative of Toronto District School Board (29).

William Evans (57 years; 2009) was charged with sexual offences claimed to have been committed against a young female nearly 30 years previously. Minutes before he was found not guilty on technical grounds (a statute of limitations issue), Evans, who had not returned to court after the lunch break, shot himself at his Florida home. He had written to the woman apologizing for his actions 8 years before he died. He had been supported in court by his wife, daughter, son-in-law and brother (30).

Ivan Bennett (64 years; 2010) hanged himself in jail in Nevada while awaiting a court appearance on charges of possessing child

pornography. He had been convicted seven years earlier for possession of child pornography and eighteen years earlier for molestation of a child. Bennett had served 22 years in the US Army and was married with three children (31).

Richard Dyde (47 years; 2010) was born and gained his first degree in the UK, before emigrating to Canada in 2001. He was arrested on charges of child pornography, arising from Project Sanctuary, a year-long investigation into child pornography which saw 57 men arrested world-wide. The day after he was released on bail, he jumped to his death. Dyde was married, held a PhD in neuroscience, and worked as a researcher at York University, Toronto. He was popular, witty and multitalented - he had recently written a biography of the pioneer aviator, George Cayley (32).

Chad Hunt (42 years; 2011) was a well-regarded family man, who had been employed as an athletics coach at the Clear Creek High School (League City, Texas) for 5 years. He was interviewed by police due to an allegation of "improper contact" with a young (16 years old) female, and placed on administrative leave by his employer, pending the outcome of the investigation. He was interviewed by police and was to be interviewed again the following day. The police found that he did not appear to be emotionally unstable. Before the second interview, he shot himself in his car. There were many expressions of sympathy and students and staff have stated that Hunt was popular and will be greatly missed (33).

Melvin Levine (71 years; 2011) was a famous pediatrician with a special interest in learning disabilities. His book, "A Mind at a Time", had been number one on the New York Times bestseller list, and he had frequently appeared on radio and television. Married, Levine died in the woods near his Rougemont, North Carolina home, of a self-inflicted gunshot wound, a day after a class-action sexual abuse and malpractice suit was filed against him. The lawsuit charged that Dr Levine performed unnecessary genital examinations on 40 boys while at Children's Hospital, Boston from 1966 to 1985 (34).

Robert Carlson (68 years; 2011), founder and president of Penobscot Community Health Care, was a married, highly respected figure in the Greater Bangor area. Three days before plunging to his death from a bridge above the Penobscot River, police had commenced investigating an allegation that Reverend Carlson had sexually abused a young boy while he was pastor at East Orrington Congregational Church, a position he had held from the late 1970s for 25 years. Hours before his death, Carlson had met with the alleged victim, now a middle-aged adult living in the area (35).

Andrew Bourne (46 years; 2012), married with two children, was a surgeon at Mammoth Lakes Hospital, California. Bourne's body was found in the region of a fish hatchery near his home in the Eastern Sierra resort area, a fortnight after bail had been posted for alleged sex crimes against a 14 years old girl. His attorney stated, "I believe these (sex) charges led to his suicide." Bourne's body was located by his GPS tracker bracelet after he had gone missing. The method of suicide was not revealed (36).

Analysis

Twenty case histories were identified from 8 countries (Table 1). The average age was 53 years, with an age range from 26 to 71 years. Details of how long these men had been conducting illegal actions is not clear in every case, but in 4 cases evidence suggests this was in the order of 30 years (Harcourt, Lucan-Roberts, Brown, Collins and Levine), and in another 4 cases, for at least 15 years (Hocquart, Rabet, Nehls and Bennett). In no report, was there mention of mental disorder, but in every case there had been actual public exposure, threat of public exposure and/or legal punishment/threat of legal punishment.

Discussion

Critics of the use of the public record for this type of study claim that journalists are not clinicians and may overlook clinical material. However, journalists have a responsibility to discover all relevant facts and are keen to expose failings of clinicians and health systems. They actively seek evidence from relevant health authorities and it is reasonable to accept that, generally speaking, if mental disorder is present, some evidence will be discovered and presented. The alternative method of assessment, the 'psychological autopsy', is also retrospective, and conducted by researchers who may find it difficult avoiding bias (37). There are various types of psychological autopsy, leading to the belief that one cannot be compared with another (38). Further, some authors have raised concern about the validity and reliability of psychological autopsies (39–41) and "the vast majority have used ill-defined instruments" (42). In these circumstances, it is reasonable to admit 'evidence' from other sources. While perhaps lacking in some regards, journalists may be less

Table 1: Details of 20 "sex only" child sex offenders who completed suicide, 'Year' and 'Location' refer to the death

Case	Year	Family name	First name	Age (years)	Location	Method
1	1922	Harcourt	Lewis	59	London, UK	Overdose
2	1991	Hocquart	Neil	40	London, UK	Overdose
3	2000	Lucan-Roberts	William	61	Sydney, Australia	Drowning
4	2004	Brown	William	52	Karangasem, Indon	Hanging
5	2006	Crosby	Bruce	59	Bastimentos, Panama	Suffocation
6	2006	Conradt	Louis	56	Terrell, USA	Gunshot
7	2006	Priklopil	Wolfgang	44	Vienna, Austria	Train
8	2006	Rabet	Nichols	57	Pattaya, Thailand	Suffocation
9	2007	Atchison	J D "Roy"	53	Michigan, USA	Hanging
10	2007	Nehls	Helmut	64	Udon Thani, Thailand	Suffocation
11	2007	Collins	Bob	61	NT, Australia	Overdose
12	2009	Lash	Elliott	26	Nashville, USA	Jumping
13	2009	Dewees	David	32	Toronto, Ca	Train
14	2009	Evans	William	57	Florida, USA	Gunshot
15	2010	Bennett	Ivan	64	Reno, USA	Hanging
16	2010	Dyde	Richard	47	Toronto, Canada	Jumping
17	2011	Hunt	Chad	42	League City, USA	Gunshot
18	2011	Levine	Melvin	71	Rougemont, USA	Gunshot
19	2011	Carlson	Robert	68	Bangor, USA	Jumping
20	2012	Bourne	Andrew	46	Mammoth Lakes, USA	Not stated

prone than some clinicians to medicalize suicide (43).

Care was taken to exclude violent individuals. For example, Urs von Aesch (67 years, 2010, Switzerland) was excluded because he is believed to have murdered his victim. We also excluded cases of mass murder-suicides involving sects or cults (e.g. the Branch Davidians at Waco, Texas), where suspicions of child sexual abuse have sometimes surfaced but have played a very minor part in a complex set of factors bringing about the group's demise. The case of Priklopil may be challenged because, while the public record clearly states he imprisoned a 10 year old girl for 8 years, specific details of actual sexual acts have not been reported. The case of Collins may be challenged because while it is clear that he was facing extremely damaging accusations, he was also dealing with cancer. These two cases could be withdrawn without significantly altering the weight of the evidence presented. In the case of Collins, the 'predicament' may have been public humiliation and cancer, rather than public humiliation alone.

Many of these individuals were gainfully employed shortly before their deaths (Hocquart, photographer; Lucan-Roberts, teacher; Brown, teacher; Priklopil, IT specialist; Atchison, prosecutor; Conradt, prosecutor; Collins, bureaucrat; Lash, nurse; Dewees, teacher; Carlson, administrator; Bourne, surgeon), counting against the possibility of serious mental disorder other than pedophilia.

If pedophilia is accepted as a mental disorder, the question arises as to whether the pedophilia or the exposure/punishment (actual or likely) is the more telling predicament/factor in the suicide. As the described individuals appear to have 'suffered' pedophilia for years, but completed suicide within days of exposure (or threat of exposure), it is reasonable to suggest that the exposure/punishment was a potent influential factor. This is consistent with Durkheim's conception that social factors account for most cases of suicide (10). While the suicides described in this study have prominent social triggers, they do not fit snugly - individually or collectively - into any one subtype in the classification offered by Durkheim

("anomic" suicide would perhaps be closest) (10) or the classifications described by later theorists like Baechler ("escape-type" suicide is the most relevant of Baechler's subtypes) (44).

Conclusion

Without downplaying the prominent role of underlying mental illness in many cases of completed suicide, it would appear that sometimes there are other pathways to this event. Predicaments alone – in this case series, publicly exposed pedophilia – may be a sufficient trigger, and therefore warrant further attention and study.

Authors' Contribution

Conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, provision of study materials or patient, statistical expertise, and collection and assembly of data: GW, SP

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Original Article

Deep-fried *Keropok Lekors* Increase Oxidative Instability in Cooking Oils

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Abstract

Background: **This study was performed to compare the oxidative quality of repeatedly heated palm and soybean oils, which were used to fry *keropok lekors* and potato chips.**

Method: **A kilogramme of *keropok lekors* or potato chips was fried in 2.5 L of palm or soybean oil at 180 °C for 10 minutes. The frying process was repeated once and four times to obtain twice-heated and five-times-heated oils. The peroxide value and fatty acid composition of the oils were measured.**

Results: **Frequent heating significantly increased the peroxide values in both oils, with the five-times-heated oils having the highest peroxide values [five-times-heated palm: 14.26 ± 0.41 and 11.29 ± 0.58 meq/kg vs fresh: 2.13 ± 0.00, F (3,12) = 346.80, *P* < 0.001; five-times-heated soybean: 16.95 ± 0.39 and 12.90 ± 0.21 meq/kg vs fresh: 2.53 ± 0.00 oils, F (3,12) = 1755, *P* < 0.001, when used to fry *keropok lekors* and potato chips, respectively]. Overall, both oils showed significantly higher peroxide values when *keropok lekors* were fried in them compared with when potato chips were fried. In general, the heated soybean oil had significantly higher peroxide values than the heated palm oil. Fatty acid composition in the oils remained mostly unaltered by the heating frequency.**

***Conclusion: Keropok lekors*, when used as the frying material, increased the peroxide values of the palm and soybean oils. Fatty acid composition was not much affected by the frequency of frying or the fried item used.**

Keywords: *keropok lekors, peroxide value, palm oil, soybean oil, deep frying*

Introduction

Deep frying is one of the oldest practice for food preparation, during which the oil is exposed continuously or repeatedly to high temperature (160–190 °C) in the presence of air and moisture. This leads to a number of chemical reactions in the oil, such as oxidation, hydrolysis and polymerization, which may alter the compositions of the oil leading to production of various types of oxidative products (1). Hydroperoxides and aldehydes are the primary products formed in the initial stages of oxidation and are absorbed into the fried food (2). The extent of oxidation can be measured using the peroxide value of the oil (3,4).

The usage of repeatedly heated cooking oil is common amongst Malaysians who without taking into consideration the effects on health; do this in order to reduce the cost of cooking. Repeated heating can cause changes in the physical appearance of oil, such as increased viscosity, darkening in colour, increased foaming and decrease in the smoke point of the oil (5). Consumption of repeatedly heated oil has been shown to increase the risk of hypertension (6) due to impaired vascular relaxation in rats (7,8).

In many studies involving oil heating, potato chips were the most commonly used food item for deep frying (9–11). The oil quality during deep frying is determined by many factors, such as the type of frying materials and the type of oils used (1,12). In Malaysia, *keropok lekors*, or traditional fish sticks, are one of the most popular snacks that are commonly deep fried before being served. Different from potatoes, which exclusively contain starch (13), the major compositions of *keropok lekors* are protein and starch.

Therefore, the objective of this study was to compare the quality of the repeatedly heated palm and soybean oils when using *keropok lekors* and potato chips as frying items. This study used palm and soybean oil because of their wide usage as cooking oils for deep frying purposes in

Malaysia, and the difference in the fatty acid composition (14,15) and antioxidant content (16) of the two oils.

Materials and Methods

Materials

Palm oil (Lam Soon Edible Oil, Malaysia) and soybean oil (Yee Lee Edible Oil, Malaysia) were used in this study. The *keropok lekors* (Malaysian traditional fish sticks) and potatoes were bought from the same sources at a local market. The size of the food item was standardised for each frying process.

