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Letter To The Editor

Editorial

Coping with Brain Disorders using Neurotechnology

Pedro A Valdes-Sosa

Submitted: 7 Dec 2011
Accepted: 15 Dec 2011

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Abstract

Brain disorders account for more than 34% of the global burden of disease, crippling nations by decreasing their "mental capital"—with greater effect in developing countries. Early detection is the key to their management, but establishing such programmes seems nearly impossible due to the high prevalence of the dysfunctions as compared with the high cost of neuroimaging devices. Thus, at first sight, the research of the Decade of the Brain and the international Human Brain Mapping Project might seem to be condemned to benefit only a small elite. Cuba has shown that is not so by using neurotechnology for the last 3 decades to implement stratified active screening programmes for brain disorders at the population level. This experience has shown that, by the transformation of health indicators, an appropriate use of technology can be integrated with attention to the population at the primary levels of both health care and education. An essential component of neurotechnology is neuroinformatics, which—like its counterpart bioinformatics—combines databases, analysis tools, and theoretical models to craft tools for early disease diagnosis and management. Much work remains to be done and will depend critically on south–south cooperation to solve problems for countries with similar situations.

Keywords: *brain disorders, international cooperation, medical informatics, neuroimaging, neurosciences, technology*

Brain Disorders and the Mental Capital

There are many expectations with regard to the outcome of the Decade of the Brain (1). This is justly so, for not only is there the promise of solving the essential enigma of human consciousness, there is also hope of finding ways of dealing with the brain disorders, a grouping of dysfunctions that account for more than 34% of the global burden of disease measured in disability-adjusted life years (2). Consider the numbers in Figure 1 (3). These disorders cripple the development of nations, subtracting substantially from their mental capital (4)—a situation dramatically more critical in developing countries.

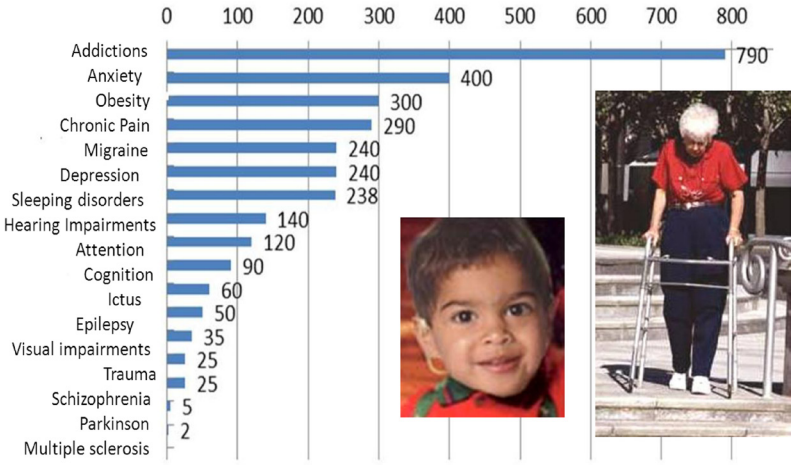

Figure 1: Brain disorders affect millions of people in the world, affecting the mental wealth of nations.

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Many look for solutions to these problems from research in the neurosciences, an effort that has reached an unprecedented level. Of note is the Human Brain Mapping Project—a gigantic exercise in systems neuroscience—that is assembling what is known of brain structure and function along the full span of levels from molecular genetics to behaviour. A critical feature is that all levels are linked by different types of neuroimages. Central to these endeavours is neuroinformatics: the use of shared databases, tools for image analysis, and theoretical modelling to develop, validate, and deploy instruments for early detection and optimal management of brain disease, as well as the support of clinical trials (Figure 2). An overview of this field can be found at the International Neuroinformatics Coordinating Facility website (5).

Current neuroscience research (including neuroinformatics) has even spawned a new industry, neurotechnology, that now rivals in growth and importance that of biotechnology. In spite of these encouraging developments, many express concern that this gigantic effort may fall short and not justify the expenditure involved. Similar misgivings have been voiced about the Human Genome Project (6). What is needed to deal with brain disorders, according to this view, is not more technology but rather more public health measures. These critics point to the high cost of neuroimaging devices and the impossibility of deploying them where most needed.

Cuba has had to deal with its own problems under tight economic constraints. This has led not to less but rather more neurotechnology—with a twist (7). This has been to insert the use of neurotechnology in a public health framework of stratified active screening of brain disorders. This approach uses the appropriate technology at each health level. A prime example of this has been the programme of early screening for hearing loss (Figure 3), initiated in 1983, which has already pushed the children detected with the problem 5 years of language development ahead of what was expected without screening (8). This is an increase of mental capital indeed! These programmes are now actively being extended to other Latin American countries.

Cuba considers that this model can be extended to other brain disorders (Figure 4) and is now embarked on projects for the early detection and appropriate management of neurodevelopmental disorders, learning disabilities, stroke, as well as several types of cognitive disorders. Conscious that combining research efforts and data is essential, our country has been active in promoting initiatives that group nations with similar problems, recent examples being the Latin American Brain Mapping Network (9) and the Chinese/Cuban Brainnettome Project.

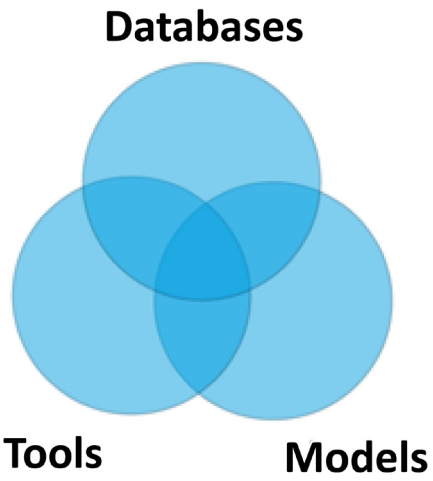

Figure 2: Neuroinformatics is the intersection of neuroimaging databases, tools for managing them, and theoretical modelling to interpret normal and pathological brain functions.

Figure 3: Cuba has used its own national neurotechnology industry to carry out stratified active screening, an example of which is the detection of hearing loss.

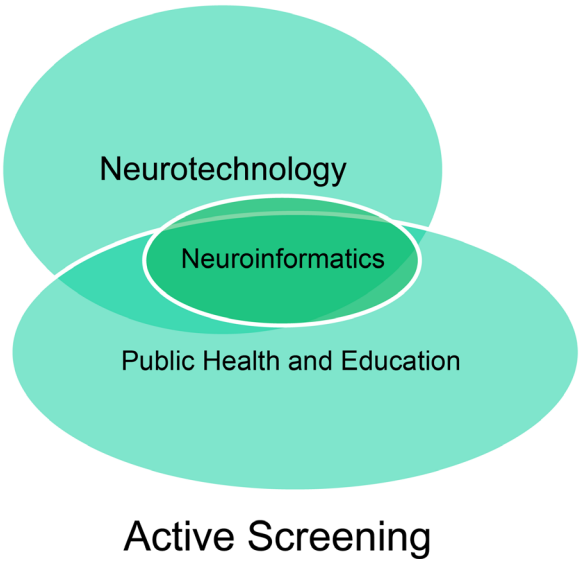

Figure 4: The active screening for brain disorders is based on the appropriate use of neurotechnology and neuroinformatics.

Malaysia is poised to play a major role in the translation of neuroscience research into health and education. It seeks to complement its recent establishment of a neuroimaging infrastructure with the development of neuroinformatics to allow the structured organisation of the imaging data gathered and its integration with other types of information from the country as well as the world. I was lucky to be able to give a recent course in neuroinformatics that was organised by Universiti Sains Malaysia. As a result, Malaysian and Cuban neuroscience groups have recently decided on a roadmap to join efforts in research, technology development, and medical evaluation geared towards collaboratively solving mental health problems of our countries and to establish links to other groups. We have a major role to play in transforming the mental health landscape of developing countries. This is an exciting time in which we who are engaged in neuroscience have the possibility and the responsibility of guaranteeing that the goal is reached.

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Review Article

The Impact of Nutrition Education Interventions on the Dietary Habits of College Students in Developed Nations: A Brief Review

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Submitted: 5 Jul 2011
Accepted: 29 Oct 2011

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Abstract

The purpose of this review is to provide a summary of studies on the effectiveness nutrition education interventions used by college students. Electronic databases such as Medline, Science Direct, CINAHL (EBSCO*host*), and Google Scholar were explored for articles that involved nutrition education interventions for college students and that were published between 1990 and 2011. Fourteen studies, which involved a total of 1668 college students as respondents, were identified and met the inclusion criteria. The results showed that there were 3 major forms of nutrition education interventions: web-based education, lectures, and supplement provisions. Dietary intake measures were used in almost all studies and were primarily collected with food records, recall, food frequency questionnaires, and dietary habit questionnaires. The outcome measures varied among the studies, with indicators such as consumption of food, nutrition knowledge, dietary habits, physical activity, and quality of life. Methodological issues were also identified. In general, college students experienced significant changes in their dietary habits after the interventions were employed. The highlighted methodological issues should be considered to improve the quality of similar research in future.

Keywords: *dietary habits, education, intervention studies, nutrition, programme effectiveness, young adult*

Introduction

College students between the ages of 18 and 24 years gain new experiences and personal freedom as well as develop a sense of identity as they ascend from adolescence to adulthood (1). Unfortunately, during this phase, the tendency to engage in unhealthy dieting, meal skipping, and fast food consumption is rather common. Minimal physical activity is also a norm (1). Poor eating habits and limited physical activity can likely increase the risk for osteoporosis, obesity, hyperlipidaemia, diabetes, and cancer later in life (1). Such an unhealthy lifestyle is further linked to health-related quality of life (HRQoL), which is related to an individual's nutritional status (2). All of these associations suggest that it is important to establish good eating habits at an early age (3).

Nutrition education is widely used for a range of population groups as a medium to deliver healthy diet and nutrition information; however, this type of intervention is still rarely implemented for college students. While there are various modes of nutrition education interventions, their effectiveness on eating habits remains unclear. This review thus intends to describe the impact of different nutrition education interventions on the dietary habits of college students by reviewing previous studies from developed nations.

Materials and Methods

Articles were identified through relevant databases (i.e., Medline, Science Direct, CINAHL [EBSCO*host*], and Google Scholar) from 1990 until 2011 using the following keywords: nutrition education, effectiveness, college/university students, and dietary habits.

The keyword-based screening strategy alone generated 52 articles, but only 14 met the specified inclusion criteria: 1) the participants were 18 to 25 years (college/university students), 2) the study design was cross-sectional, exploratory, longitudinal, or randomised controlled trials (RCT), and 3) they were available in full-text form. Studies published in languages other than English, reviews, and abstracts were excluded. The included studies were subsequently reviewed based on the study design, year, country, sample size, duration, type of nutrition intervention, and outcome measures. The selection method is summarised in Figure 1.

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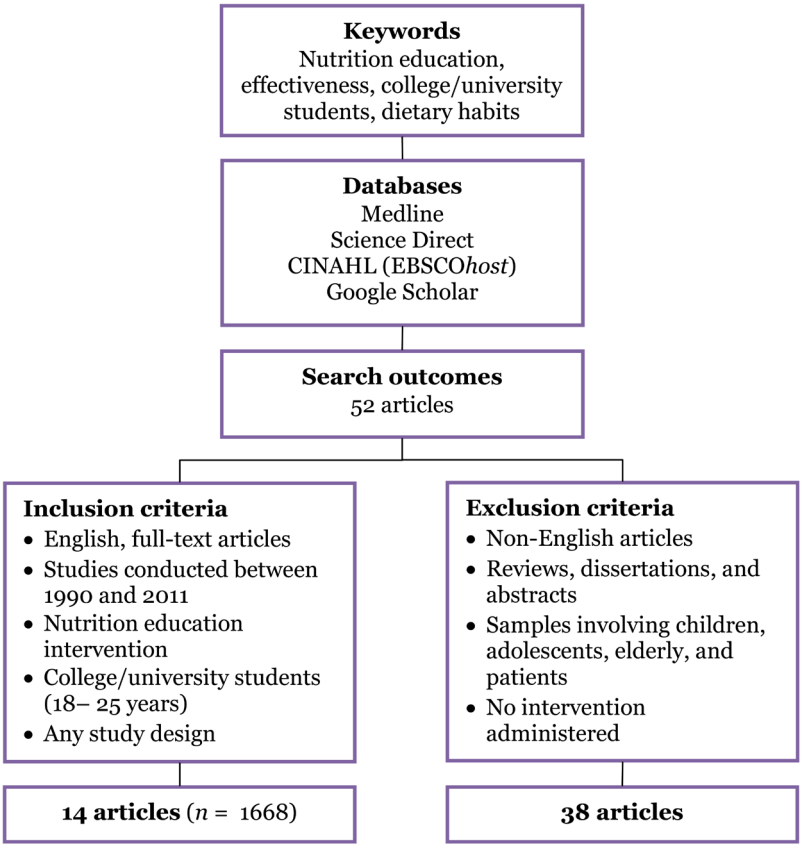

Figure 1: The process of article selection

Results

The 14 studies included 1668 participants (Table 1). These studies involved more female college students (n = 1113) than males (n = 555) and that many of the studies were conducted in the United States of America. Only 2 studies were conducted elsewhere: in Korea and in Israel.

Only 1 study was cross-sectional by design, 4 were RCTs, and 9 were longitudinal studies. Sample sizes varied greatly across the studies, ranging from 22 to 294 participants. Nine out of 14 studies reported the questionnaire validity and reliability. Survey feasibility was reported in 1 study, but the validity of other self-report measures was not indicated. The overall study duration ranged from 2 days to 3 years.

The modes of intervention also differed among the studies (Table 1). As the delivery mode, 3 studies used web-based education, 1 study provided dietary supplements, and the other studies used educational lectures. The methods of lecture differed: some studies used traditional lectures combined with hands-on activities, while others utilised debates on nutritional treatments and cooking classes. Only 2 studies employed social cognitive theory (SCT) as a theory-based intervention. In another study, sea tangle (20 g/day) was distributed as a supplement to a combination of diet therapy, exercise, and behavioural modification (4).

To measure dietary changes before and after the intervention, most studies used the food frequency questionnaire, 3-day dietary record, and 24-hour food recall questionnaire (Table 2). Data were analysed and presented as nutrient intake. In addition, dietary habit questionnaires were used, and the results showed that the total score significantly increased after an 8-week body weight control programme (4).

Only 1 study highlighted HRQoL issues in relation to nutrition education, which was assessed using the generic Short Form-36 (SF-36) (4). However, SF-36 is an instrument that has been widely used for population-based HRQoL rating (5).

Table 1: Studies using nutrition education as interventions for college students

1. Ha and Caine-Bish, 2011 (20)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** Longitudinal **Duration** 15 weeks **Sample** 80 college students	An interactive, introductory nutrition course focusing on disease prevention	• Increased whole-grain intake • A cost-effective way to modify eating habits	• Use of a convenience sample • No control group

2. Gow et al., 2010 (21)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** RCT **Duration** 3 months **Sample** 159 first year college students (CG = 40, OI = 40, FI = 39, CI = 40)	An online intervention to reduce adult obesity rates with 4 modalities: • no treatment (CG) • online intervention (OI) • weight and caloric feedback (FI) • combined feedback and online intervention (CI)	• CI group had lowest body mass index at post-test than the other groups • Online intervention for weight gain prevention was feasible and effective	• The use of self-report measures of diet and exercise is less ideal than direct measures

3. Poddar et al., 2010 (22)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** Experimental **Duration** 5 weeks **Sample** 294 college students (IG = 148, CG = 146)	A web-based intervention using an online course system (email messages, posted information, and behaviour checklists with tailored feedback)	• Increased self regulation and self-efficacy in consuming 3 servings/day of dairy products	• Short length of the intervention

4. Ha and Caine-Bish, 2009 (9)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** Longitudinal **Duration** 15 weeks **Sample** 80 college students	Class lectures covered nutrition knowledge related to prevention of chronic diseases, healthful dietary choices, increasing fruits and vegetables, promoting active lifestyles, and interactive hands-on activities	• Increased fruits and vegetables consumption	• More than half of respondents were female (88%), which may bias outcomes • No control group • No long-term effect of intervention explored • Heterogeneous group of students (i.e., different majors)

5. Ha et al., 2009 (8)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** Longitudinal **Duration** 15 weeks **Sample** 80 college students	A class-based nutrition intervention combined with traditional lectures, interactive hands-on activities, and dietary feedback	• Decreased soft drink consumption • Increased total milk and fat-free milk consumption • Decreased whole milk and low-fat milk consumption	• More than half of respondents are female (88%), which may bias outcomes • No control group • No long-term effect of intervention explored • Heterogeneous groups of students (i.e., different majors)

6. White et al., 2009 (3)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** Randomised and longitudinal **Duration** 3 years **Sample** 144 college students	• The Students Teaching Alcohol and Drug Responsibility peer health educators target alcohol- and drug-related topics • Healthy Eating and Living peer health educators target eating and nutrition topics • Sexual Health and Relationship peer health educators target sexual health topics	• Peer health education plays an important role in promoting healthy behaviours concerning alcohol and drug use, as well as eating and nutrition	• No control group • Long study duration

7. You et al., 2009 (4)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
Korea **Design** Longitudinal **Duration** 8 weeks **Sample** 22 Korean female college students	Nutrition education (diet therapy, exercise, and behavioural modification) and supplementation (sea tangle)	• Significant reductions in body weight, body fat mass, percentage of body fat, waist-hip ratio, and body mass index	• No control group (i.e., without supplementation) • Small sample size

8. Franko et al., 2008 (1)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** RCT **Duration** 6 months **Sample** College students from 6 universities in the States (Experimental I = 139, Experimental II = 148)	MyStudentBody.com-Nutrition (MSB-N) Internet-based nutrition and physical activity education program	• Effective Internet based nutrition education for promoting changes in health behaviours	

9. Endevelt et al., 2006 (11)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
Israel **Design** Cross-sectional **Duration** 10-hour nutrition workshop (2 days) in 2003 and 2004 **Sample** 122 second-year medical students (1st and 2nd class)	Four topics: • nutritional policy • dietary assessment • nutritional recommendations • obesity Method: dietary intake interviews, debates regarding nutritional treatments, and in-class activities	• It appeared to be beneficial to work with the students on personal issues as a way to enhance their nutritional experiences	• No nutrition knowledge test before and after workshop

10. Abood et al., 2004 (23)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** Longitudinal **Duration** 8 weeks **Sample** 30 college female athletes (IG = 15, CG = 15)	Focused on nutrition knowledge, self-efficacy in making healthful dietary choices, and dietary practices to demonstrate treatment effect Social Cognitive Theory in 8 educational sessions (1 hour per session)	• Significant improvement in nutrition knowledge, self-efficacy and the overall number of positive dietary changes	• Small sample size

11. Levy and Auld, 2004 (24)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** Exploratory **Duration** 3 months **Sample** 65 first-semester college students (IG = 33, CG = 32)	Demonstration versus hands-on cooking classes	• Significant improvement in attitudes and cooking related knowledge and behaviours • Cooking classes can be an effective tool for improving participants' attitudes, behaviours and knowledge regarding cooking	• Small sample size • An effective cost-saving strategy to enhance attitudes and knowledge

12. Matvienko et al., 2001 (25)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** RCT **Duration** 16 months **Sample** 40 first-year female college students (IG = 21, CG = 19)	College course composed of both lectures and laboratory exercises	• Weight gain was prevented for at-risk college students	• Small sample size

13. Winzelberg et al., 2000 (26)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** RCT **Duration** 3 months **Sample** 43 female students (IG = 23, CG = 20)	Internet-based, computer-assisted health education programme	• This programme improved women's body satisfaction	• Small sample size • No long-term evaluation of effects

14. Aaron et al., 1995 (27)			
Country	**Intervention**	**Result/conclusion(s)**	**Comment(s)**
United States **Design** Experimental **Duration** 2 weeks **Sample** 90 college students (IG = 65, CG = 25)	Provision of nutrient intake information at lunch at college	• IG respondents had significantly increased total energy, fat, and carbohydrates, but decreased protein intake and protein-based energy over time	• Short duration of intervention exposure

Abbreviation: RCT = randomised controlled trial, IG = intervention group, CG = control group

Table 2: Measurement instruments and corresponding outcomes

No.	Authors	Measurement instrument	Outcomes
1.	Ha and Caine-Bish (20)	• Anthropometry • 3-day dietary records (2 weekdays and 1 weekend day) • Social Cognitive Theory concepts	• Whole-grain consumption
2.	Gow et al. (21)	• Anthropometry • International Physical Activity Questionnaire • Three Factor Eating Questionnaire • Eating Behaviours Questionnaire • Binge Eating Scale • Block Food Screener • Body Rating Scale • Eating Disorder Inventory • Eating Disorder Screening Questions • Smoking Items	• Body mass index • Eating and weight-related attitudes and behaviours
3.	Poddar et al. (22)	• 7-day food records • Social Cognitive Theory questionnaires	• Self-efficacy, self- regulation, and intake of dairy products
4.	Ha and Caine-Bish (9)	• Anthropometry • 3-day dietary records (2 weekdays and 1 weekend day) • Social Cognitive Theory concepts	• Fruit and vegetable consumption • Effectiveness of 15-week basic nutrition class
5.	Ha et al. (8)	• Anthropometry • 3-day dietary records (2 weekdays and 1 weekend day) • Social Cognitive Theory concepts	• Soft drink and milk consumption • Beverage choice alteration
6.	White et al. (3)	Questionnaires based on the following health behaviours: • Alcohol and drug consumption • Negative consequences of alcohol and drug use • Weight management • Fat talk • Safer sex behaviour • Sex under the influence of alcohol/drug	• Health behaviours (knowledge, attitudes and behaviours)
7.	You et al. (4)	• Anthropometry (anthropometer and bioelectrical impedance) • Dietary habit questionnaires (10 items) • 24-hour dietary recall • 3-day records • Short Form-36	• Effectiveness in body composition • Dietary habits • Serum lipid profiles • Nutrient intake • Quality of life
8.	Franko et al. (1)	• The Food Frequency Questionnaire • Stages of dietary and physical activity change • Nutrition knowledge test • International Physical Activity Questionnaire • Social support, encouragement, and self-efficacy for dietary changes • Exercise benefits and barriers	• Nutrition knowledge • Health behaviours • Physical activity • Social support and attitudes
9.	Endevelt et al. (11)	• Multiple-choice questionnaire (knowledge of nutritional issues) • Mark Spilsbury's Measuring The Effectiveness of Training	• Nutrition knowledge • Perception of programme effectiveness