Frying procedure

The potatoes were peeled before being thinly sliced. Two and a half litres of palm oil or soybean oil were used to fry 1 kg of *keropok lekors* or potato chips in a stainless steel wok for 10 minutes at 180 °C. The frying process was repeated once or four times to obtain twice-heated or five-times-heated oils, respectively, with a cooling interval of 24 hours between repetitions (17). The food quantity was reduced proportionately with the amount of vegetable oil left till the fifth heating. No fresh oil was added between the frying processes. After heating, 5 samples per group were obtained from different batches for the peroxide value measurement.

Determination of peroxide value

Peroxide values of the heated oils were determined according to American Oil Chemists' Society (AOCS) Official Methods Cd 8–53 (18). Briefly, 5 g of the oil sample was transferred into a 250 mL flask before adding 30 mL of acetic acid-chloroform (3:2). The flask was swirled and then 0.5 mL of saturated potassium iodide was added. Then, the solution was swirled again for 1 minute and 30 mL of distilled water and a few drops of starch solution (10%) were added. The solution was titrated against 0.01 N sodium thiosulphate solution ($Na_2S_2O_3$), which had been previously standardised using potassium dichromate and potassium iodide, until the blue colour disappeared. The peroxide value was expressed in miliequivalents of peroxide per kg of the sample calculated as:

$$\text{Peroxide value (meq/kg)} = [(Va\text{-}Vb)\ N \times 1000]/W$$

Where;

Va = volume of sodium thiosulphate solution (mL)

Vb = volume of sodium thiosulphate solution (mL) used for the blank

N = normality of sodium thiosulphate

W = weight of the test portion (g)

Fatty acid composition determination

Fatty acid composition of the fresh and heated oils was analysed using gas chromatography (GC-17A, Shimadzu, Japan) with nitrogen at a flow rate of 0.40 mL/min as the carrier gas. The chromatography system consisted of a flame ionisation detector and a BPX 70 capillary column (30 m × 0.25 mm × 0.25 μm), with programmable injector temperature set at 250 °C and detector temperature set at 280 °C. The oil samples (100 μL) were first transesterified to fatty acid methyl ester using 1 mL of 1 M sodium methoxide in 1 mL hexane prior to injection (1 μL) into the gas chromatographic system. Authentic standards were used in the identification of fatty acid methyl ester peaks by comparing their retention times. The readings were obtained based on one sample per group. The fatty acid composition in the oils was expressed as the percentage of the total fatty acids.

Statistical analysis

The data are expressed as mean ± standard error and were analysed using Statistical Package for Social Science version 19. To compare the effect of heating frequency, the data were analysed using a within-subjects analysis of variance for repeated measure. Student's t test (independent samples) was used for comparison between the type of oil and the food item used for frying at the same heating frequency. Differences were considered significant if $P < 0.05$.

Results

The results presented in Table 1 show a highly significant increase in peroxide values of palm oil with increasing frequency of heating (once heated: 4.84 ± 0.43 and 3.68 ± 0.26; twice heated: 9.90 ± 0.44 and 4.70 ± 0.16; five times heated: 14.26 ± 0.41 and 11.29 ± 0.58 meq/kg, when used to fry *keropok lekors* and potato chips, respectively) compared with the fresh oil (2.13 ± 0.00 meq/kg) [$F(3,12) = 346.80$, $P < 0.001$]. The same trend was observed in the soybean oil groups [once heated: 6.54 ± 0.19 and 2.74 ± 0.26; twice heated: 7.86 ± 0.07 and 7.12 ± 0.30; five times heated: 16.95 ± 0.39 and 12.90 ± 0.21 meq/kg, when used to fry *keropok lekors* and potato chips, respectively; $F(3,12) = 1755$, $P < 0.001$). Oils heated five

times had significantly higher peroxide values than the oils heated twice, which in turn had higher peroxide values than the oils heated once. The peroxide values were significantly higher in heated palm and soybean oils that were used to fry *keropok lekors* than in the oils used to fry potato chips ($P < 0.01$). The peroxide values of all soybean oil groups (except oils heated only once to fry potato chips) were significantly higher than those of all palm oil groups regardless of the frying materials used.

Fatty acid composition of the fresh and heated palm and soybean oils is shown in Figure 1. The percentages of saturated, monounsaturated and polyunsaturated fatty acids in fresh and heated palm oils were similar. Both oils contained about 43% saturated, 49% monounsaturated and 8% polyunsaturated fatty acids. In soybean oil, fresh and heated oils contained similar composition of saturated and monounsaturated fatty acids, which were about 17% and 25%, respectively. It seemed that the polyunsaturated fatty acid content in the five-times-heated soybean oil (42%) was slightly reduced compared to that in other soybean oil groups (50–52%). Generally, palm oil contained higher proportions of saturated and monounsaturated fatty acids but a lower proportion of polyunsaturated fatty acids compared with soybean oil.

Discussion

The peroxide value is often used as an indicator for the oxidative stability of fats and oils (3,4,19). It was found to be highly correlated with the total concentration of major odorants (including 1-pentanal, pentanoic acid, hexanoic acid and 1-nonanal) and the total concentration of five unsaturated aldehydes (t-2-heptenal, t-2-octenal, t-2-decenal, t-2-undecenal and t,t-2,4-decadienal) in oils. These odorants and

Table 1: Peroxide values in (meq/kg) when *keropok lekors* and potato chips were fried in repeatedly heated palm and soybean oils

	Fresh	Once	Twice	Five times
Palm oil				
Keropok lekors	2.13 ± 0.00*	4.84 ± 0.43*	9.90 ± 0.44**	14.26 ± 0.41**
Potato chips		3.68 ± 0.26*#	4.70 ± 0.16**#	11.29 ± 0.58**#
Soybean oil				
Keropok lekors	2.53 ± 0.00*	6.54 ± 0.19**§	7.86 ± 0.07**§	16.95 ± 0.39**§
Potato chips		2.74 ± 0.26**#§	7.12 ± 0.30**#§	12.90 ± 0.21**#§

Values represent mean ± sem ($n = 5$). Significantly different from other groups in the same type of oil and food used with a within-subjects test (*$P = 0.002$, **$P = 0.0001$), #significantly different from the keropok lekors group fried in the same oil at the same heating frequency ($P < 0.05$), §significantly different from the palm oil groups at the same heating frequency and same type of food ($P < 0.05$).

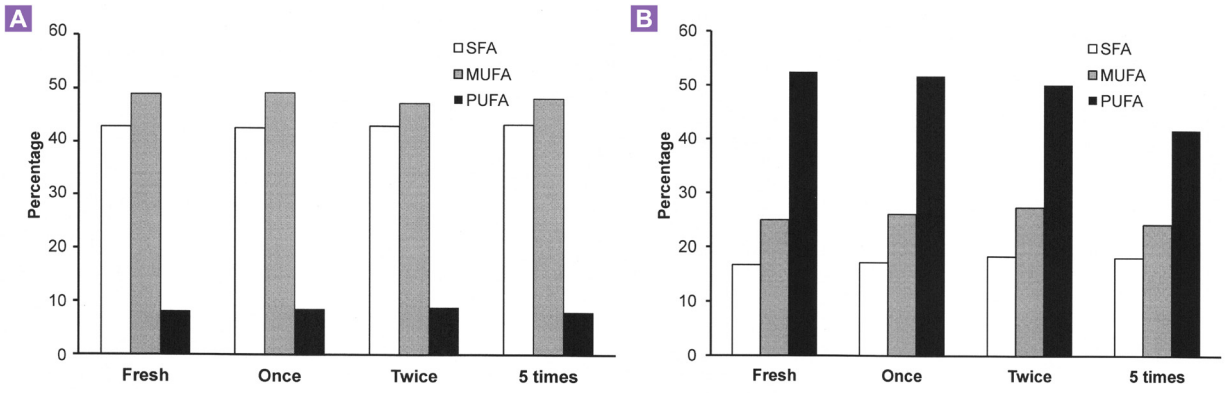

Figure 1: Composition percentage of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in fresh, once-, twice-, and five-times-heated palm (panel A) and soybean (panel B) oils, 216 × 227 mm (300 × 300 DPI).

aldehydes showed strong cytotoxicity in the heated oils. Moreover, it is relatively easier to measure the peroxide value compared to the direct measurement of odorants in the oils (20). Therefore, the peroxide value is of significance in assessment of quality and stability of foods with high oil content. There are several factors that can affect the quality of oil during the deep frying process, such as the types of food being fried, composition of the oils used, frying temperature, length of frying time, use of a continuous or intermittent frying method and replenishment of fresh oil (1). However, in this study, we investigated only the effect of different types of food items and different types of vegetable oil for the deep frying process on the quality of repeatedly heated oil.

In this study, repeated heating increased the peroxide values of palm and soybean oils. The value increased with the increasing frequency of heating. This indicated that repeated heating of the oils augmented the formation of lipid peroxidation products in the oil, which are potentially harmful to health. Consumption of repeatedly heated vegetable oils has been shown to have detrimental effects on health (7,8,17). A study by Rueda-Claussen et al. (21) demonstrated that repeatedly heated (10 and 20 times) olive, palm and soybean oils had similar acute detrimental effect on the endothelial functions in healthy young subjects. This is contrary to a report by Shuid et al. (22) who found that bone properties of ovariectomized rats fed repeatedly heated palm oil (once and five times) was better than those fed repeatedly heated soybean oil. The discrepancy between these studies could be due to the difference in the heating frequency.

Palm and soybean oils that were used to fry *keropok lekors* had significantly higher peroxide values compared to the ones that were used to fry potato chips. *Keropok lekors* are primarily made from a combination of pounded fish, mainly mackerel or sardines (about 55–60%) and sago or starch flour (about 40–45%). On the other hand, potatoes only contain about 16% starch, 2% protein and a very small amount of lipids (< 1%) (13). As a marine product, *keropok lekors* are rich in fish oil. Fish oil is very susceptible to autoxidation because of the high degree of polyunsaturated fatty acids (23). Autoxidation of the fish oil in the *keropok lekors* during the frying process might have accelerated the oxidative degradation of both vegetable oils and fish oil with the higher production of peroxide content compared to that in the oils used to fry potato chips. This may explain the higher peroxide values of the oils used to fry the *keropok lekors*. Even in our daily cooking chores, we notice that frying salted fish produces blackish discolouration of the oil.

Previously, no similar study has reported the use of *keropok lekors* in deep frying. Many studies have used potato chips to suit the Western context to measure the oxidative stability of cooking oils, such as corn and olive oil, either using deep frying or microwave heating (9–11,24). *Keropok lekors* are one of the popular snacks in Malaysia, which are commonly consumed deep-fried and can be easily be found in night markets. Due to the low level of awareness amongst night market food outlet operators regarding the detrimental effects of the use of repeatedly heated cooking oil (25), there is a big possibility that *keropok lekors* found at the night markets are fried in repeatedly heated cooking oils. This kind of practice could render bad health effects to the public.

Generally, for both types of food (*keropok lekors* and potato chips), higher peroxide values were found in soybean oil than in palm oil after repeated heating. This suggests that soybean oil undergoes more oxidative modification compared to the palm oil on repeated heating. The unique composition of palm oil allows it to withstand heat better than soybean oil. Palm oil is rich in saturated and monounsaturated fatty acids (MUFA) but has low levels of polyunsaturated fatty acids (PUFA) compared to soybean oil, as shown in the present study. Vegetable oils which are rich in PUFA are more prone and less stable to oxidation compared to those which are rich in MUFA, whereas oils that are rich in MUFA, such as palm oil and olive oil, can better withstand oxidation and form less degradation products on repeated heating (26). In the present study, the percentage of PUFA in five-times-heated soybean oil was 10% less compared to the fresh, once-heated and twice-heated soybean oils. This finding suggests that the unsaturated bonds in PUFA were oxidised due to heating. Previously, the frequency of heating has shown to reduce the unsaturation of fatty acids (27). However, an increase in the saturated fatty acids and MUFA percentage was not obviously seen in the five-times-heated soybean oil. The characterisation of individual fatty acids (such as oleic, linoleic and linolenic acids) was not done in this study. Oils that are repeatedly heated at high temperature for a long period will undergo thermal oxidation process with configuration changes in fatty acid from cis to trans isomer (28). Some studies have shown that food containing high trans fatty acid can increase the risk of cardiovascular disease (29) by inducing a proinflammatory response in the endothelial cells (30). The increase in

the percentage of saturated fatty acids may be attributed to the cholesterol raising effect of the heated oils.