No.	Authors	Measurement instrument	Outcomes
10.	Abood et al. (23)	• Nutrition knowledge and self-efficacy • 3-day diet record • Social Cognitive Theory concepts	• Nutrition knowledge • Self-efficacy • Dietary practices
11.	Levy and Auld (24)	• Eating habits survey • Cooking survey • 72-hour food preparation recall	• Changes in attitudes, knowledge, and behaviours regarding cooking
12.	Matvienko et al. (25)	• Anthropometry • Food Frequency Questionnaire (116 items) • Multiple choice and short answer questions (overall knowledge, nutrition knowledge, physiologic knowledge, and metabolic knowledge)	• Body weight • Nutrient intakes • Knowledge
13.	Winzelberg et al. (26)	• Body Shape Questionnaire • Eating Disorder Inventory: Drive for thinness and bulimia subscales • Eating Disorder Examination Questionnaire: Weight concerns and shape concerns subscales	• Body image • Eating disorder
14.	Aaron et al. (27)	• Dutch eating behaviour questionnaire • Debriefing questions	• Changes in energy and fat content

Discussion

This brief review compiles evidence on the effectiveness of nutrition education interventions that have been used for college/university students in developed countries. Methodological issues, types of nutrition education interventions, dietary habits, related outcomes, and suggestions for future investigations are highlighted.

Because females out-numbered the males with a ratio of 2:1 across all studies, the overall sample composition may be imbalanced. This higher rate of female participation may be related to the increasing proportion of women in tertiary institutions (6). A recent report in Malaysia indicates that the proportion of female to male students has increased to a current ratio of 65:35 (7), and the same trend is believed to occur in other countries. Regarding attitudes toward nutrition, females exceeded their male counterparts. Similar findings have been previously discovered, such that females reported more positive attitudes towards healthy eating and greater health-consciousness than males did (8,9). These results imply that female students are highly motivated and are more interested in their health, body weight, and body image than male students are. Furthermore, the transition from home to college has often been identified to be a potentially critical period for weight gain among young adults, and in comparison to men, women have especially been eager to change their body shape and weight to conform with current fashion trends (10). Consequently, female students are more likely to be respondents in weight- and body shape–related programmes involving nutrition education interventions.

Regarding the methodological issues, most techniques seem to require several improvements. The common usage of cross-sectional design (11) in most investigations has its drawbacks, such that group differences can only be gauged at one time point and temporal changes could not be assessed. This methodological challenge may prevent experimentally conclusive and sustainable evidence. The sample sizes in several studies were also rather small, ranging from 22 to 43 participants. Thus, the findings may be limited and may lack generalisability because the data could only be analysed using less powerful statistical techniques and the study samples were likely not representative of the more general population . In addition, the reliability and validity of the assessment tools were not comprehensively reported, which is a methodological weakness because these indicators are essential for determining the effectiveness of the interventions (12).

A variety of outcomes have been reported across the interventions studied. Encouraging and positive results with improved health outcomes have been demonstrated in most studies. Nonetheless, more than half of the studies have not reported any preliminary evaluations of newly developed interventions. Such initial evaluations are crucial because they can facilitate subsequent modifications to ensure that an intervention is feasible and acceptable for use in an actual study (12). As a result, later experimental investigations may be less exposed to methodological flaws and may thus provide stronger outcomes.

The results for dietary habits showed that the combination of nutrition education and supplement provision was significantly beneficial in improving body composition, dietary habits, daily nutrient intake, and quality of life in a sample of Korean students (4). Supplements have been commonly administered to either healthy or unhealthy Korean populations (13). Furthermore, a few studies have also reported changes in dietary habits after interventions involving educational lectures as a nutrition improvement tool. For instance, Ha and Caine-Bish (9) have successfully showed an increased consumption of fruits and vegetables after nutrition interventions. Because dietary habits could worsen during university years, any undesirable dietary norm should be addressed at earlier ages and preferably through individuals' routine learning environments (9). Hence, nutrition education is a well-suited technique to improve both students' dietary habits and their awareness of overall health.

HRQoL data have been universally used to assess populations with illness and disability, to identify health disparities and needs and to monitor health changes over time (5). HRQoL refers to an individual's satisfaction or happiness with the domains of life that are affected by health (5). Based on our review, HRQoL as related to dietary habits was not directly or extensively studied among college students. Because HRQoL represents a vital and holistic parameter for population healthcare needs, future investigations should include nutrition-related HRQoL as an outcome measure.

Additionally, research should focus on the development of nutrition education tools, which are not only effective but also interesting and practical for the current generation of students. For example, the effectiveness of the short messaging system has been demonstrated in smokers, diabetics, and bulimia nervosa patients (14). Another recommendation is to target nutrition education for first-year university students, who may still be adjusting to the collegiate environment and experiencing independence in life for the first time.

Several drawbacks of this review deserve attention. In particular, our limited accessible online databases generated only 14 articles that met the inclusion criteria from Medline, Science Direct, CINAHL (EBSCO*host*), and Google Scholar. With a small number of reported RCTs and lacking studies from developing countries, we could not provide a more comprehensive, potentially less-biased review. We did find 5 investigations from developing countries, which included Malaysia and Indonesia, but they unfortunately did not conform to the aims of our review. These studies were either focused on primary school children (15–17) or the elderly (18,19), which did not meet our main target sample of college/university students. Future studies should also enrol larger samples, with the provision of sample size calculations, and a more balanced gender representation. With the majority of respondents being women across the studies, we acknowledge that this review may be biased toward female nutritional habits. Because publications in languages other than English were excluded, additional information from these studies could complement the existing research findings.

Conclusion

Despite several methodological limitations, we found that significant and beneficial changes in dietary habits have been found for college students after the implementation of nutrition interventions via various techniques. In particular, nutrition education and its combination with supplement provision appeared to be the best methods for enhancing students' eating habits and promoting healthier diets and lifestyles. Nonetheless, these findings are more representative of the female populations in developed nations, and we suggest that further trials of similar nature, with improved methodology and in less-developed countries, are highly important.

Authors' Contributions

Conception and design, critical revision of the article: LPP
Collection, assembly, analysis, and interpretation of the data, drafting of the article: WPEWD
Final approval of the article: LPP, WPEWD

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Original Article

Cloning of a Recombinant Plasmid Encoding Thiol-Specific Antioxidant Antigen (TSA) Gene of *Leishmania major* and Expression in the Chinese Hamster Ovary Cell Line

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Abstract

Background: **TSA (thiol-specific antioxidant antigen) is the immune-dominant antigen of *Leishmania major* and is considered to be the most promising candidate molecule for a recombinant or DNA vaccine against leishmaniasis. The aim of the present work was to express a plasmid containing the TSA gene in eukaryotic cells.**

Methods: **Genomic DNA was extracted, and the TSA gene was amplified by polymerase chain reaction (PCR). The PCR product was cloned into the pTZ57R/T vector, followed by subcloning into the eukaryotic expression vector pcDNA3 (EcoRI and HindIII sites). The recombinant plasmid was characterised by restriction digest and PCR. Eukaryotic Chinese hamster ovary cells were transfected with the plasmid containing the TSA gene. Expression of the *L. major* TSA gene was confirmed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and Western blotting.**

Results: **The plasmid containing the TSA gene was successfully expressed, as demonstrated by a band of 22.1 kDa on Western blots.**

Conclusion: **The plasmid containing the TSA gene can be expressed in a eukaryotic cell line. Thus, the recombinant plasmid may potentially be used as a DNA vaccine in animal models.**

Keywords: *CHO cells, gene expression, genetics, Leishmania major, plasmid, recombinant DNA*

Introduction

Leishmaniasis is a parasitic disease caused by several species of the genus *Leishmania*. The disease is prevalent in many parts of the world, with about 12 million total cases worldwide. As many as 1.5–2 million new cases of cutaneous leishmaniasis and 500 000 cases of visceral leishmaniasis are reported every year (1–3).

Treatment of leishmaniasis is complicated due to its toxicity, side effects, and resistance to available drugs (4). Development of new anti-*Leishmania* drugs is needed, but a *Leishmania* vaccine would offer an attractive alternative. Immunity against reinfection is acquired following cutaneous infection with *Leishmania* spp., suggesting that prophylactic immunisation is feasible (5).

In recent years, significant progress has been made in the identification of vaccine candidates capable of inducing a protective response against *Leishmania*. However, no protective, effective anti-*Leishmania* vaccine is presently available, despite several tested protocols. Thiol-specific

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antioxidant antigen (TSA) has been shown to be a potentially suitable candidate molecule for a vaccine (6–9).

The Friedlin strain *L. major* genome is approximately 34 Mb and is distributed over 36 chromosomes. Its G+C content is estimated to be approximately 63%. TSA is the *L. major* recombinant protein homologue of eukaryotic thiol-specific antioxidant protein. This 22.1 kDa protein is composed of 200 amino acids and is located on chromosome 15. TSA is expressed in *L. major* promastigotes and amastigotes (10) and was chosen as a vaccine candidate because it elicits a good Th1 response in *L. major*-infected BALB/c mice. In previous studies, TSA DNA–vaccinated mice showed excellent and strong protection compared with mice vaccinated with other DNA vaccines. The TSA vaccinated mice had high titres of specific IgG1, IgG2a antibodies, high levels of IFN-γ, low levels of IL-4, and phenotypic markers of Th1 responses (1,3,11).

The aim of the present study was to construct a pcDNA3 eukaryotic expression vector containing the TSA gene of *L. major*. The ability of the construct to induce protein expression in mammalian cells was confirmed using Chinese hamster ovary (CHO) cells.

Materials and Methods

In a previous study, genomic DNA was extracted from MRHO/IR/75/ER of *L. major*, and the TSA gene (Accession number: LmjF15.1080) was amplified by PCR. The PCR product was then cloned into the pTZ57R/T vector and transformed into *Escherichia coli* (TG1 strain) (12).

Plasmid construction

To subclone TSA into the pcDNA3 plasmid, the gene was cloned with linkers to join it to the HindIII and EcoRI sites of pcDNA3 (Invitrogen, US) to produce the recombinant eukaryotic expression plasmid pcTSA. The upstream primer for the TSA gene contained a HindIII site and the ATG start codon, and the downstream primer contained an EcoRI site and the TAA stop codon. Competent *E. coli* cells (TG1 strain) were transformed with the ligation mixture by the heat shock method (13). The plasmid was then purified using a plasmid extraction kit (Bioneer, DE) according to the manufacturer's instructions and sequenced. DNA concentrations were measured by absorbance at 260 nm. The OD260/280 ratios for the purified DNA were 1.80–1.95. The plasmid recovered from the recombinant bacterial colony was sequenced by Takapou Zist Co. (IR) to confirm the presence of the TSA gene (12).