Palm oil also has an abundant content of vitamin E, which may play an important role in its ability to withstand thermal oxidative changes. Inclusion of α-tocopherol to frying oil was found to render PUFA more resistant to oxidation (31). Vitamin E, which effectively protects fatty acids in the oil from oxidation, deteriorates after each frying episode (16). Therefore, repeated heating of frying oils destroys the vitamin E content and exposes the fatty acids to oxidation. The vitamin E content of palm oil mainly consists of tocotrienols, while the vitamin E in soybean oil mainly consists of tocopherols (16). Tocotrienols have better antioxidant capacity than tocopherols (32) and this may have contributed to the better resistance of palm oil to oxidative changes due to repeated heating.

It was also noted that up to two heatings, the peroxide values of the oils were still below the maximum limit of the peroxide value (10 meq/kg), according to the Malaysian Food Act and Regulation 1985 and the AOCS (33). However, if the Food Sanitation Law of Japan guideline (peroxide value ≤ 30 meq/kg oil) is used instead, all fresh and heated oils can be considered safe for human ingestion. In Malaysia, it is very common to use repeatedly heated vegetable oil for frying to cut cost and only be discarded when the colour of the heated oil has darkened. The level of awareness of the general public in this country regarding such usage is influenced by the socioeconomic status, with higher level of awareness in higher income and education level groups (34).

Conclusion

In the present study, it can be concluded that the use of food item with high contents of fish oil, such as *keropok lekors*, may further increase the peroxide value in thermally oxidised oil, which is influenced by the heating frequency and the type of oil used during the frying process. It is recommended that vegetable oils should not be heated more than two times for safe consumption.

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Authors' Contribution

Conception and design, final approval of the article: AA, HMSQ,MFNA,KJ
Analysis and interpretation of the data: YK,AA, MFNA,KJ
Conception and design, drafting of the article, critical revision of the article for the important intellectual content: YK
Provision of study materials or patient, Obtaining of funding: KJ
Analysis and interpretation of the data, final approval of the article, drafting of the article, collection and assembly of data: SS, MJN, SYK, NASH

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Original Article

Hearing Threshold, Loss, Noise Levels and Worker's Profiles of an Open Cast Chromite Mines in Odisha, India

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Abstract

Objectives: **The aims of the study were to describe the noise levels at an open cast chromite mine in Odisha, India, and the hearing threshold of its workers and to associate their hearing loss with their age, work station and length of employment at the mine.**

Method: **We performed a cross-sectional study of the hearing threshold of chromite mine workers. Audiometric data from 500 subjects was collected at the mines' hospital in the Sukinda Valley of Jajpur, Odisha, India. The latest audiometry data available for the period 2002 to 2008 was used in the analysis. Audiometric screening was performed using an audiometer (TRIVENI TAM-25 6025A) in a quiet environment by qualified technicians, audiologists or physicians. Tests were conducted on the subjects after they had completely rested for 16 hours or more after their day shift.**

Results: **A maximum of 262 subjects (52.4%) were employed in the work zone area and a minimum of 2 subjects (0.4%) had less than 5 years working experience. The age of the subjects ranged from 29 to 59 years and their working experience ranged from 4 to 37 years. The subjects' average mean hearing thresholds at 4, 6 and 8 kHz were 21.53 dBA, 23.40 dBA and 21.90 dBA, respectively. The maximum L_{eq} and L_{90} levels exceeded the prescribed limits for commercial, residential and silence zones. The maximum Leq levels exceeded 95 dBA for large and medium heavy earth moving machineries (HEMMs), both outside and at the operator's position. Hearing loss due to the subjects' work experience was found to be greater than that attributable to age and workstation.**

Conclusion: **In our study population, the maximum noise levels for large and medium HEMMs and inside the cabins of HEMMs were found to be more than 95 dBA. This indicates that operators in this particular chromite mine at Odisha, India were exposed to noise levels exceeding 95 dBA for more than 10% of the monitoring time. The subjects' hearing loss was also found to increase for every 10-year age interval and that for every 5 years of work experience at high fence. The subjects' age and experience are significantly associated with hearing loss at all levels for frequencies of 4.0, 6.0, and 8.0 kHz, with older and more experienced workers having a higher incidence of hearing loss.**

Keywords: *hearing loss, noise, presbycusis*

Introduction

Hearing loss is associated with numerous factors (1–8), primarily age (9–11), exposure to various sources of noise (12,13) and length of time exposed to noise (14,15). It has been reported that when male steelworkers are exposed to 90–99 dBA noise levels, their hearing ability is significantly affected (1), with a mean shift of 6.8–7.8 dB after 6–8 years. The incidence of presbycusis (9) in subjects aged 65 years and older is 37.8% and 8.3% for the ≥ 27 dB HL criterion and the ≥ 41 dB HL criterion, respectively. There is also a significant difference in the hearing threshold of men and women aged 65 years and older. The noise-induced hearing loss (NIHL) is significant at 4 kHz, a well-established clinical sign (4,7,12,14). This frequency is also considered the typical notch frequency where hearing loss has its maximum dip when compared with other high fence frequencies. The degree of association is even stronger when the intensity of the noise and the temporary hearing threshold shift are high (16).

The aims of the study were to describe the hearing threshold based on audiometry data and noise levels in various areas of an open cast chromite mine in Odisha, India. The study also

sought to find an association between hearing loss and the various profiles of workers at an open cast chromite mine from 2002 to 2008.

Materials and Methods

Study area

The mine site is located in the Sukinda valley of Jajpur, Odisha, India. The mine produces chromite ore in both friable and lumpy varieties and has a chrome ore beneficiation (COB) plant. The mine is located 160 km from Bhubaneswar, the state capital of Odisha, 65 km from National Highway 5 (NH-5) and 52 km from JK Road, the nearest railway station.

Study design

A cross-sectional study of the hearing threshold of the chromite mine workers was conducted with the aim of gaining insight into the factors associated with hearing loss. Audiometric data from 500 subjects were obtained from the mine hospital's records. Subjects with audiometry data for 0.5, 1, 2, 4, 6, and 8 kHz frequencies for the period 2002 to 2008 were included in the study and divided into five broad categories as shown in Table 1. The audiometry data for the above period was used in the statistical analysis.

Audiometry test

Screening audiometry was performed using an audiometer (TRIVENI TAM-25 6025A) in a quiet environment by qualified technicians, audiologists, or physicians. Tests were conducted on the subjects after they had completely rested for 16 hours or more after their day shift. Audiometric air conduction tests were performed by presenting a pure tone to the ear through an earphone. The hearing threshold (dB) was recorded at the frequency at which a particular tone was perceived 50% of the time. The better ear was first tested at 1 kHz and then at 2, 4, 6, 8, and 0.5 kHz, in that order. Retests were performed at 1 kHz in the first ear. When the test value exceeded 5 dB or was more acute than the original, a retest was performed at the next frequency and so on. Audiometry tests were conducted in the opposite ear in the same manner except for retesting at 1 kHz. The duration of the presented tone was 1–3 seconds. The same duration was maintained between the tones. The total time required to perform the audiometry test by a subject was 3–5 minutes.

Noise measurements

A digital sound level metre (M & K, Bruel & Kjaer, Denmark) was used throughout the entire noise survey. The sound level metre was placed 1.2 to 1.5 m above the surface of the ground and 6 m away from the side of the road, avoiding obstacles and reflecting objects. The air temperature varied between 19.38 and 34.31 °C, and the wind velocity was less than 1.02 m/s. Measurements were taken under clear skies and sustained wind conditions to avoid any background noise level differences greater than 10 dBA (17).

Ambient noise

Systematic ambient noise monitoring was performed at all stations in the summer (June

Table 1: Area code, category of area, and work settings

Area Code	Category of Area/ Zone	Subjects Working at/in	Number of Subjects
W	Work zone	Mine quarry, chrome ore beneficiation plant (COBP), lumpy ore processing plant (LOPP), and operation of HEMMs	262
A	Industrial area	Maintenance of equipments, store yard (loading), quality control-COBP and LOPP and sewerage treatment plant	128
B	Commercial area	Administrative Buildings (It is located near the Mine Quarry area), Mining Weigh Bridge, Project & Construction and Airfield	65
C	Residential area	Main Gate of the Plant, Canteen, Guest Houses and Vocational Training Centre	20
D	Silence zone	Hospital and Arm Guards	25

2008) and winter (November 2009) between 0700 and 2200 hours. For blasting operations, the survey was conducted half an hour before and after the blasting operations at a distance of 100 m from the blasting site for three consecutive days in April, 2010. As shown in Table 1, the working areas were categorized based on the individual administrative records. Table 2 shows the descriptive statistics of 500 subjects in the demographic categories of age (4 groups), experience (8 groups) and working area (5 groups). Table 3 shows a summary of the various noise parameters in the work zone, the industrial area, the commercial area, the residential area and the silence zone. A time gap of 60 seconds was observed during the first monitoring between two consecutive readings and 15 seconds during the second and third noise survey.

Noise parameters

The noise levels were quantified in terms of various sound levels, with L_{10}, L_{90}, and L_{eq} defined as follows:

L_{10} : maximum noise level measured for more than 10% of the monitoring time.

L_{90} : minimum noise level measured for more than 90% of the monitoring time, also designated as background noise.

L_{eq} : the equivalent noise level over a particular monitoring time period.

The following equation was used to estimate L_{10}, L_{90} and Leq values (18):

$$L_{av} = 10 \log_{10} \sum 10^{Li/10} \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

Where ;

L_{av} = average noise level of L_{10}, L_{90}, and L_{eq} in dBA

L_i = the i_{th} sound pressure level in dBA

i = 1, 2, 3, ..., N

N = the number of readings of a particular parameter

In the present study, audiometric data from 500 subjects was obtained for the period 2002–2008 to evaluate the possibility of a dip or a notch at high fence frequencies (i.e. at 4, 6, and 8 kHz) due to the subjects' exposure to different levels of noise. The subjects' ages varied between 29 and 59 years and their working experience ranged from 4 to 37 years.

Data were entered and cleaned using SPSS version 16.0 for Windows. We described the data using means, standard deviations, frequencies and percentages where applicable. Line plots were used to depict the hearing thresholds for different frequencies. The associations between the subjects' age, workstation and work experience and their hearing loss were assessed using the Chi-square test. The results were deemed significant if the *P*-values were less than 0.05 (2 tailed).

Results

The codes and categories for the various areas and the work settings of the open cast chromite mine are shown in Table 1. The profiles of the 500 subjects with respect to age, work experience and hearing threshold are shown in Table 2.

Equation 1 was used to evaluate the different noise parameters (viz., L_{10}, L_{90}, and L_{eq}) at each station. The summary of these noise parameters is presented in Table 3. We found that the maximum L_{eq}, and L_{90} levels exceeded the prescribed limits (19) in commercial, residential and silence zones. The maximum noise levels were found to be more than 90 dBA (19), the warning limit for large and medium HEMMs, both at 7 m away from the equipment and at the operator's position. The maximum value of L_{10} was found to be 100.92 dBA inside the cabin. Almost all of the subjects had been exposed to this type of noise; thus, without personal ear protection equipment, a change in hearing threshold from their normal hearing is unavoidable.