Transfection of recombinant pcTSA into eukaryotic cells

CHO cells were grown to 60%–70% confluence at 37 °C and 5% CO_2 in 35-mm wells in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, UK) containing 100 IU/mL penicillin, 100 µg/mL streptomycin, and 10% foetal calf serum (FCS). The cells were washed in serum-free medium. Transfection was then performed using a transfection kit (Genejuice Transfection Kit, Novagen, US) according to the manufacturer's instructions, and cells were incubated overnight at 37 °C in 5% CO_2. Serum-free DMEM medium was mixed with Genejuice reagent and incubated for 10 min, and then the recombinant plasmid was added and incubated for 15 min. After addition of DMEM medium with FCS, the mixture was added to cells and incubated overnight at 37 °C in 5% CO_2.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis and Western blot analysis

Transfected and untransfected cells were cultured for either 48 h or 72 h following respectively, and were harvested and lysed in sample buffer. After sonication and freezing-thawing (10 times using liquid N_2 and a 37 °C water-bath), the cells were centrifuged. The protein profile of the supernatant was resolved using 12.5% reducing sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) according to the method of Laemmli (14) and stained with Coomassie blue. An additional SDS–PAGE was performed for Western blotting, and proteins were transferred to nitrocellulose membranes.

Membrane strips were blocked with 1% bovine serum albumin in phosphate-buffered saline–Tween 20 solution (BSA–PBST20) overnight and sequentially incubated with *Leishmania* antibody-positive mouse serum (provided by Dr MZ Hassan, Department of Immunology, Tarbiat Modares University, Tehran, Iran) and anti–mouse IgG horseradish-peroxidase secondary antibody (Sigma, US) diluted in 1% BSA–PBST20 (1/10 000). In addition, some strips were incubated with anti-His–tagged horseradish peroxidase (1/2 000 dilution, Qiagen, US). Specific binding was shown with diaminobenzidine (DAKO, DK) (13,14).

Results

In this study, we constructed a novel plasmid containing pcTSA and transfected it into eukaryotic cells. After ligating PCR-amplified gene into the mammalian expression vector pcDNA3 and performing a restriction digest with EcoRI and HindIII, 2 bands were observed by agarose gel electrophoresis for the plasmid containing the insert, compared with 1 band for pcDNA3 digested with the same enzymes (Figure 1).

Next, SDS–PAGE and Western blot analysis were performed to confirm expression of the TSA protein. A band of 22.1 kDa was observed in the transfected cell lysates by SDS–PAGE (Figure 2). Furthermore, Western blotting using both *Leishmania* antibody-positive mouse serum and anti-His–tagged antibody revealed a band at about 22.1 kDa in cells transfected with pcTSA (Figure 3).

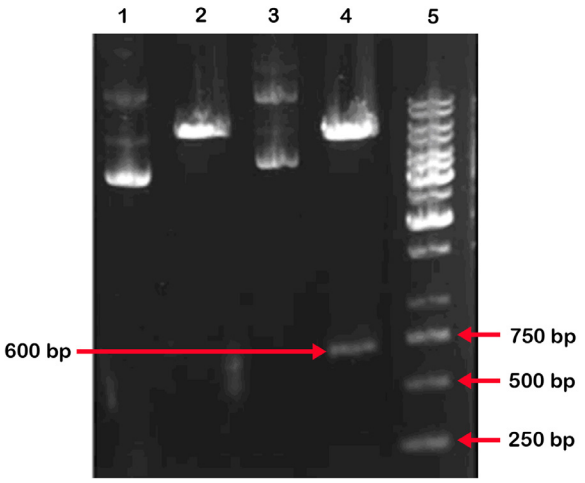

Figure 1: Electrophoresis of purified pcDNA3 and pcTSA plasmids before and after digestion with enzymes. Lanes 1 and 2: pcDNA3 (uncut and cut, i.e., digested by EcoRI and HindIII). Lanes 3 and 4: pcTSA (uncut and cut). Lane 5: O'GeneRuler 1 kb DNA ladder. Lane 1 is undigested supercoiled plasmid pcTSA. Supercoiled plasmid is denser than linear plasmid and thus migrates through the gel faster than the ladder. The 2 bands in Lane 4 correspond to the 600 bp TSA gene and the vector backbone of more than 5 000 bp.

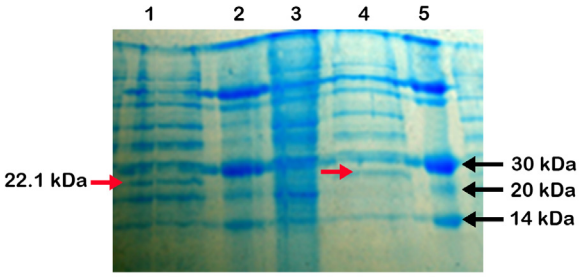

Figure 2: SDS–PAGE analysis of expressed gene product. Lane 1: CHO cells 72 h after transfection with TSA. Lane 2: Untransfected CHO cells after 72 h. Lane 3: Untransfected supernatant CHO cells after 48 h. Lane 4: CHO cells 48 h after transfection with TSA. Lane 5: Low molecular weight markers (14, 20, 30, 43, 67, and 94 kDa).

Discussion

Leishmania can cause a wide range of human diseases, ranging in severity from spontaneously healing skin lesions to fatal visceral disease. Although measures may be taken against vectors and reservoirs, and identification of new drugs is a desirable goal, particularly in view of emerging drug resistances, the development of safe and efficient vaccines remains the best hope for definitively controlling disease (6). Immunity against reinfection is acquired following cutaneous infection with *Leishmania* spp., suggesting that prophylactic immunisation is possible (5,11). *Leishmania* vaccine strategies have evolved from crude parasite preparations to defined molecules administered as recombinant proteins or DNA vaccines. DNA vaccines have some notable features compared with traditional vaccines: they are easy to produce, relatively inexpensive, homogeneous, heat stable, and believed to be safer than subunit or viral vector-based vaccines. DNA vaccines can induce strong, long-lasting and powerful humoural and cellular immunity. They also have the potential to increase immunogenicity through modifications of the vector or incorporation of adjuvant-like cytokine genes (15–17). DNA vaccines may be especially useful for protection against cutaneous leishmaniasis because the progress of naturally acquired immunity from a primary exposure to *L. major* has recently been shown to depend on both CD4+ and CD8+ T cells.

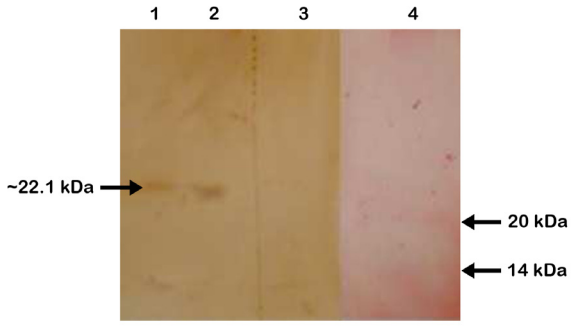

Figure 3: Western blot analysis of expressed gene product . Lane 1: Cell lysates probed with anti-His–tagged horseradish peroxidise. Lane 2: Cell lysates probed with *Leishmania* antibody-positive pooled mice sera. Lane 3: Protein extract of untransfected CHO cells probed with anti-His–tagged horseradish peroxidise. Lane 4: Low molecular weight markers (14, 20, 30, 43, 67, and 94 kDa).

In recent years, significant advances in determining the best kind of vaccine to induce an optimal immune response have been achieved. For example, antigens such as LACKp24, TSA, LmSTI1, and CPA have been tested as candidate DNA vaccines for *Leishmania*. All of these antigens were able to elicit relatively protective effects, but none could confer complete protection.

Recently, type I, II, and III cysteine proteinases have become attractive candidates as vaccine antigens against visceral leishmaniasis (7,11,18–21). In an attempt to develop a DNA vaccine against cutaneous leishmaniasis, we focused on the gene encoding TSA because previous studies have shown that immunisation with TSA peptides, proteins, or DNA can elicit a broad range of humoural and cellular immune responses in animals infected with *L. major*. *Leishmania* TSA protein is known to be antigenic in both murine and human systems and is constitutively expressed in both promastigote and amastigote life stages. Moreover, this antigen is abundant and homogeneously distributed on the surface of both extracellular and intracellular promastigotes and amastigotes (10).

Immunisation of BALB/c mice with TSA DNA vaccine results in high levels of protective immunity (humoural and cellular), induces cytotoxic lympocytes activity, and robust protection, and stimulates high titres of specific IgG1 and IgG2a antibodies. It also induces strong IFN-γ production, with low levels of IL-4 and phenotypic markers of Th1 response (1,3,11). Both LmSTI1 and TSA antigens confer outstanding protection in both murine and nonhuman primate models of human cutaneous leishmaniasis. The recombinant TSA protein, along with IL-12 , has also been shown to induce good protection in murine models. As it induces a CD8 response and specific immunity, which can suppress parasite numbers, TSA DNA may be considered the vaccine of choice (2).

Thus, the production of TSA protein is a critical basis for further research into and development of a sophisticated, effective vaccine against leishmaniasis.

Conclusion

In this study, we successfully expressed recombinant plasmid containing TSA gene of *L. major* in eukaryotic cells, further demonstrating its potential to be used as a DNA vaccine in animal models.

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Authors' Contributions

Conception and design, obtaining of funding, provision of study materials, collection, assembly, analysis, and interpretation of the data, statistical expertise, drafting of the article: FG, FT
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Original Article

Effect of Repeatedly Heated Palm Olein on Blood Pressure–Regulating Enzymes Activity and Lipid Peroxidation in Rats

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Abstract

***Background*: Oxidative stress is associated with the pathogenesis of cardiovascular diseases. The process of deep-fat frying in dietary cooking oil plays a role in the generation of free radicals. In this study, palm olein heated to 180 °C was tested for its effect on the activity of blood pressure–regulating enzymes and lipid peroxidation.**

***Methods:* Forty-two adult male Sprague-Dawley rats were equally assigned into 6 groups. The first group was fed with normal rat chow as the control group, and the subsequent groups were fed with rat chow fortified with 15% weight/weight of the following: fresh palm olein, palm olein heated once, palm olein heated twice, palm olein heated 5 times, or palm olein heated 10 times. The duration of feeding was 6 months. Fatty acid analyses of oil were performed using gas chromatography. Peroxide values were determined using standard titration. Plasma was collected for biochemical analyses.**

***Results:* Repeatedly heated palm olein increased the levels of peroxide, angiotensin-converting enzyme, and lipid peroxidation as well as reduced the level of heme oxygenase. Fresh palm olein and palm olein heated once had lesser effects on lipid peroxidation and a better effect on the activity of blood pressure–regulating enzymes than repeatedly heated palm olein.**

***Conclusion:* Repeatedly heated palm olein may negatively affect the activity of blood pressure–regulating enzymes and increase lipid peroxidation.**

Keywords: *angiotensin-converting enzyme, heating, heme oxygenase, nutrition, oxidative stress, palm oil*

Introduction

Palm oil obtained from the *Elaeis guineensis* mesocarp exhibits good frying performance, which contributes to its widespread use in deep-frying applications (1). Due to the rising demand and increase in fat intake, palm oil is the major oil in the world's oil and fat market, and palm oil is projected to remain the most influential fat source through 2016 (2). The refined, bleached, and deodorised palm olein, which is fractionated from palm oil, is commonly used as cooking oil. It offers better resistance to oxidation at high temperature during frying as well as natural antioxidants from the vitamin E group, the tocotrienols (3). Tocotrienols, which have an unsaturated side chain, have greater antioxidant properties than the saturated tocopherols (4). In addition, palm olein contains almost 50% saturated fatty acids (SFA), 50% monounsaturated fatty acids (MUFA), and low levels of polyunsaturated fatty acids (PUFA) under normal conditions (5), which reduce susceptibility to oxidation.