Figures 1 to 3 indicate the variation in hearing loss for all subjects at all test frequencies with respect to age, experience and work station. The audiograms indicate bilateral hearing loss, no hearing loss below low fence frequencies (0.5, 1, and 2 kHz), moderate flat sloping hearing loss from 2 to 6 kHz, a small notch at 6 kHz and then a slight recovery at 8 kHz for almost all subgroups. However, there is no clear sign of a dip or a notch at the characteristic 4 kHz frequency. Therefore, the Pearson Chi-square test was used to estimate the association of hearing loss with different subject groups.

Table 4 describes the Chi-square test for the various subject categories. The hearing threshold levels for all subjects were divided into two groups (viz., ≤ 25 dB HL and > 25 dB HL) to identify the degree of hearing loss at 4, 6 and 8 kHz and to form an 'n x k' table for the three demographic categories separately. We found that the expected number in the cell was less than 5 in the 20–30 age group, 0–5, 5–10, and > 35 years experience groups at 4, 6, and 8 kHz and also the residential and silence zone at 4 and 6 kHz. As the Pearson

Table 2: Age, experience, and hearing threshold of subjects, n = 500

Category	Subjects		Age (years)		Experience (years)		Hearing Threshold Levels (dB HL)					
							4.0 kHz		6.0 kHz		8.0 kHz	
	n	%	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)												
20–30	013	02.6	29.92	0.28	10.54	0.78	16.15	3.00	18.08	4.35	15.77	5.34
30–40	168	33.6	36.02	2.61	12.85	3.17	18.45	6.19	20.74	7.53	17.59	7.28
40–50	208	41.6	45.38	2.72	18.04	4.73	22.16	6.63	24.18	7.33	23.09	8.19
50–60	111	22.2	53.87	2.36	26.81	7.60	25.60	8.53	26.40	7.64	27.15	8.29
Experience (years)												
0–5	02	0.4	37.50	3.54	4.00	1.41	20.00	7.07	22.50	3.54	25.00	0.00
5–10	56	11.4	35.21	5.41	9.911	0.29	17.50	5.52	19.46	7.44	15.89	5.76
10–15	174	34.6	39.73	5.77	13.09	1.39	20.11	6.83	22.13	7.94	19.22	8.35
15–20	127	25.4	44.91	4.81	17.63	1.34	22.72	8.23	24.06	7.10	23.65	7.96
20–25	59	5.8	47.73	3.75	22.61	1.39	22.54	6.46	24.41	6.95	23.90	7.94
25–30	29	11.8	51.00	2.38	27.59	1.48	25.17	8.61	27.93	9.40	27.41	9.03
30–35	45	9.0	54.67	2.44	33.29	1.31	23.78	6.17	25.56	6.18	27.60	7.44
> 35	08	2.4	55.63	2.67	36.50	0.53	29.38	6.78	30.00	9.26	29.38	7.29
Working Area/Zone												
W	262	52.4	42.53	7.08	16.82	6.29	20.95	6.95	22.77	7.55	21.25	8.53
A	128	25.6	44.41	8.15	19.62	8.52	22.34	8.57	23.83	8.61	22.66	9.40
B	65	13.0	45.71	7.45	19.45	8.07	21.92	7.32	24.15	7.84	21.95	8.41
C	20	04.0	47.40	7.13	20.85	7.77	23.50	7.86	24.50	6.63	25.00	8.02
D	25	05.0	44.44	5.42	16.82	4.91	21.00	4.75	24.20	6.74	23.00	6.71
Total	500	100	43.72	7.45	18.05	7.28	21.53	7.42	23.40	7.80	21.90	8.67

Table 3: Noise levels (in dBA) of different areas of the mines

Category of Area/ Zone	L_{10}				L_{90}				Le_q			
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
Industrial	58.15	84.37	68.56	10.7	52.78	70.64	60.03	7.62	53.31	72.29	60.94	7.97
Commercial	71.62	90.58	78.78	8.16	56.79	77.30	64.64	8.68	58.33	78.65	66.13	8.58
Residential	66.60	88.83	75.04	8.16	55.81	72.07	63.02	6.95	57.91	72.86	64.25	6.86
Silence Zone	64.76	73.69	69.48	4.49	58.78	66.01	61.69	3.82	59.46	67.02	62.58	3.95
Work Zone[a]												
Large HEMMs	72.29	104.04	84.65	10.5	65.47	96.47	77.91	9.03	65.88	97.23	78.72	8.98
Medium HEMMs	87.18	100.72	93.86	6.28	76.42	94.50	85.34	6.76	77.50	95.12	86.19	6.69
Light HEMMs	82.15	89.52	84.79	4.27	74.50	82.74	78.22	3.54	74.53	83.42	78.76	3.83
Blasting area	74.04	79.50	76.39	2.81	52.66	63.92	58.38	5.63	54.79	65.51	60.16	5.36
Haul Roads	–	79.51	–	–	–	69.24	–	–	–	70.28	–	–
COBP area	65.63	83.54	73.04	9.35	58.56	73.81	67.46	7.94	54.79	74.79	67.82	7.70
Cabin of HEMMs	60.62	100.92	87.93	13.5	56.01	100.14	84.75	15.3	56.48	100.56	85.03	15.0

[a] Large HEMMs: Pay Loaders, JCB, Shovel with Rock Breaker, Poclain, and Giant Excavators; Medium HEMMs: Dozers, Dumpers, and Trucks and Small HEMMs: All Drilling Machines.

Chi-square test is robust enough for this sample size, there is no serious disadvantage in the present study (1).

The Pearson Chi-square test was performed for the subject categories by assuming the following hypothesis: The age group, experience group and the working group are independent of hearing loss at the 4, 6, and 8 kHz test frequencies.

Given that $P < 0.01$, the hypothesis was rejected at the 1% level of significance for the age group and experience group at the 4, 6 and 8 kHz test frequencies. However, the Pearson Chi-square test was rejected at the 5% level of significance for the working area group at 6 kHz because $P < 0.05$ but was accepted at 4 and 8 kHz.

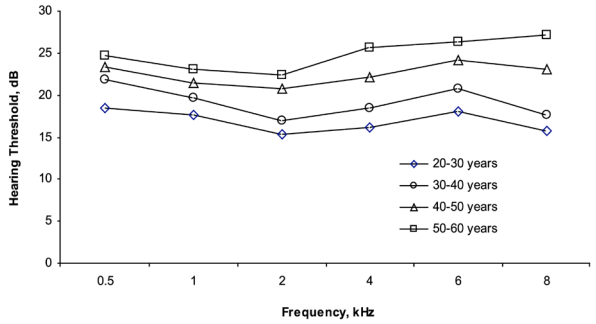

Figure 1: Hearing Threshold Vs. age.

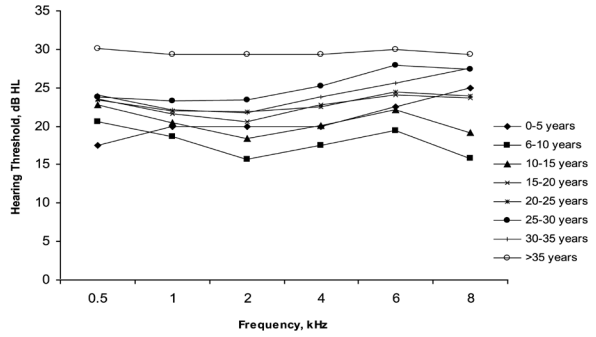

Figure 2: Hearing Threshold Vs. experience.

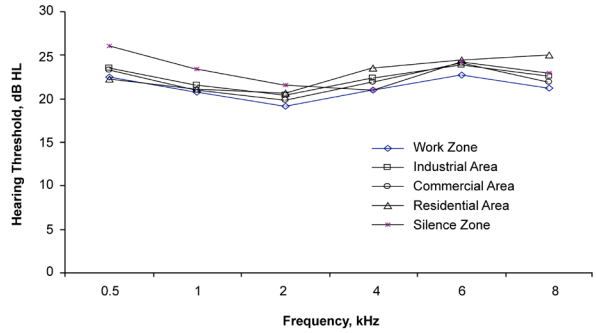

Figure 3: Hearing Threshold Vs. work stations.

The Pearson Chi-square test revealed an association between hearing loss and the age groups for all test frequencies (4, 6, and 8 kHz), and the degree of association varied from 0.22 to 0.27. There was also a relationship between hearing loss and the years of work experience, with the degree of association between 0.22 and 0.30. Similarly, the hearing threshold was also associated with working at the various workstations, and the value of the association was between 0.08 and 0.14. Hearing loss was associated with age and work experience for the 4, 6, and 8 kHz frequencies and with the subjects' different working areas for the 6 kHz frequency only.

Discussion

Table 3 shows that the maximum Leq level exceeded 90 dBA for all areas except the industrial area as did L_{90}, the background noise level. Similarly, the maximum noise levels were found to be more than 90 dBA for large and medium HEMMs, both at 7 m away from the equipment and at the operator's position. It may be inferred from this that the subjects, particularly the HEMMs operators, are overexposed to noise during the course of their working shift. In addition, the L_{10} value for the large HEMMs was found to be more than 100 dBA. Therefore, it may be inferred that the subjects are exposed to such high noise levels that they may suffer from hearing loss during the work shift at different areas of the mine.

The maximum association between hearing loss and age for 8.0 kHz implies that hearing loss increases with age and noise frequency. The maximum association between hearing loss and work experience for 4.0 kHz indicates a dip at the characteristic frequency. The maximum association between hearing loss and workstation occurred at 6.0 kHz instead of at the characteristic 4 kHz frequency. Therefore, it may be inferred that a number of the subjects may have been exposed to areas with high noise levels in the 6.0 kHz frequency.

As indicated in Table 4, the Chi-square test of independence revealed that hearing loss and age were dependent, with a degree of association of 0.27 at 8 kHz. Franks (20) has shown that 90% of coal miners and 49% of metal/non-metal miners undergo a hearing loss by the age of 50. Johansson et al., (11) have also shown a strong

Table 4: Pearson Chi-square test of the subjects (n = 500)

Category	% age of subjects at		$\chi 2^*$	P (2-tailed)
	≤ 25 dB HL	> 25 dB HL		
a. Subjects profiles and status of hearing loss at 4.0 kHz				
Age (years)				
20–30	100.0	0.0	32.82	0.001
30–40	94.1	5.9		
40–50	81.2	18.8		
50–60	69.4	30.6		
Experience (years)				
0–5	100.0	0.0	44.92	0.001
5–10	96.4	3.6		
10–15	89.1	10.9		
15–20	77.2	22.8		
20–25	79.7	20.3		
25–30	79.3	20.7		
30–35	82.2	17.8		
> 35	12.5	87.5		
Working Area/Zone				
W	84.7	15.3	3.15	0.534
A	79.7	20.3		
B	81.5	18.5		
C	85.0	15.0		
D	92.0	8.0		
b. Subjects profiles and status of hearing loss at 6.0 kHz				
Age (years)				
20–30	92.3	7.7	23.80	0.001
30–40	87.5	12.5		
40–50	71.6	28.4		
50–60	64.9	35.1		
Experience (years)				
0–5	100.0	0.0	24.68	0.001
5–10	87.5	12.5		
10–15	83.9	16.1		
15–20	70.1	29.9		
20-25	71.2	28.8		
25–30	62.1	37.9		
30–35	68.9	31.1		
> 35	37.5	62.5		
Working Area/Zone				
W	81.3	18.7	9.89	0.042
A	71.1	28.9		
B	72.3	27.7		
C	60.0	40.0		
D	68.0	32.0		

Category	% age of subjects at		$\chi 2$*	*P* (2-tailed)
	≤ 25 dB HL	> 25 dB HL		
c. Subjects profiles and status of hearing loss at 8.0 kHz				
Age (years)				
20–30	92.3	7.7	35.5	0.001
30–40	90.5			
40–50	75.0			
50–60	61.3			
Experience (years)				
0–5	100.0	0.0	33.39	0.001
5–10	92.9	7.1		
10–15	85.6	14.4		
15–20	71.6	28.4		
20–25	74.6	25.4		
25–30	65.5	34.5		
30–35	62.2	37.8		
> 35	37.5	62.5		
Working Area/Zone				
W	79.0	21.0	3.24	0.518
A	75.0	25.0		
B	81.5	18.5		
C	65.0	35.0		
D	76.0	24.0		

NB: * Chi-square value.

association between hearing threshold levels and age. They also demonstrated that reductions in hearing threshold levels start more rapidly in the 50-year age group for frequencies over 3 kHz. Edwards (21) demonstrated a strong association between hearing loss and age in a study of gold miners. Furthermore, it has been shown that the average deterioration in the pure tone threshold of gold miners is 14.16 dB for every ten years at 6 kHz. Thus, it can be concluded that the subjects' hearing threshold is positively associated with age for the 8 kHz frequency. This finding also indicates that hearing loss continues every 10 years up to the age of 50 to 60 years.

There is also a relationship between hearing loss and the length of time of job exposure at the 1% level of significance, with a maximum degree of association of 0.30 at 4 kHz, the characteristic frequency. Celik et al., (4) have found that workers at a hydroelectric power plant demonstrate hearing loss within the first 10 years of noise exposure and that there is a slight progression in the following years in the frequency range of 4 to 6 kHz. Abbate et al., (13) are in agreement with the present study and found that noise-induced hearing loss is observed in occupational exposure exceeding 17 years at 4 kHz in two bottling plants. However, the present study reveals that subjects' hearing loss increases with every 5 years of working experience in an open cast chromite mine at 4 kHz.

Similarly, hearing loss and working at different stations are dependent at the 5% level of significance, with a maximum association of 0.14 at 6 kHz, where the notch is found. The work zone was found to be the most significant factor affecting the subjects' hearing loss for 6 kHz at the 5% level of significance. Spencer et al. (22) agree with these findings and have shown that there is a strong association between noise exposure and heavy construction equipment operators. Edwards (21) showed that there is also a strong association between hearing loss and rock drillers, the most severely affected of whom are gold mine workers.

Conclusion

In our study population, the maximum noise levels for large and medium HEMMs and inside the HEMM cabins were found to be more than 95 dBA. This indicates that the operators in that particular chromite mine of Odisha, India, were exposed to noise levels exceeding 95 dBA for more than 10% of the monitoring time. The subjects' hearing loss was also found to increase for every 10-year age interval and for every 5 years of work experience exposed to high fence frequencies. The subjects' age and experience were significantly associated with hearing loss at all levels for 4.0, 6.0, and 8.0 kHz frequencies, with a higher percentage of older and more experienced workers experiencing hearing loss.

Authors' Contribution

Conception and design, analysis and interpretation of the data, drafting of the article, provision of study materials or patients, and collection and assembly of data: SK
Conception and design, analysis and interpretation of the data, critical revision of the article for important intellectual content, and statistical expertise: RG
Conception and design, analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article, provision of study materials or patients, and statistical expertise: SB

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Case Report

Hodgkin Lymphoma Mimicking a Large Soft Tissue Sarcoma of The Shoulder: The Essential Role of Immunohistochemistry in Histopathological Diagnosis

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Abstract

The shoulder and axillary regions contain various complex anatomical structures in close proximity, many of which can give rise to neoplasms. Determining the origin and hence the exact diagnosis of advanced (diffuse) tumours in this region may become problematic. In view of the tumour morphology and the affected location in this case, we highlighted the importance of Hodgkin lymphoma immunohistochemistry interpretation in a tumour which was initially suspected to be a soft tissue sarcoma.

Keywords: *hodgkin, immunohistochemistry, sarcoma*

Introduction

Immunohistochemistry (IHC) is a collective term used for various methods utilized to identify tissue constituents as antigens by means of corresponding colour-tagged antibodies. The result enables pathologists to recognize the tissue cells origin. In addition, it may specify cells' function in vivo if one poses the right questions by means of selecting the correct antibodies.

The complex anatomy of the shoulder and axillary regions results in the presence of numerous tissue types located in close proximity. When a diffuse neoplasm arises in this area, there may be difficulty in concluding both the clinical and histopathological diagnosis.

Soft tissue sarcomas and lymphomas are among the malignant neoplasms which can be seen in these regions. Differentiation between these tumour types is based on clinical findings, radiological features, and histopathology.

Hodgkin lymphoma (HL) most often occurs at latero-cervical region (75%), followed by mediastinal, axillary, and para aortic region. Peripheral extranodal involvement is very rare (1). While the onset of HL is typically nodal, it can secondarily affect extranodal tissue and surrounding organs. This presentation may mimic a soft tissue sarcoma (1). It typically displays a bimodal age distribution with the first peak occurring at 10 to 35 years of age (1). The site of involvement and the age at presentation generally pose a suspicion of HL.

The histopathological distinction between lymphoma and soft tissue sarcoma is routinely determined by IHC. Leucocyte common antigen (LCA) (lymphoid marker) and vimentin (mesenchymal marker) are commonly used for this purpose. However, in the case of HL, the malignant cells display a peculiar IHC pattern.

Non-Hodgkin lymphomas (NHL) are classically composed of a clonal monomorphic population of neoplastic lymphoid cells. This appearance is quite different to HL which comprises of a minority of neoplastic cells in a majority background reactive inflammatory cells. The diagnosis of HL is primarily based on the identification of characteristic multinucleated giant neoplastic cells within an inflammatory milieu. These cells are called Reed-Sternberg (RS)

cells or Hodgkin cells in their mononuclear forms. The RS cells and their variants only constitute between 1% to 10% of the entire cell population. The IHC patterns displayed by HL are quite different to that of NHL. Unlike most lymphoid malignancies, RS cells and Hodgkin cells are not commonly reactive towards LCA. However, they are reactive towards vimentin which is a feature commonly seen in neoplasm of mesenchymal origin especially most soft tissue sarcoma.

In this report, we highlighted the important role of IHC in the diagnosis of HL which occurred in the shoulder region, an area predominantly occupied by soft tissue.

Case report

A 35-year-old man presented with a diffuse right shoulder and anterior chest mass. It had been present for the past one year with progressive enlargement over recent months and was associated with occasional mild pain. On physical examination, the mass was poorly defined with a smooth outer surface (Figure 1). The mass was warm with prominent dilated cutaneous veins and was fixed to the underlying structures. He suffered significant limitation of shoulder movement. Clinically, a soft tissue sarcoma infiltration was suspected.

MRI revealed a diffuse infiltrative tumour mass involving right shoulder musculatures (rotator cuff, deltoid, pectoralis, trapezius, and rhomboids muscles) (Figure 2a). The right subclavian vessels were encased by the tumor with extension along the brachial plexus. Other findings include erosion to the adjacent scapula, right humerus medullary involvement, and multiple lymphadenopathy (cervical, axillary, and mediastinal). During admission, the patient's condition was complicated by recurrent symptomatic pleural effusions which required several pleural aspirations.

Figure 1: The arrows pointed to a large and diffuse swelling involving the right shoulder and the anterior chest wall. The swelling displayed smooth outer surface with ill-defined outer margin. Congested cutaneous veins were evident on the outer skin.

Biopsy of the right shoulder lesion showed scattered atypically large mononuclear and pleomorphic neoplastic cells with an inflammatory background (Figure 2b). Initial IHC showed neoplastic cells reactivity towards vimentin but non-reactive towards LCA (Figure 3). Further IHC markers showed the neoplastic cells were reactive towards CD30 and CD15 (Figure 3). The cells were however, non-reactive towards anaplastic lymphoma kinase (ALK) protein. The final diagnosis of Mixed cellularity Hodgkin lymphoma (MCHL) variant was made. The neoplastic cells were also noted to be non-reactive towards cytokeratin, cytokeratin 7, epithelial membrane antigen (EMA), smooth muscle actin, desmin, HMB45 (melanoma marker), CD31 (vascular marker), CD34 (fibrohistiocytic marker), CD1a (dendritic cell marker), CD_3 (T cell marker), and CD_{20} (B cell marker).

Discussion

Classical HL composes 95% of all HL cases (1). In general, classical HL may start unifocally by affecting a single lymph node or a single group of lymph nodes. It commonly spreads by contiguity as seen in this case with cervical, axillary, and mediastinal lymphadenopathy. It can also exhibit focal involvement within a lymph node (2).

About 15 to 25% of classical HL is MCHL. This histotype commonly affects the whole or large areas of lymph nodes with disruption of the normal lymph node architecture (2). The invasion process is typically diffuse rather than nodular (2). It is possible that in this case the tumour had totally destroyed the underlying lymphoid tissue and invaded the surrounding soft tissue. This pattern of invasion may closely mimic sarcomatous infiltration. At this region, Meterissian et al. noted various sarcoma subtypes including malignant peripheral nerve sheath tumour, desmoids tumour, malignant fibrous histiocytoma, myxoid liposarcoma, chondrosarcoma, and fibrosarcoma (3).

The patient's age at presentation was a clinical pointer in arriving at the final diagnosis. HL demonstrates bimodal age distribution curve with the first peaks at 15 and 34 years and second

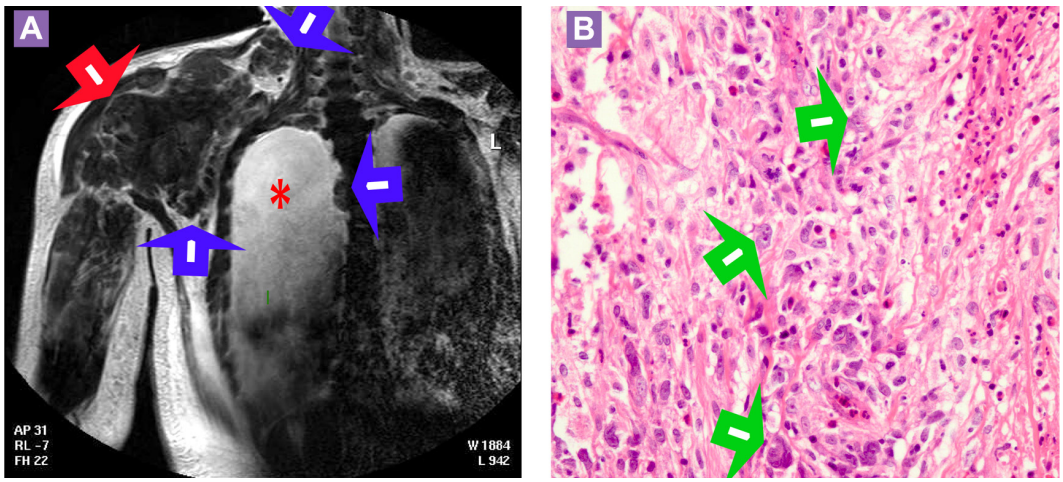

Figure 2: (A) Coronal T2-weighted MR image shows a large diffuse soft tissue mass involving the right shoulder musculatures (red arrow). The mass was associated with marrow involvement of the adjacent right humerus (not shown) and multiple cervical, mediastinal, and axillary lymph nodes (blue arrows). There is also thoracic involvement with presence of pleural effusion (asterisk) and multiple lung nodules on chest radiograph (not shown). (B) Microscopic picture of the tumour mass biopsy. The green arrows point to Hodgkin cells scattered amongst mixed population of inflammatory cells background. The neoplastic cells are mainly mononuclear plump and large with moderate amount of cytoplasm. Nuclear pleomorphism, irregular nuclear membrane, and prominent single large nucleolus are noted. No typical Reed-Sternberg cells noted. The inflammatory cells include lymphocytes, eosinophils, and neutrophils. The pleomorphic nature of the neoplastic cells may mimic pleomorphic sarcoma cells (H & E 40× magnification).