The main economic factor considered in fried food products is the cost of oil because oil is one of the major ingredients in these products. Therefore, very often the oil is repeatedly used to minimise the expense of food preparation.

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During the reheating process, the oil undergoes various physical reactions, such as formation of foam, increases in viscosity, darkening of colour, and deterioration of flavour. These changes may affect the organoleptic qualities, such as the odour and taste, and the nutritional value of the fried food (6). Furthermore, repeated heating causes chemical reactions, such as hydrolysis, oxidation, and polymerisation, that alter the chemical structure of triacylglycerol molecules, of which PUFA molecules are affected the most (7).

Thermally oxidised oils, such as those produced by repeated frying, contain a complex mixture of products, such as oxidised monomers, dimers, and polymers. These products have been reported to be the substances mainly responsible for changes in the physicochemical properties of fats (8). When frying oil is heated at high temperatures, toxic products, such as hydroperoxides and aldehydes, are formed, absorbed by the food, and subsequently absorbed into the gastrointestinal system and introduced into systemic circulation after consumption (9). The practice of reusing frying oil causes harmful health effects, such as an increased risk of hypertension (10,11), disturbance of endothelial function (12,13) and histological abnormalities (14,15). Free radicals generated during frying process could damage lipids by initiating lipid peroxidation. Malondialdehyde (MDA), one of the major secondary oxidation end products of peroxidised PUFA, has been shown to be of biological significance (16).

Heme oxygenase (HO), the rate-limiting factor in heme catabolism, produces free ferrous ion, biliverdin, and carbon monoxide (CO). Biliverdin is further converted to bilirubin, which acts as an antioxidant (17). Furthermore, CO has vasodilatation, anti-proliferation, and anti-inflammation properties (18,19). Among the isoforms of HO (HO-1, HO-2, HO-3), HO-1 has been suggested to contribute to the control of blood pressure (BP). It is inducible and highly sensitive to various stimuli that are involved in oxidative and haemodynamic damages (20).

On the other hand, angiotensin-converting enzyme (ACE) plays a vital role in the regulation of BP via hydrolysis of the inactive form of angiotensin I (Ang I) to the active form, angiotensin II (Ang II). ACE is mainly located on the surface of endothelium and epithelium involved in the constriction of blood vessels, which leads to elevation of BP. Effects of Ang II can be observed via 2 types of receptors, AT_1 and AT_2, which both have different pharmacological and biochemical characteristics.

Ang II is an important factor in cardiovascular homeostasis (21). It induces oxidative stress via activation of nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidases and the production of reactive oxygen species (ROS) (22), which cause hypertension and cardiac failure (23,24). In addition, Ang II increases lipid peroxidation (25) and stimulates the production of pro-oxidant cytokines (26,27) that eventually increase BP.

Our laboratory has previously shown that heated oils increase BP and lead to an impairment in vasorelaxation (10,12). Therefore, in this study, we determined whether heated oils affect BP by increasing vascular reactivity due to a reduction in nitric oxide (NO) content or by affecting the BP-regulating enzymes, ACE and HO. The effect of heated oils on the activity of BP-regulating enzymes and lipid peroxidation was studied in rats.

Materials and Methods

Palm olein and diet preparation

Palm olein was purchased from a local market. The oil was used as either the fresh or heated form according to a modified method (28), which was also used in our previous studies (10,12). Briefly, the oil was used to fry potato chips as follows. First, sweet potatoes were peeled and cut into slices. Next, the slices were deep-fried using fresh palm olein (FPO) for 10 min at 180 °C. The oil was then cooled to room temperature and used to fry another batch of sweet potatoes. The frying process was performed without the addition of fresh oil. The same process was repeated to obtain palm olein heated 5 times and 10 times. Standard rat chow (Gold Coin, Selangor, MY) was ground to coarse powder and fortified with 15% weight/weight (w/w) of the prepared oils. The mixture was subsequently dried in a 70 °C oven overnight. The diet was stored in a closed cabinet and prepared weekly.

Animals and study protocol

Three-month-old male Sprague-Dawley rats (200–280 g) were used in the study. Forty-two rats were obtained from the Animal Source Unit, Universiti Kebangsaan Malaysia, with prior ethical clearance (UKMAEC: FP/FAR/2008/KAMSIAH/9-APR/220-APR-2008-FEB-2011). All animal management and handling procedures were performed according to the recommended guidelines. The animals were equally and randomly divided into 6 experimental groups. The experimental groups were as follows:

a. Group 1 (control): normal rat chow
b. Group 2: 15% w/w FPO mixed with chow
c. Group 3: 15% w/w palm olein heated once (1HPO) mixed with chow
d. Group 4: 15% w/w palm olein heated twice (2HPO) mixed with chow
e. Group 5: 15% w/w palm olein heated 5 times (5HPO) mixed with chow
f. Group 6: 15% w/w palm olein heated 10 times (10HPO) mixed with chow

All rats were kept in stainless steel cages in a room maintained at 27 °C (SD 2) with a 12-hour light-dark cycle. The animals had free access to water and food throughout the study. The rats were fed daily with the oil diet based on their respective groups for 6 months after 1 week of adaptation. Blood was collected through the orbital sinus prior to treatment and at the end of the study. The blood was then centrifuged to obtain plasma. Aliquots of the plasma were stored at −70 °C and used later for the enzyme activity and lipid peroxidation studies.

Fatty acid composition

Fatty acid methyl esters were prepared from fresh or heated palm olein by transesterification with sodium methoxide (NaOMe, 1 M) in hexane prior to analysis. A gas chromatograph (Shimadzu GC-17A, Kyoto, JP) equipped with a flame ionisation detector was used for fatty acid profiling. Nitrogen was used as the carrier gas at a flow rate of 0.40 mL/min through a BPX 70 capillary column (30 m × 0.25 mm × 0.25 µm film thickness, SGE, New Jersey, US). The injector temperature was programmed to 250 °C, and the detector temperature was set to 280 °C. Injection volume was 1 µL. Retention times obtained from gas chromatography were compared with those of individually purified standards subjected to the same conditions for the identification of fatty acid methyl ester peaks.

Peroxide measurement

The peroxide content of palm olein was determined by the Official Method of American Oil Chemists' Society (Cd 8-53).

Biochemical measurements

The HO-1 (Assay Designs, Michigan, US) and ACE (USCNLife, Wuhan, CN) activities were analysed in plasma samples using commercially available kits. The coloured end products of these 2 enzymes were measured in a microplate reader (Molecular Devices, California, US) at 450 nm. These measurements were performed according to a previous protocol (12) and following the manufacturers' instructions.

Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation in the plasma samples, as determined by the MDA levels, was measured using thiobarbituric acid. The plasma TBARS levels were estimated following a previously described method (29) with some modifications. Briefly, 2.5 mL of 1.22 M trichloroacetic acid/0.6 M hydrochloric acid was used to acidify 0.5 mL of the plasma sample, which was incubated at room temperature for 15 min. Next, 1.5 mL of 0.67% thiobarbituric acid/0.05 M sodium hydroxide was added, and the samples were incubated for 30 min in a 100 °C water bath. After the mixture had cooled to room temperature, 4 mL of *n*-butanol was added. The mixture was then centrifuged for 10 min at 1500g. The supernatant was analysed against *n*-butanol (Ex: 515, Em: 553) using a spectrofluorometer (Shimadzu RF500, Kyoto, JP).

Protein content

The protein content of the plasma was determined using a method described in Lowry et al. (30) with some modifications. About 5 mL of a 100:1:1 mixture of 2% sodium carbonate, 2% sodium or potassium tartrate, and 1% copper sulphate solution was added to 0.5 mL plasma. Next, the mixture was incubated at room temperature for 15 min, and 0.5 mL of diluted Folin–Ciocalteau phenol reagent was then added. After 35 min, the absorbance of the mixture was measured at 700 nm with a spectrophotometer (Shimadzu UV-160A, Kyoto, JP). The results are expressed as TBARS/protein (nmol/mg protein).

Statistical analyses

The results for the BP-regulating enzymes activities and lipid peroxidation levels were presented as percentages of the baseline values. All data analyses were conducted using SPSS version 13.0 (SPSS Inc., Chicago, Illinois, US). Normality of the data was determined by Kolmogorov–Smirnov test. The peroxide values among the dietary groups were compared using one-way analysis of variance (ANOVA) with Tukey's Honestly Significant Differences post-hoc test for differences between pairs of means when applicable. To analyse the differences in the BP-regulating enzyme activities and levels of lipid peroxidation among the experimental groups, the Kruskal–Wallis and Mann–Whitney tests were performed. Statistical significance was defined as $P < 0.05$. Data are expressed as means (SD).

Results

Fatty acid composition of palm olein

Fatty acid analyses of fresh oil and oil subjected to different frying levels are shown in Table 1. All of the main fatty acids were present in the oil regardless of the frequency of frying.

Peroxide content of palm olein

There was a significant increase ($P < 0.001$) in the peroxide index for 2HPO (4-fold increase), 5HPO (4-fold increase), and 10HPO (5-fold increase) compared with the value of fresh oil (Table 1).

Activity of blood pressure-regulating enzymes

All groups, including the control, exhibited a reduction in plasma HO-1 levels after 6 months of feeding. HO-1 activity was significantly lower ($P < 0.05$) in the heated palm olein groups (Figure 1). The activity of ACE was also found to be significantly increased ($P < 0.05$) in the heated palm olein groups. The percentage increase was significantly higher in the 10HPO group compared with the FPO and other dietary groups (Figure 2).

Lipid peroxidation

The control and the experimental groups showed increases in the plasma TBARS level at the end of study. The percentage increase was significantly higher ($P < 0.05$) in heated palm olein groups compared with the control and FPO groups (Figure 3).

Discussion

Palm oil is unique in terms of its ratio of SFA to unsaturated fatty acids, which is close to one. Furthermore, it is rich in the antioxidant vitamin E. Due to its availability and affordable price, palm oil is widely used as a dietary cooking oil in daily food preparation. Therefore, palm oil (olein) was chosen for our present study. Frying remains one of the most popular methods for food preparation. The various frequencies of frying were used in this study to simulate the cooking conditions used by street vendors and most households.

Deep-frying oil contained relatively more SFA with less unsaturated fats, as observed in the present study. Initially, the PUFA composition increased and then decreased as palm olein was repeatedly heated. However, due to the other findings shown in this work, such as the peroxide values, enzymes activities and lipid peroxidation values, we do not think that the oil was improved after being heated twice. Generally, heating at high temperatures has a negative effect on the fatty acid composition. The presence of unsaturated bonds in the fatty acid chains render it accessible to attack from the free radicals produced during frying process. Fats with higher numbers of unsaturated bonds are prone to oxidation. The increased peroxide and TBARS values shown in our present study may be attributed to the destruction of double bonds by oxidation and polymerisation. Heat treatment causes oxidative rancidity, which may increase free fatty acids (31). Hence, the fatty acid composition was analysed to observe the degradation of fatty acids during the frying process.

The peroxide value is used to indicate the extent of oil degradation. It measures the amount of peroxides formed in the cooking oil during the process of oxidation. From the results obtained, the extent of oxidation rancidity was influenced by the number of frying episodes. The more frequently the oil was reheated, the higher the peroxide index. Nevertheless, compared with our previous report, soy oil had a higher peroxide value when it was repeatedly heated under the same frying conditions (12). As an increased peroxide value indicates that oil is not suitable

Table 1: Fatty acid compositions and peroxide values of fresh and heated palm olein

Composition	FPO	1HPO	2HPO	5HPO	10HPO
SFA (%)	42.87	42.64	43.03	43.25	43.28
MUFA (%)	48.94	49.24	47.32	48.21	50.64
PUFA (%)	8.18	8.52	8.87	7.97	6.08
Peroxide (mEq O_2/kg)[a]	2.22 (0.44)[b]	6.41 (0.26)[b]	8.35 (0.16)[b]	9.18 (0.11)[b]	11.76 (0.40)[b]

[a] Values are the averages of 3 estimations in mean (SD). [b] Analysis of variance indicates significant difference between groups ($P < 0.05$). Abbreviations: FPO = fresh palm olein, 1HPO = palm olein heated once, 2HPO = palm olein heated twice, 5HPO = palm olein heated 5 times, 10HPO = palm olein heated 10 times, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

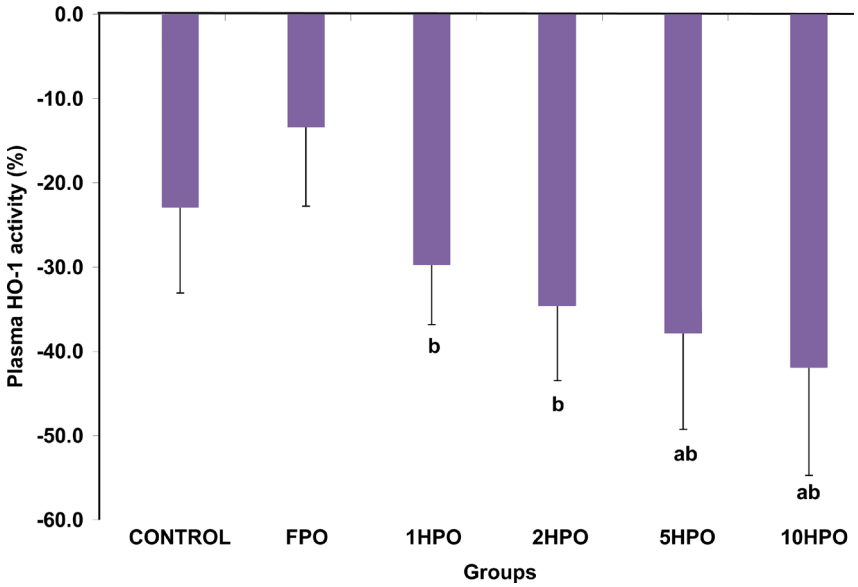

Figure 1: Percentage of changes in the plasma heme oxygenase-1 (HO-1) activity after 6 months of feeding with the basal diet (control), fresh palm olein (FPO), palm olein heated once (1HPO), palm olein heated twice (2HPO), palm olein heated 5 times (5HPO), or palm olein heated 10 times (10HPO). The bars represent mean, and the error bars, SD, with $n = 7$ in each group. Significant differences ($P < 0.05$) were observed in the heated palm olein groups compared with the [a] control or [b] FPO groups.

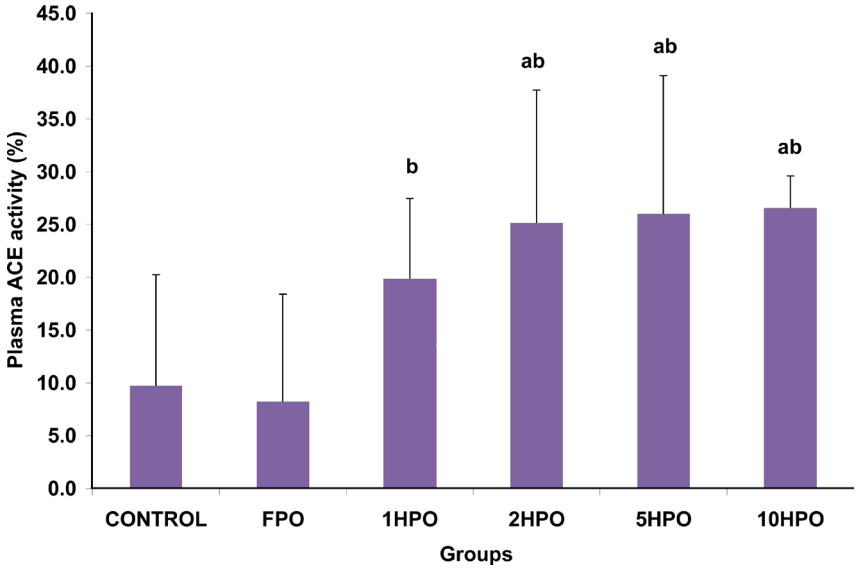

Figure 2: Percentage of changes in plasma angiotensin-converting enzyme (ACE) activity after 6 months of feeding with the basal diet (control), fresh palm olein (FPO), palm olein heated once (1HPO), palm olein heated twice (2HPO), palm olein heated 5 times (5HPO) or palm olein heated 10 times (10HPO). The bars represent mean, and the error bars, SD, with $n = 7$ in each group. Significant differences ($P < 0.05$) were observed in the heated palm olein groups compared with the [a] control or [b] FPO groups.

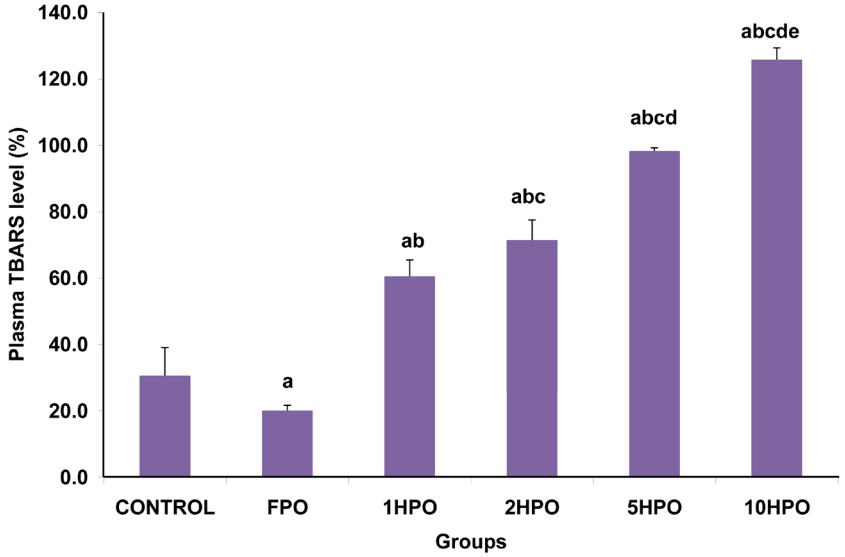

Figure 3: Percentage of changes in the plasma thiobarbituric acid reactive substances (TBARS) level after 6 months of feeding with the basal diet (control), fresh palm olein (FPO), palm olein heated once (1HPO), palm olein heated twice (2HPO), palm olein heated 5 times (5HPO) or palm olein heated 10 times (10HPO). The bars represent mean, and error bars, SD, with $n = 7$ in each group. Significant differences ($P < 0.05$) were observed in the palm olein groups compared with the [a] control, [b] FPO, [c] 1HPO, [d] 2HPO, or [e] 5HPO groups.

for deep-frying, our recommendation concerning safety is that the oil should not be reheated more than once.

A higher peroxide value indicates lower chemical stability of the oil. Naghshineh et al. (32) postulated that a high content of SFA increases the chemical stability of oils. However, the peroxide value alone is not sufficient to assess the extent of oxidation and chemical stability of an oil because the peroxides and hydroperoxides generated during the frying process are unstable and easily decompose to other compounds, which reduces the peroxide index (33).

In this study, the changes in the peroxide value of the oil may be associated with a significant increase in plasma MDA. MDA is a major end product of PUFA peroxidation and is often used as an indicator of cell injury. This association may indicate that repeated heating increases oil oxidation, which subsequently increases lipid peroxidation. Although lipid peroxidation may be initially prevented by antioxidants, such as vitamin E, in the oil, repeated heating also decreases the vitamin E content (34).

HO is important in the modulation of BP and vascular tone. Biliverdin and CO, the by-products of HO, have been reported to have cytoprotective effects against oxidative damage (35). Past research has shown that high expression of HO increases the HO enzyme activity and reduces BP (36). Due to the similarity of the effect exerted by HO-1 and its by-products to those of endothelium-derived NO, there is a possible relationship between the HO-1 and NO pathways. Hence, HO-1 and heme degradation products can improve vascular function by compensating for the loss of NO bioavailability (37). The effects of the HO-1 enzyme, including the depletion of pro-oxidative heme and promotion of the antioxidant function of bilirubin and the signalling action of CO, may also contribute to the prevention of endothelial dysfunction when peroxynitrite is formed from the reaction of superoxide with NO. Peroxynitrite is highly reactive and has negative effects on vascular function and structure (38).

Endothelial HO-1 induction by NO may act as a feedback mechanism to preserve NO-mediated endothelial regulation of vascular function. It has

been reported that increasing HO-1 activity in vivo has protective effects on the NO regulation of vascular function and are associated with an upregulation of other important antioxidant systems that protect the vasculature, such as extracellular superoxide dismutase and plasma catalase activities (39). Thus, antioxidant effects of NO-elicited increases in HO-1 expression may participate in preventing endothelial dysfunction. Endothelial dysfunction as observed in vascular disease is often associated with a loss of NO-mediated vasodilatation. Our study shows that the HO-1 level decreased in the animals fed the heated oil, most prominently in the 10HPO group animals. The reason for the decrease in HO is not clear. We postulate that more peroxides are formed during frying episodes and have a direct detrimental effect on the endothelial function. It has been suggested that hypertension is characterised by a decline in endothelial function (40). In addition, the peroxides formed may have affected the HO enzyme structure, thereby leading to denaturation and destruction of the resulting malfunctional enzyme (41).

Our study shows that the ACE level is significantly elevated in the rats fed heated palm olein. Increases in the ACE level that leads to Ang II synthesis may contribute to the elevation of BP. Our finding was in contrast with Yen et al. (42), who reported no changes in the ACE level after consumption of heated vegetable oil. The discrepancy might be due to the duration of the study and the type of oil and animal used in the experiment.