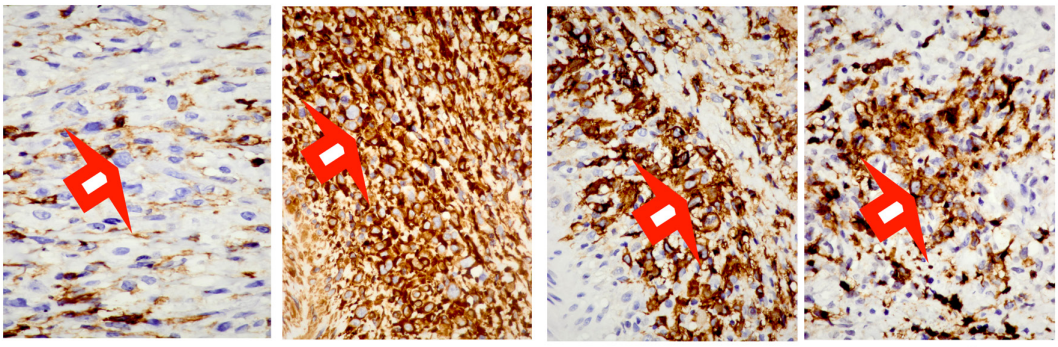

Figure 3: Immunohistochemistry staining on the neoplastic cells. The arrows points to the Hodgkin cells which lacked of LCA staining despite the lymphoid nature of the cells. Strong reactivity of the Hodgkin cells together with the underlying connective tissue for vimentin was observed even though the Hodgkin cells were non-mesenchymal in origin. CD30 and CD15 immunohistochemistry staining on the Hodgkin cells showed typical membraneous and paranuclear globules positivity pattern. CD30 and CD15 reactivity with lack of ALK protein positivity (not shown) pointed to Hodgkin lymphoma diagnosis.

peaks at 54 years (2). In this case the patient's age together with the site of involvement were consistent with the diagnosis of MCHL. Most soft tissue sarcomas occur in patients of older age group.

The MRI findings were consistent with reported features of skeletal muscle lymphoma infiltration. These features include muscle enlargement and mass formation, long segmental involvement along muscle fascicles, intramuscular traversing vessels, intermediate signal intensity on T2-weighted image, and diffuse infiltrative nature involving multiple muscle groups (4). The striking feature of this tumour mass was the hypointensity on T2-weighted images. T2 hypointensity can be seen in fibrosarcoma and lymphoma due to their fibrotic component and hypercellularity. The associated lymphadenopathy which is rarely seen in soft tissue sarcoma favours lymphoma as the diagnosis.

The presence of RS cells and Hodgkin cells is considered a sine qua non for the diagnosis of HL (2). These cells however, may show morphological variations and some cells may demonstrate similarity to them in some unrelated conditions. These conditions include infectious mononucleosis, toxoplasmosis, post-vaccinial lymphadenitis, and some neoplasms (2). Thus, the presence of an appropriate cellular inflammatory background and the IHC pattern are the basis for the diagnosis.

The CD45 clusters of antibodies recognized a family of protein expressed on the surface of almost all hematolymphoid cells called LCA (1,5). In classical HL, immunoreactivity towards LCA is rare (5). On the other hand, vimentin expression has been traditionally accepted as specific for cells derived from mesenchymal origin. The Hodgkin cells immunoreactivity towards vimentin may mistakenly lead to the diagnosis of a sarcoma infiltration. In our case, the Hodgkin cells were also noted to be non-reactive towards B and T cell markers.

With respect to the mixed inflammatory cells background, other neoplastic and inflammatory conditions may exhibit features that closely mimic HL. Inflammatory fibrosarcoma, anaplastic large cell lymphoma, T cell/histiocyte rich large B cell lymphoma, primary mediastinal large B cell lymphoma, Lennert's lymphoma (peripheral T cell lymphoma), chronic granulomatous inflammation, and chronic mediastinitis may cause diagnostic problem (1). This problem is further aggravated if the Hodgkin cells are predominantly of mononuclear variant rather than the classical mirror-image RS cells. Nevertheless, with strict morphological and IHC criteria for recognition of Hodgkin cells, this problem can be avoided (2).

CD30 and CD15 are not entirely specific for RS or Hodgkin cells. Expression of CD30 and CD15 molecules by Hodgkin cells are seen in more than 98% and about 80% of all classical HL (1). CD30 helps to identify Hodgkin cells by its distinctive IHC staining pattern. The typical positive staining pattern is membranous with strong paranuclear globule in the region of Golgi together with weak cytoplasmic staining (5). Cytoplasmic staining alone may be spurious as it can be seen in several non-hematolymphoid neoplasms. This should not be considered as positive staining. One should be aware of other CD30-positive neoplasms such as anaplastic large cell lymphoma, immunoblastic lymphoma, mycosis fungoides, some peripheral T cell lymphoma, plasmacytoma, Langerhans cell histiocytosis, and embryonal carcinoma (5). Anaplastic large cell lymphoma which may resemble HL is conversely reactive towards LCA, EMA, and ALK protein. CD15 staining is commonly non-reactive in this disease.

As in CD30, CD15 staining on Hodgkin cells also displays distinctive membrane and cytoplasmic staining with globular juxta-nuclear staining of Golgi. Almost all RS cells and mononuclear Hodgkin cells variants are reactive towards CD15. The characteristic CD15 positivity pattern is crucial in excluding other differential diagnoses. Other CD15 positive neoplasms include myeloid leukaemias, some low-grade B cell lymphoma, granulocytic sarcoma, and some carcinomas (5).

The evaluation of HL may be further aided by other IHC markers. These markers include TNF receptor-associated factor 1 (TRAF1) protein, fascin (a marker for dendritic cells), and EBV infection membrane protein 1 (LMP1). HL is likely to be EBV-positive in very young patients, very old patients, and HIV infected patients (2). These markers were not used in our setting due to practical constraints.

The overlapping immunoreactivity features in IHC necessitate utmost consideration in every histopathological diagnosis. Several neoplasms may show peculiar clinical and histomorphoplogical pattern regardless the cell-lineage origin of the tissue. The definitive diagnosis must take into account the clinical presentation and correlations with the radiological imaging features.

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Conception and design, drafting of the article, and critical revision of the article for important intellectual content: ZAI
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Case Report

Spontaneous Reversibility of an Iatrogenic Orthodontic Elastic Band-induced Localized Periodontitis Following Surgical Intervention – Case Report

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Abstract

Orthodontic elastic bands are an important iatrogenic etiologic factor in the causation of periodontal attachment apparatus breakdown. Appropriate diagnosis and a well constructed treatment plan tailor-made to suit the requirements of the particular patient is imperative for management of periodontal lesions induced by subgingival retention of rubber band. There are conflicting reports regarding the reattachment and regeneration of lost periodontal supporting tissues in such cases. The present case report highlights the spontaneous reversal and correction of periodontal destruction due to iatrogenic orthodontic elastic band displacement deep into the subgingival tissues.

Keywords: *corrective orthodontics, periodontitis, remission, spontaneous*

Introduction

Orthodontic elastic bands are non-metallic compounds of foreign body type (1), implicated in the production of localised periodontitis when inadvertently allowed to remain around teeth (2). Reports citing orthodontic elastic separators as a major iatrogenic cause for loss of periodontal bone support dates back to more than 127 years (3). Several cases have demonstrated the apical slippage of orthodontic elastic bands along the roots resulting in deep periodontal pockets, marked tooth mobility and severe bone loss, placing the teeth in serious jeopardy (2,4). The phenomena of gingival reattachment and possible regeneration are major issues in case of elastic band-induced periodontitis. A review of literature revealed a total of only five reports to date, concerning nine molars out of which five mandibular molars were treated, and only three of them showed promising results and superior periodontal health after treatment (2,3,5–7). The duration of periodontitis ranged from 10 days to one year in all these cases (8).

This case report highlights the spontaneous correction of localised periodontitis involving a mandibular molar, initiated by inadvertent displacement of an orthodontic elastic band deep into the subgingival area.

Case Report

A 20-year-old female patient reported to the Department of Periodontology and Oral Implantology with a chief complaint of swelling of gums in relation to the lower right back tooth region and associated dull, aching nature of pain in the same region since two days. A detailed case history was recorded. The dental history revealed that the patient had undergone oral prophylaxis and orthodontic therapy had been initiated one week back. The patient's orthodontist was

contacted and information was obtained regarding placement of orthodontic elastic band separators in relation to all the first molar regions. The medical and family history was non-contributory.

On clinical intraoral examination, the oral hygiene status of the patient was satisfactory with an oral hygiene score of 0.6 according to the oral hygiene index by Greene and Vermilion (9). A gingival swelling was detected on the gingiva over the lateral aspect of the root surface of the mandibular right first molar with an associated deep periodontal pocket of 8 mm on the disto-buccal aspect as measured using a William's graduated periodontal probe (Hu-Friedy, US). The six point probing depth recordings revealed a pocket depth of 5 mm on the midbuccal, distolingual aspects and 3 mm on the mesio-buccal, mid lingual and mesio-lingual aspects of the mandibular right first molar. The six point probing depths on the mandibular right second molar were 6 mm on the mesio-buccal aspect and 3 mm on the mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual aspects. The six-point pocket depth recordings of all the other teeth revealed a normal gingival sulcus depth of 2–3 mm. Purulent exudates and severe bleeding on probing was present in the region associated with the abscess with a Muhlemann and Son sulcus bleeding index (10) recordings of scores 2 and 1 for mandibular right first and second molars respectively. There was an absence of bleeding on probing around the remaining teeth. Vitality test provided negative result and confirmed the absence of pulpal involvement. The gingiva in all the other areas appeared clinically healthy with no detectable local deposits. Exploration of the deep pocket with an explorer (#23 Shepherd's Hook explorer, Hu-Friedy, US) failed to reveal the presence of subgingival calculus or foreign bodies. The orthodontic band was also missing in that region. The apical displacement of the orthodontic band deep into the subgingival area was suspected as a possible etiological factor for the abscess formation and associated pain in that localized region. Intraoral periapical radiographic evaluation of the mandibular right first and second molar region revealed horizontal bone loss extending up to the middle one third of the roots in the interdental area between the two teeth (Figure 1). Since the orthodontic bands are radiolucent, it was not discernible radiographically. A provisional diagnosis of iatrogenic orthodontic elastic band-induced periodontal abscess and localized periodontitis in relation to 46 was made. Since, the elastic band lacked radiopacity and was not readily detectable with an explorer, the presence, although suspected, could not be proven without a surgical exploration. The procedure was explained in detail to the patient and a written informed consent was obtained from the patient.

Under 2% lignocaine hydrochloride local anaesthesia (LOX, Neon Laboratories LTD, Andheri, Mumbai, India) containing 1:200 000 adrenaline, incision and drainage of periodontal abscess was established and the patient was placed on an antibiotic and analgesic regimen of Amoxicillin 500 mg and diclofenac respectively, for 8 days. When the patient reported for the next appointment the following week, the periodontal abscess had completely subsided but the pain persisted in that region. In order to establish a definitive diagnosis and effectively treat the lesion, an access flap surgical procedure was decided upon.

The surgical site in the mandibular right quadrant was anesthetized by 2% lignocaine hydrochloride (LOX, Neon Laboratories LTD; Mumbai, IN) with 1:200 000 adrenaline. Using crevicular and interdental incisions a localized mucoperiosteal flap, extending from the distal aspect of mandibular right second premolar to the distal aspect of mandibular second molar region. A thorough debridement and curetting was performed and the elastic band was finally retrieved from the depth of the pocket, close to the alveolar bone (Figure 2). The area was irrigated with normal saline and carefully inspected to ensure complete removal of the foreign body and other irritants. Flap was sutured in position with interrupted sutures, using non-resorbable 3–0 silk (CENTENIAL Surgical Suture LTD, Thane, IN). Postoperative instructions were given and the patient was instructed in using chlorhexidine gluconate mouthrinse 0.12% (Peridex, Zila Pharmaceuticals, Phoenix, US).

One week post surgery, the suture removal was performed and the patient reported to be completely free of pain and discomfort. A clinical examination at this visit revealed a clinically healthy gingiva and an intraoral periapical radiograph obtained showed complete bone fill and restoration of the bone height to normal healthy levels (Figure 3). The patient was referred back to the Department of Orthodontics and Dentofacial Orthopedics for continuation of the necessary orthodontic treatment. The patient has been put on regular recall regime to review the gingival and periodontal health status.