ACE is required for the conversion of inactive Ang I to Ang II, a potent vasoconstrictor. Ang II-induced hypertension is associated with increased vascular superoxide production and impaired vasorelaxation to acetylcholine (22). Ang II exacerbates oxidative stress, and the increase in the superoxide level could result in endothelial dysfunction via scavenging NO and decreasing NO bioavailability (43). NO generated from the endothelium plays a contributory role in determining the balance between relaxation and contraction of vascular smooth muscle. Hence, the NO–Ang II imbalance may be an important component in the vascular pathophysiology of hypertension.

Our previous work has shown that intake of heated palm oil increases low-density lipoprotein (LDL) cholesterol levels (44). In addition, we have also shown that oxidised LDL (ox-LDL) is cytotoxic, causing ultrastructural changes in the rat aorta (45). Ang II mediates most of the biological effects of the renin-angiotensin system (RAS), such as vasoconstriction and cell proliferation, via stimulation of the Ang II type 1 (AT_1) receptor. The AT_1 receptor plays an important role in the pathogenesis of atherosclerosis and hypertension. Like ox-LDL, Ang II decreases NO synthase expression and stimulates the generation of ROS (22). In addition, Ang II has been suggested to cause an increase in ox-LDL uptake, eventually causing endothelial cell injury (46). On the other hand, it has also been suggested that ox-LDL upregulates AT_1 receptor expression (47). Previous work has shown that hypercholesterolemia is associated with enhanced AT_1 receptor expression (48). These observations may indicate the presence of a relationship between ox-LDL and RAS in hypertension. Together, the 2 systems may be responsible for endothelial dysfunction.

Our earlier findings showed that soy oil heated 10 times caused a more significant increase in BP compared with 10HPO (49). Other parameters also showed more severe effects using soy oil compared with palm olein, such as bone histomorphometric properties (50) and lipid peroxidation (16,51) of ovariectomised rats. In the results obtained from those studies, palm olein is more resistant to repeated heating than soy oil. This might be due to the unique fatty acid composition and vitamin E content of palm olein.

Conclusion

Consumption of thermally oxidised palm olein may affect the functions of enzymes involved in the regulation of BP by increasing the ACE level and decreasing HO activity, which contributes to the development of hypertension. In addition, heated palm olein increases lipid peroxidation. Hence, palm olein should not be reheated more than once in view of its deleterious effect on health.

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Authors' Contributions

Conception and design, obtaining funding: KJ
Provision of study materials: JS, KJ
Collection and assembly of data: XFL, JS
Analysis and interpretation of the data, drafting of the article, statistical expertise: XFL
Critical revision and final approval of the article: XFL, JS, MRM, KJ
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Original Article

Effect of Calabash Chalk on the Histomorphology of the Gastro-Oesophageal Tract of Growing Wistar Rats

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Abstract

Background: **Calabash chalk is a naturally occurring mineral consumed by members of some Nigerian communities for pleasure and by pregnant women as a remedy for morning sickness. The consumption of this geophagic material motivated our interest on the effect of the chalk on the histomorphology of the gastro-oesophageal tract.**

Methods: **Twenty-eight young Wistar rats, 4 weeks old, were divided into 4 groups of equal size. Group 1 animals served as controls and received 1 mL of distilled water. Groups 2, 3, and 4 received orally 1 mL of a Calabash chalk suspension containing 40 mg/mL for 14, 21, and 28 days, respectively. Upon completion of the treatments, the animals in groups 2, 3, and 4 were sacrificed on days 15, 22, and 29, respectively, and the control group animals were sacrificed on day 29. All animals were euthanised using chloroform anaesthesia. The oesophagus and the stomach of each animal were dissected out and routinely processed for histological studies.**

Results: **There was oedema with haemorrhages in the mucosa of the stomach, and acanthosis, hyperkeratosis, and koilocytic changes were observed in the mucosa of the oesophagus of the groups treated with 40 mg/mL of Calabash chalk suspension.**

Conclusion: **Calabash chalk caused histological changes to the stomach and the oesophagus that may lead to other pathophysiological conditions.**

Keywords: *Calabash chalk, gut, stomach, oesophagus, histology, Wistar rats*

Introduction

Geophagia is defined as the practice of eating earth, including soil and chalk (1). People engage in geophagia for a variety of reasons including religious beliefs, medicinal purposes, or as part of a regular diet (2). One geophagic material is Calabash chalk, which is popularly consumed by members of Nigerian and West African communities for pleasure and by pregnant women as a remedy for morning sickness (3,4).

Calabash chalk, also known as Calabar stone, Poto, La craie, Argiles, Mabele, Nzu, and Ndom, is available in a variety of forms including powder, moulded shapes, and blocks. Though native to Africa, it is available in the United Kingdom in ethnic stores and markets. The practice of eating Calabash chalk is also observed among women of African descent in the southern United States, especially in Georgia (4). In addition to the already mentioned uses, Calabash chalk is also used in facial masks and soaps (5). Although this chalk is consumed by both sexes and by many age groups, the prevalence of chalk consumption is greater among women, especially during pregnancy (3).

This chalk is a naturally occurring substance made up of fossilised sea shells but can be prepared artificially (5). It is prepared using a combination of clay and mud, with other ingredients such as sand, wood ash and sometimes salt. This mixture is moulded and then heated to produce the final product (5).

Calabash chalk has the same structure as aluminium silicate hydroxide, which is a member of the kaolin clay group with a possible formula $Al_2Si_2O_5(OH)_4$ (6). Multi-elemental analysis using energy dispersive X-ray fluorescence spectroscopy identified 22 elements in Calabash chalk, including lead and aluminium, and persistent organic pollutants (6).

Another report established the presence of arsenic in Calabash chalk (7). Lead and other toxic elements present in the chalk have been reported to be associated with numerous gastrointestinal

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disorders including nausea, ulcers, and gastritis. (8). This geophagic material is consumed by the oral route, and several pollutants have been reported to be present in Calabash chalk. It is based on this background that this research was carried out to determine if this chalk has an effect on the oesophagus and the stomach, as these organs serve as the conduit for food entering into the body.

Materials and Methods

Twenty-eight 4-week-old Wistar rats (males and females) were used for the experiment. They were purchased and kept in the Animal House, College of Health Sciences, University of Uyo, Nigeria. The temperature of the room was maintained at 26–28 °C with a 12-hour light/12-hour dark cycle.

The animals were cared for according to the international regulations governing the use and care of laboratory animals. They were housed in wooden cages and maintained on standard feeds pellets (growers marsh, Vital Feed, Grand Cereal, Nigeria). Drinking water was allowed ad libitum. The animals were allowed to acclimatise for 1 week. Each animal was weighed prior to the commencement of the experiment and every week thereafter.

Blocks of non-salted Calabash chalk were purchased from a local market in Calabar, Nigeria. The blocks were ground into powder using a manually operated grinder. A 40-g sample of the powder was dissolved in 1000 mL of distilled water, and the mixture was stirred continuously. The chalk was administered as a suspension and was stirred prior to administration. Each millilitre of the suspension contained 40 mg of Calabash chalk.

The rats were weighed and divided into 4 groups of 7 animals each. Group 1 served as the control group and received 1 mL of distilled water for 28 days. Groups 2, 3, and 4 were the test groups and received 1 mL of the Calabash chalk suspension for 14, 21, and 28 days, respectively (Table 1). The rats were humanely sacrificed using chloroform anaesthesia the day after the end of their treatment: rats in groups 2, 3, and 4 were sacrificed on days 15, 22, and 29, respectively, and the controls were sacrificed on day 29. The oesophagus and the stomach from each animal was immediately removed and preserved in 10% buffered formalin for 1 week. These samples were then routinely processed using the haematoxylin and eosin staining method.

Results

The change in weight relative to the control was smaller in groups 2 and 3 but higher in group 4 . The mean weekly weights of the animals in each group are given in Table 2.

Histomorphological observations

Stomach

The stomach sections of group 1 (control) were normal. A preserved cyto-architecture of the mucosa consisting of simple columnar epithelium forming folds and glands lined by mucus-secreting goblet cells, parietal cells, and chief cells, in addition to endocrine cells having coarse cytoplasm, was observed. The submucosa consisted of a fibro-collagenous stroma, glands, and plexuses. The muscle layer was made up of smooth muscle fibres, and the serosa was composed of mesothelium (Figure 1a).

Group 2 had normal mucosae with inflammatory cell infiltrates consisting mainly of lymphocytes and plasma cells. The submucosa, muscle layer, and serosa were intact (Figure 1b).

Group 3 had oedematous mucosal linings with haemorrhages and mononuclear inflammatory cell infiltrates consisting mainly of plasma cells and lymphocytes. The submucosa and the serosa were intact, whereas the muscle layer was thickened and hypertrophic compared with that in the control (Figure 1c).

Group 4 had oedematous mucosae close to the basement membrane with haemorrhages and mononuclear inflammatory cell infiltrates consisting mainly of lymphocytes and plasma cells. The submucosa, muscle layer, and serosa were intact (Figure 1d).

Table 1: The treatment schedule in the experimental groups

Group	Dosage	Duration (days)
1 (control)	1 mL of distilled water	28
2	1 mL of suspension (40 mg/mL of Calabash chalk)	14
3	1 mL of suspension (40 mg/mL of Calabash chalk)	21
4	1 mL of suspension (40 mg/mL of Calabash chalk)	28

Table 2: Weekly weight of Wistar rats in the control and the test groups treated with 40 mg/mL of Calabash chalk suspension for 14, 21, or 28 days

Group	Weight (g)					Change in weight
	Week 0	Week 1	Week 2	Week 3	Week 4	
1 (control)	99.26 (6.22)	120.29 (7.80)	126.57 (10.00)	135.14 (11.32)	136.57 (9.95)	37.31
2	81.43 (9.92)	101.57 (10.34)	109.57 (11.88)	NA	NA	28.14
3	107.43 (8.36)	120.86 (12.01)	129.00 (15.11)	136.29 (16.72)	NA	28.86
4	99.14 (10.16)	118.14 (9.87)	136.43 (16.46)	139.14 (14.87)	142.86 (14.63)	43.72

Results are presented as the mean (SD). Each group consisted of 7 rats. The Week 0 weight represents the weight before the commencement of the experiment. Abbreviation: NA = not applicable.