Figure 1: Pre-operative intraoral periapical radiograph of the mandibular right first and second molar region showing horizontal bone loss extending up to middle one thirds of the roots of the teeth.

Figure 2: Surgical exposure of the elastic band retrieved from the depth of the pocket.

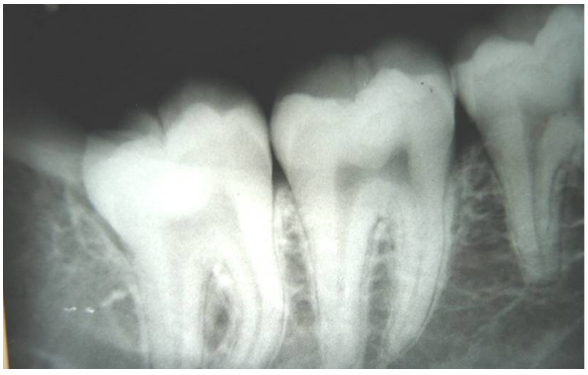

Figure 3: Post-operative intraoral periapical radiograph of the mandibular right first and second molar region showing complete bone fill and restoration of the bone height to normal healthy level.

Discussion

The findings of previous studies have painted a mixed picture. According to one study, the the elastic band-induced periodontal lesion is not spontaneously reversible and complete regeneration of periodontal attachment apparatus is not probable (12). However, another study demonstrated the possibility of achieving complete regeneration of the lost bone upon removal of the offending foreign body (11). Also, another case report involving maxillary central incisors published that a complete reattachment was achieved in one week following treatment and removal of the apically displaced multiple orthodontic bands (8).

A literature search has revealed a total of 22 case reports to date, of periodontitis associated with accidental apical displacement of orthodontic elastic band over the past 127 years and the duration of periodontitis extended between 10 days to one year, hence confirming the possibility of spontaneous reversal of periodontal lesions (8,11). Compared to the case reports of occurrence of iatrogenic elastic band induced periodontal lesion in the anterior region, only a negligible number of cases have been reported of the similar conditions in the mandibular molar area, making this a rather rare occurrence. Also, in the past, various non-surgical as well as surgical treatment modalities have been attempted for the management of these lesions, however, the best treatment protocol is not yet found in the literature (8).

The radiolucency seen in the pre-operative radiograph can be confirmed to be the result of alveolar bone destruction in that region due to foreign body reaction and not mere presence of the displaced band as the orthodontic bands in all the cases reported till date were discernible radiographically (1–8). Only one case report identified the presence of orthodontic elastic band in the radiograph as the authors of that case reported that the band appeared as a radio-opaque, rectangular-shaped mass in the interproximal region indistinguishable from subgingival calculus (13).

Conclusion

The results of the present study indicate that the lost alveolar bone can be completely regenerated within a short span of time, upon absolute removal of the aberrant foreign body and by adopting appropriate treatment modalities thus making the iatrogenic orthodontic elastic

band–induced localized periodontitis lesion a spontaneously reversible one. Locating orthodontic elastic bands that have been displaced deep into the supporting periodontal tissues can be an arduous task. Attention to detail, careful monitoring, discriminate use and patient education are the best deterrents to damage caused by orthodontic elastics (4). The development and manufacture of orthodontic elastics that are radiopaque would definitely minimize the incidence and severity of such cases.

Authors' Contribution

Conception and design, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, and collection and assembly of data: NS
Conception and design, analysis and interpretation of the data, drafting of the article, final approval of the article, and obtaining of funding: NSK
Critical revision of the article for important intellectual content, provision of study materials or patients, and administrative, technical, or logistic support: KKK
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Case Report

Penile Augmentation with Resultant Foreign Material Granuloma and Sequalae

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Abstract

Throughout history, a proportion of men appear to correlate penis size and dimensions directly with physical fitness and sexual prowess. Foreign materials, such as paraffin oil, paraffin balm, mineral oils, and silicone, have been used to promise an improvement in penile shaft contour and dimensions. These materials are injected directly into the penis; inducing granuloma formation to achieve increased penis length and girth. However, the result is a severely disfigured and swollen penis, which cannot achieve erection. Local complications of penile lipogranuloma include infection, ulceration, local migration, and cavernosal invasion; leading to functional impairment. Meanwhile, systemic complications include foreign body embolization, organ infarct, and death. Penile lipogranuloma is best treated surgically. Granulomatous skin needs to be completely excised; wound closure with a scrotal skin flap, Cecil's inlay operation and split thickness skin graft commonly used options. Our case series has shown that penile lipogranuloma, induced by subcutaneous foreign body injections into the penile shaft, and its subsequent adverse outcomes to patients and their partners.

Keywords: *augmentation, foreign body reaction, granuloma, silicone, penis*

Introduction

Throughout history a proportion of man appear to correlate penis size and dimensions directly with physical fitness and sexual prowess. Foreign materials, such as paraffin oil, paraffin balm, mineral oils, and silicone, have been used to improve penile shaft contour and dimensions. Various dubious establishments offer treatments where foreign material is injected directly into the penis to achieve an increase in length and girth. Our case series has shown that this results in adverse outcomes to patients and their partners.

Case Report

Three men aged between 32 to 59-years-old were given subcutaneous foreign body injections into their penile shafts by non-medical practitioners (Table 1). Two were given silicone and one was given paraffin oil. They volunteered to take the injections in a non-sterile environment, with their motivation being a larger penile size. They denied influence from their sexual partners. They began to notice abnormal reactions to their penises, about 14 months after the injection. Over this time, their penile shafts gradually became deformed, swollen, and hard. Each patient was able to achieve an erection. However, erections and sexual intercourse were painful to both the patient and their partner, due to the irregular hard lumps around the penile shaft. In all cases, treatment was sought between four to six years after the injection.

Physical examinations showed similar changes for all three patient's penises. The penile shafts were swollen and disfigured, with irregular semi-mobile masses extending into the scrotum (Figure 1). Masses did not involve the corpus cavernosum and both testes. The overlying skin was diffusely dark with no cutaneous ulceration.

After full history taking and physical examination, followed by patient counselling, the decision was made to proceed with surgical excision and reconstruction. Full skin excision was required in these patients, because the granulomas were extensive; involving subcutaneous tissue of the penile shaft with extension into the

overlying penile skin. The different approaches available, including immediate skin excision or replacement, as well as, trial at skin preservation, will be discussed below. The fibrotic skin and subcutaneous tissue were circumferentially excised from the corona distally to the scrotum proximally down to the level of Buck's fascia. Thick split skin grafts, harvested from the inner thigh, were used to cover the denuded penile shaft in all three cases. All three grafts were minimally fenestrated to prevent hematoma formation under the grafts.

All three patients were discharged five to seven days post-operatively, with full graft take. Wounds were fully healed at the outpatient review, one month post-operatively (Figure 2). All three patients were able to achieve full erection with normal sexual intercourse with their partners after the operation. Histopathological examination results were consistent with lipogranuloma; where two were induced by silicone and one by paraffin oil.

Discussion

Mineral oil injections, used to enlarge penis contours, were popular in early 1900. Currently, penile augmentations through foreign material injections are still practice worldwide by non-medical practitioners; despite the severe destructive effects that have been recognized and described above. Local complications include infection, ulceration, local migration, and cavernosal invasion; which usually leads to functional impairment. Systemic complications include foreign body embolization, organ infarction, and death. Rollins et al. reported a sudden death case, secondary to disseminated lipogranulomas to the lung, causing acute severe pulmonary oedema (1).

Granuloma formation is caused by a natural host response to walled off exogenous substances with multinucleated giant cell and chronic inflammatory cells. However, they are too large to be broken down naturally. Immunological and

Table 1: Patient Demographics

Patient	Age	Foreign material used	Initial indication	Occupation	Location of procedure	Time of presentation
1	32	Silicone	Group trial	Construction workers	Friend's house	5 years
2	46	Silicone	Own initiative	Businessman	Hotel	5 years
3	59	Parafine Oil	Own initiative	Government servant	Hotel	4 years

Figure 1: Pre-operative image showing grossly swollen, disfigured penis with masses. The overlying skin w as diffusely dark.

Figure 2: Post-operative image showing the skin graft was fully taken and healthy. The penis appeared grossly normal.

inflammation reaction, induced by silicone and paraffin oil, are similar; as shown in our case series. Granulomatous and fibrotic reactions occur in the subcutaneous fat from the injection of silicone or mineral oil. This is known as lipogranulomas, due to the recipient tissue involved. Physically, the lipogranulomas appears as firm, disfiguring subcutaneous masses, with surrounding skin fibrosis, and thickening. The patients were told that this expected augmentation would be a result of immunological reaction. However, the actual outcome was disastrous, resulting in a severely disfigured and swollen penis, which was unable to achieve erection.

The main aim of treatment is to restore penis function, as a conduit for bodily fluids and as a sexual organ; with an acceptable cosmetic appearance. The medical treatment of sclerosing lipogranuloma involves the use of antibiotics and topical and/or systemic steroids. Corticosteroid therapy is effective in treating primary sclerosing lipogranuloma (2). However, for foreign body induced lipogranuloma, no corticosteroid treated cases have been reported.

Penile lipogranuloma is best treated surgically. Granulomatous skin needs to be completely excised. Primary closure offers the best cosmetic and functional outcome, but is usually impossible, due to the large defect size. Wound closure using a scrotal skin flap, Cecil's inlay operation, and a Split thickness Skin Graft (SSG), are the more commonly used options (3). Penile split thickness skin grafts demonstrate good graft survival and remains the most successful management technique for a denuded penis (4). Our series showed excellent reconstructive outcomes using SSG. Lee et al described using a scrotal skin flap supplied by posterior branch of internal pudendal artery for wound closure in their series of 19 patients; of which 17 patients were treated successfully (3). If the granulomas are not extensive, granulomas mass may be excised via circumferential subcoronal incision with skin preservation; which was demonstrated by Shaeer et al., (5). This technique is not possible for our patients, because the granulomas extended to the penile skin circumferentially. Any remaining skin (following degloving) would be at a high risk of being compromised vascularity; leading to eventual necrosis.

Penile augmentation using foreign body injections can be a misleading concept that has been capitalised by unethical practises. From case series reported worldwide, the majority of foreign body injections were performed by non-specialist personnel. For this reason, we should increase public awareness on the detrimental effects of such injections. In an ideal world, strict legal action would limit unethical procedures taking place, but this calls for continuous widespread education and enforcement from various organisations.

Authors' Contribution

Conception and design, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, and collection and assembly of data: FXE
Conception and design, analysis and interpretation of the data, drafting of the article, final approval of the article, and obtaining of funding: FHI, RIH, ZMZ
Critical revision of the article for important intellectual content, provision of study materials or patients, and administrative, technical, or logistic support: FHI, RIH, ZMZ
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Case Report

Infant with a Sudden, Large, Post-Extubation Subglottic Cyst

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Abstract

Acquired subglottic cyst in infancy is almost always associated with episodes of early life intubation. Most cases typically presented late, usually days to months after extubation. We report a case of a subglottic cyst with different presentation than the norm. This case highlights that subglottic cyst can present acutely, and rapidly enlarging soon after the airway extubation. As the management of a large subglottic cyst can be challenging, a close observation for early diagnosis and intervention are recommended post extubation in the high-risk cases, such as in the premature infant.

Keywords: *subglottic cyst, acquired cyst, premature infant, endotracheal intubation, airway obstruction*

Introduction

Subglottic cyst is a rare cause of stridor in infants. The largest series of subglottic cyst to date is by Lim et al., who reported 55 cases (2.6%) from 2055 series of upper airway endoscopy (1). Agada et al. found only 7 cases of subglottic cysts (0.05%) in a large cohort of 12 240 premature newborn, while Watson et al. mentioned that subglottic cyst represents only 6.8% of upper airway obstruction in their laryngotracheobonchoscopy series (2,3).

Most of the reported cases presented late, usually days to months after the trauma. We report a unique case of an infant with a large subglottic cyst, presented differently from the norm, and highlight the importance of close observation following extubation in the high risk infant.

Case Report

A 4-month-old baby girl presented with stridor two hours after airway extubation. Earlier, she had assisted ventilation via an endotracheal intubation following the diagnosis of severe pneumonia. She had a size 3.0 non-cuffed endotracheal tube (ETT) inserted, and the intubation process was reportedly uneventful. She was ventilated for four days, and at one time on day one of ventilation in the pediatric intensive care unit, the tube had dislodged; and required re-intubation with similar sized ETT. The infant had a history of premature birth at 30 weeks, with a birth weight of 1.85 kg. Pregnancy, delivery, and immediate post-natal period prior to the admission were unremarkable.

The infant became progressively breathless and the stridor was worsened within the next 4 hours. Attempts to re-intubate the baby were failed, because of an unexplained resistance when passing the tube. The baby was immediately transferred to the operation theatre, where efforts to ventilate the airway via a masked ventilation or jet ventilation were neither successful at sustaining the blood oxygen saturation for a long period. A rigid endoscopy (0-degree Hopkin's rod telescope) of the larynx revealed a large subglottic cyst totally occluding the airway (Figure 1). Subsequently, a tracheostomy was performed in the emergency setting. A suspension laryngoscopy was performed, and the cyst was aspirated with a spinal needle to yield a clear mucoid fluid (Figure 2). The cyst wall was marsupialized with

a laryngeal micro scissor and a cupped forcep (Figure 3).

The patient was prescribed intravenous dexamethasone for three days. The infant was decannulated in the operation theatre 3 weeks later, after laryngeal endoscopy showed no evidence of cyst recurrence. A repeat endoscopy at 6 months and at one-year post-operatively revealed no recurrence of the subglottic cyst (Figure 4).

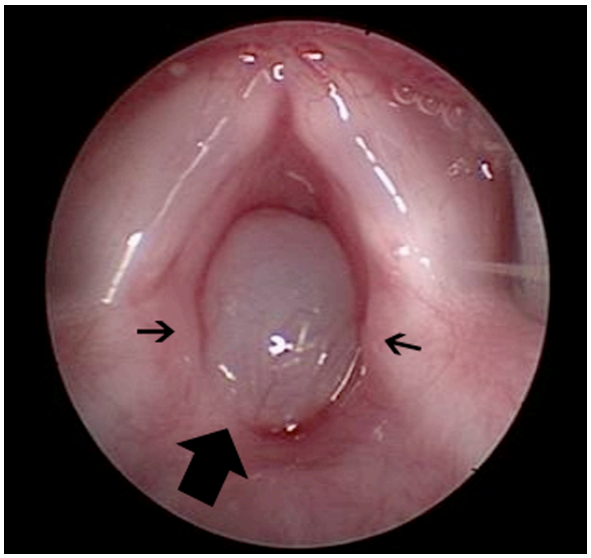

Figure 1: Endoscopic picture of the larynx showing a large subglottic cyst totally obstructing the airway. The broad-based stalk arised from the posterior wall of the left subglottis (thick arrow), just below the edematous vocal cords (thin arrow).

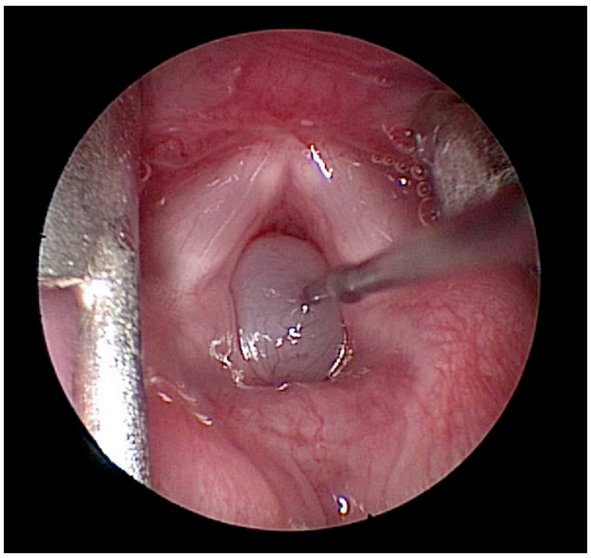

Figure 2: Endoscopic picture of the larynx showing the cyst was aspirated using a spinal needle.

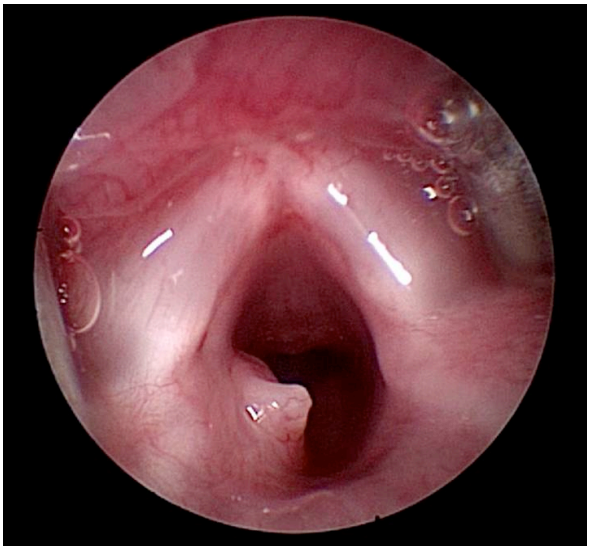

Figure 3: Endoscopic picture of the larynx after the cystic content was aspirated. The cyst wall was then excised using a microscissor and a cupped forcep.

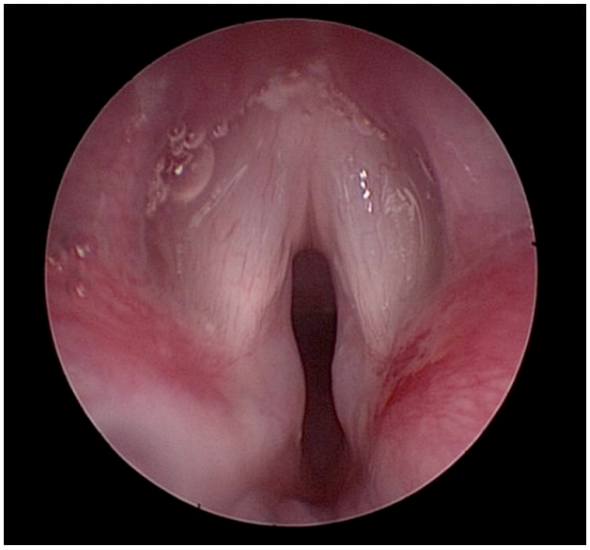

Figure 4: Endoscopic picture of the larynx at one year post-operation showing no recurrence at the subglottic region.

Discussion

Subglottic laryngeal cyst can be of congenital in origin, although it is difficult to establish if the cyst is acquired or congenital in the newborn because respiratory distress requires intubation generally without prior endoscopy. Acquired subglottic cyst is linked with prematurity and endotracheal intubation. A review of some of the largest series of subglottic cysts in the literature showed that this condition always resulted from endotracheal intubation (1–7). More than 93 percent of the cases developed in infants who were born prematurely (1,3,6). The duration of intubation does not appear to be a predictive risk factor, for these cysts can develop even after periods of intubation of less than 24 hours (6,7). Majority of the subglottic cysts, including our case, were found arising from the posterior wall of the subglottic larynx, because the area is more exposed to a contact injury from intubation.

In many of these reported series, the infants showed symptoms only after weeks to months of extubation (1,3,5,7). Our case provides unique evidence that the cyst can develop and rapidly growing following the iatrogenic injury to the mucosa in a shorter period of time. Subepithelial fibrosis as a result from intubation injury to the mucosa may obstruct the ducts of mucus glands leading to the formation of subglottic cysts (9). Traumatic intubation, multiple recurrent attempts intubation, and inappropriate size ETT used can lead to damage to the mucosa and subsequent cyst develop. The rate of cyst growth may be influenced by the severity of the injury and the abundance of mucus glands in the affected area.

Subglottic cyst is often treatable, but, if large enough can cause total airway obstruction and fatal cases have been reported (5,9). Management of the airway in infants with large subglottic cysts emphasizes on the challenges and anaesthetic considerations. In smaller lesions where the airway is partially compromised, the airway can usually be maintained by inhalational anaesthesia or jet ventilation while the airway was instrumented with a telescope by the ENT surgeon. The cyst can be decompressed by needle puncture and aspiration, and even in a very big cyst this should be the standard choice of treatment. Tracheostomy may be unavoidable in some cases, like ours, when the airway was not sustainable due to the large cyst and near total airway occlusion.

Successful management of a subglottic cyst involves early diagnosis and intervention when there is a clinical evidence of disease progression. Early intervention when the cyst is small would have avoided the need for a tracheostomy and decrease the morbidity in this case. Excision of the cysts will often be sufficient to correct the obstructed airway; however, the recurrence rate of 40% has been reported (1). Therefore, patients with subglottic cysts should be on a long-term follow up.

In conclusion, early diagnosis and intervention are vital to successfully manage a subglottic cyst. The key to early diagnosis is a strong index of suspicion; hence, we recommend a close observation period after extubation, in a child with a history of premature delivery.

Authors' Contribution

Conception and design, analysis, and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, and provision of study materials or patients: ZAA
Conception and design, analysis and interpretation of the data, final approval of the article, provision of study materials or patients: SY
Analysis and interpretation of the data, drafting of the article, final approval of the article, provision of study materials or patients: AF
Final approval of the article, provision of study materials or patients: MAZ

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Letter to Editor

Letters to the Editor

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Dear Editor,

This is in reference to the survey published in your esteemed journal titled 'The Prevalence of Cardiovascular Risk Factors in the Young and Middle-Aged Rural Population in Sarawak, Malaysia' (Malays J Med Sci. Apr-Jun 2012; 19(2): 27–34). It was an important survey identifying the factors associated with cardiovascular diseases.

I like to point to an issue. I find that persons with hypertension, myocardial infarction, or angina pectoris were excluded in the above survey. Thus, the persons surveyed will not be a representative sample of the population studied. This could be the reason for the lower prevalence of hypertension, smoking, hypercholesterolemia, and obesity. These results would be more dependent on the actual prevalence of the excluded section (from survey) of the population. An approach where in these 'excluded people' were also included in the survey and then analysed separately to find the prevalence of risk factors in non-diagnosed (hypertension, myocardial infarction, or angina pectoris) persons would have avoided this fallacy. Thus, the survey is compromised on internal validity due to exclusion bias (1).

Exclusion bias is a type of sampling bias which results from exclusion of particular groups leading to a non-representative sample. Horwitz et al. (2), as early as 1985 told the world on the importance of exclusion bias when he showed that an association between reserpine and breast cancer was due to the exclusion of women with cardiovascular disease among only controls and not cases.

Houle (3) observed that exclusion of certain groups like imprisoned inmates can substantially alter observed national disparities between race/ethnic and education groups. His research explored the effects of excluding inmates from US national obesity estimates based solely on the non-incarcerated population. Among younger men with less than a high school education, national obesity prevalence including inmates reduced estimates for non-Hispanic Whites and blacks. Estimates were not substantially altered for older men by including inmates due to the relatively small size of the older incarcerated population.

A reason given for exclusion in prevalence surveys is to get a better estimate. Thurnham and colleagues (4) proposed that in surveys that rely upon plasma or serum retinol concentrations to estimate the prevalence of vitamin A deficiency, excluding all individuals with elevated acute phase proteins improves the accuracy of the prevalence estimates due to the variation in serum retinol due to infections. However, Maqsood (5) et al., among children in Republic of the Marshall Islands found that the method of excluding individuals with elevated acute phase proteins from a survey of vitamin A deficiency results in sampling bias and a prevalence estimate that was based upon a very non representative population.

Exclusion bias is a serious type of sampling bias in observational research. It is important for public health agencies and researchers to consider omitted groups when understanding health outcomes and inequities (3).

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