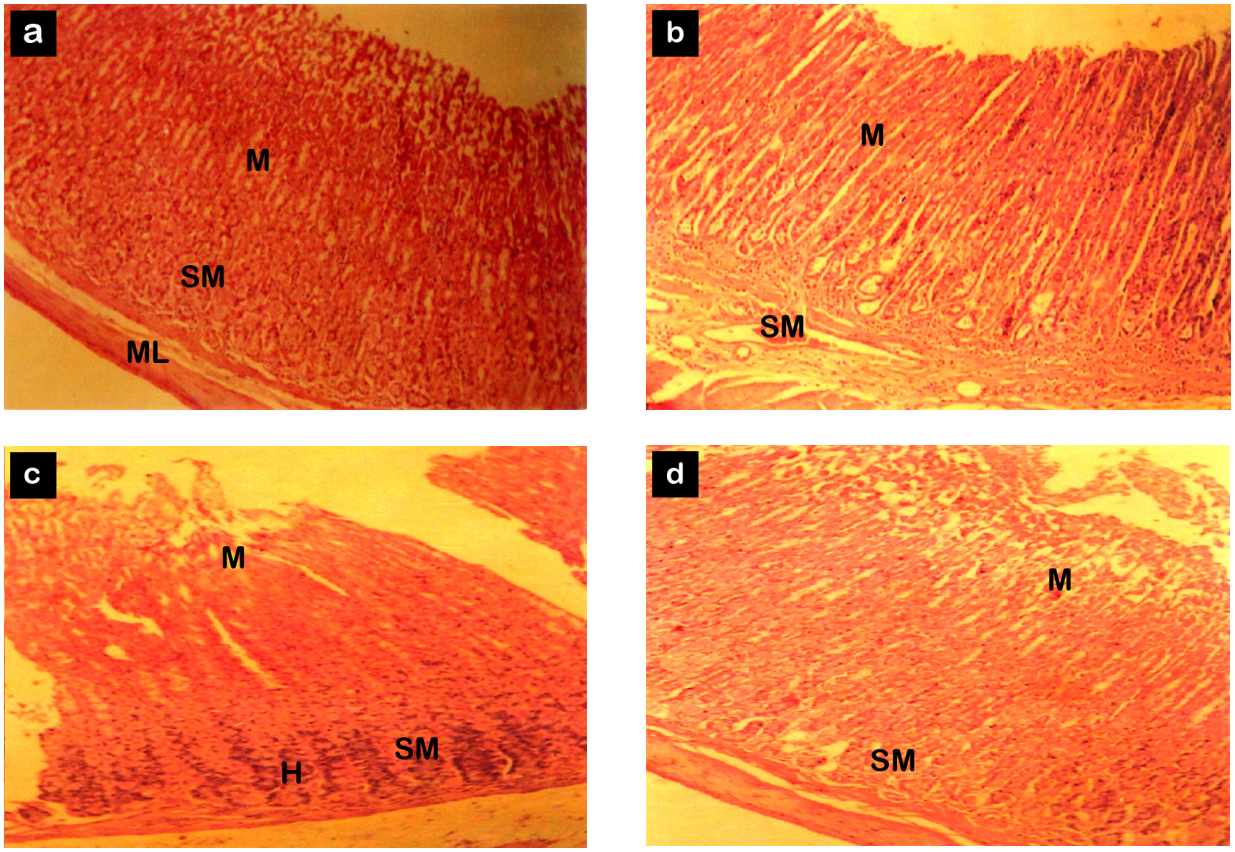

Figure 1: Photomicrograph of the stomach of rats from (a) group 1 (control), (b) group 2 treated with 40 mg/mL of Calabash chalk for 14 days, (c) group 3 treated with 40 mg/mL of Calabash chalk for 21 days, and (d) group 4 treated with 40 mg/mL of Calabash chalk for 28 days (haematoxylin and eosin staining, 100× magnification). Both groups 1 and 2 showed normal mucosal and submucosal layers, whereas groups 3 and 4 showed oedematous morphological changes in the mucosal and submucosal layers. Abbreviation: M = mucosa, SM = submucosa, ML = muscle layer, H = haemorrhage.

Oesophagus

Group 1 (control) had a stratified squamous non-keratinised epithelium overlying loose connective tissue made up of blood vessels, glands, and plexuses. The submucosae contained glands. The muscle layer was made up of smooth muscle fibres, and the serosa was intact (Figure 2a).

Group 2 showed keratinised squamous epithelial cells displaying hyperkeratosis, acanthosis, and koilocytosis, and these cells overlaid a loose connective tissue consisting of blood vessels. The submucosa, muscle layer, and serosa were prominent (Figure 2b).

Group 3 showed stratified squamous keratinised epithelial cells displaying acanthosis, hyperkeratosis, and koilocytosis. The granular cell layers were prominent, and the basement membrane was intact. The submucosa, muscle layer, and serosa were unremarkable (Figure 2c).

Group 4 showed a prominent stratified squamous keratinised epithelial cell displaying hyperkeratosis, koilocytosis, and acanthosis. The granular layer was prominent, and few histiocytes and melanocytes were observed. The submucosa, muscle layer, and serosa were unremarkable (Figure 2d).

Discussion

The oesophagus and the stomach are vital organs that serve as conduits and temporary storage spaces for substances that enter the body via the oral cavity. The stomach, which churns and temporarily stores ingested substances, can absorb some substances, such as alcohol (9,10). Substances that are highly toxic may cause morphological changes in the mucosae of both the oesophagus and the stomach.

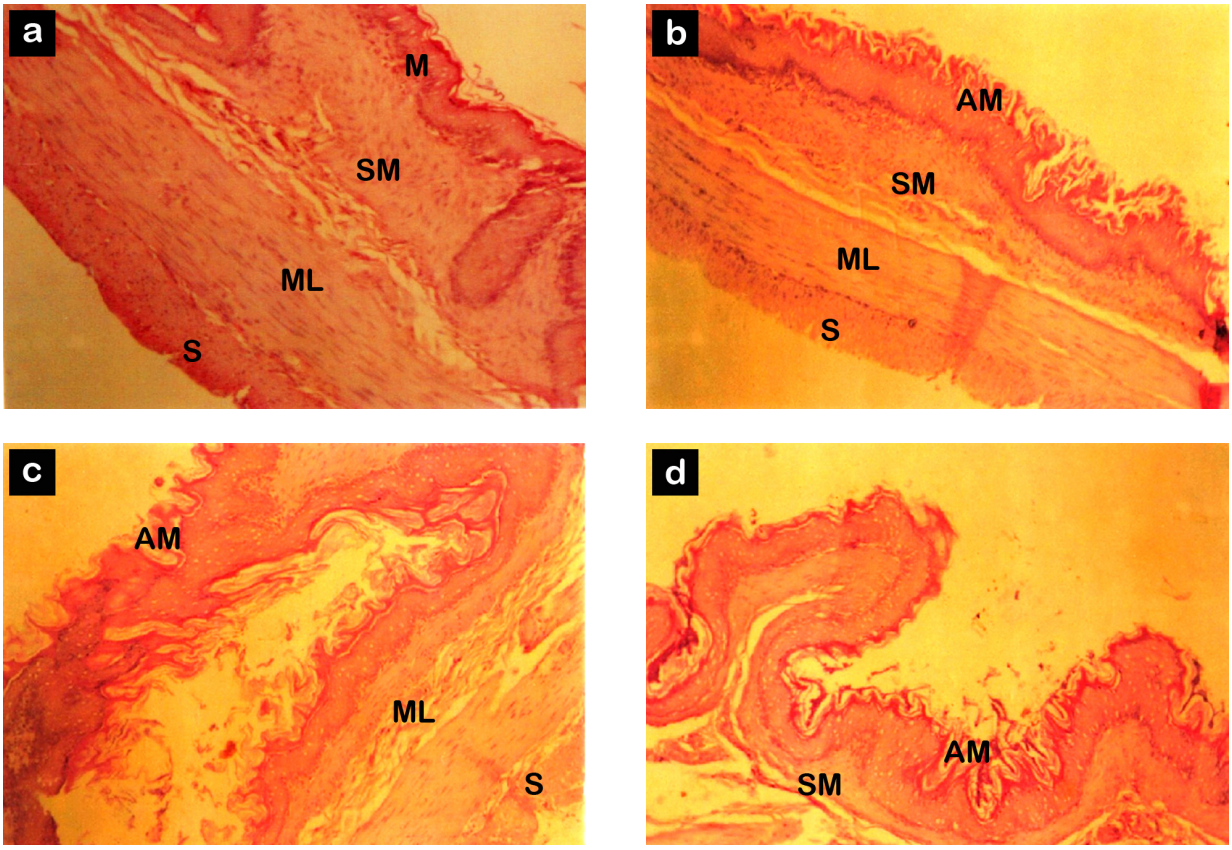

Figure 2: Photomicrograph of the oesophagus of rats from (a) group 1 (control), (b) group 2 treated with 40 mg/mL of Calabash chalk for 14 days, (c) group 3 treated with 40 mg/mL of Calabash chalk for 21 days, and (d) group 4 treated with 40 mg/mL of Calabash chalk for 28 days (haematoxylin and eosin staining, 100× magnification). Group 1 showed no histomorphological changes in the layers of the oesophagus, whereas groups 2, 3, and 4 showed changes in the mucosal layer. Abbreviation: M = mucosa, SM = submucosa, M = muscle layer, S = serosa, AM = acanthosis mucosa.

The results of this study revealed oedema and haemorrhages in the mucosa of the stomach, and acanthosis, hyperkeratosis, and koilocytic changes were found in the mucosae of the oesophagus of the groups treated with 40 mg/mL of Calabash chalk suspension. These findings indicate that the chalk may compromise the integrity of the gastro-oesophageal tract.

Calabash chalk contains poisonous substances such as lead, arsenic, aluminium, and alpha lindane (5,6). Lead intake has been implicated in gastritis, nausea, vomiting, and constipation (7) and has also been found to cause ulcers when large amounts are consumed (11,12). Arsenic has been implicated in gastric distress and stomach upset (13), and aluminium has been shown to be associated with constipation, impaction, colicky pain, anorexia, nausea, and gastrointestinal irritation (14,15).

In addition to the poisonous substances whose adverse effects have so far been reported (5,6), the chalk is reported to be largely composed of kaolin, a substance known to coat the gastrointestinal tract. Kaolin adsorbs drugs and other substances including toxins, which, in addition to reducing their bioavailability, initiates diarrhoeal episodes, thus providing bulk to the stool (16,17). The histological changes in the oesophagus and the stomach observed in this study may also arise as a result of kaolin. The oesophagus is prone to injury (18), and the presence of these substances may increase the susceptibility to injury. This increased susceptibility to injury may lead to further physiological and histomorphological damage to the stomach and the oesophagus due to the increased vulnerability to other toxins that may be consumed.

Superficial injury to the gastric mucosa has been reported to trigger an acute inflammatory response, characterised by an increase in blood flow, plasma exudation, and the recruitment of leukocytes into the mucosa. The objective of this response is thought to be to minimise tissue injury, facilitate the repair of the damaged tissue, and prevent the entry of foreign substances, including microbes and microbial products, into the systemic circulation (19). This inflammatory response is coordinated via the release of an array of soluble mediators from cells such as mucosal mast cells that act as "sentinels" within the mucosa (20). This could have been a reason for the results observed in this study. Morphological changes to the gastrointestinal tract can lead to pseudoneoplasms, a worrisome outcome of the gut's response mechanisms to injury (21).

A previous study (22) has shown that the Calabash chalk affected the liver, as there was fragmentation of the parenchymal cells, a reduction in the number of hexagonal hepatic lobules and dilated sinusoids when Wistar rats were treated with this substance. Another study (23) found that this chalk alters the normal concentration of haemoglobin, the red blood cell count, the platelet count, and the erythrocyte sedimentation rate in rats.

Conclusion

Calabash chalk caused histomorphological changes to the stomach and the oesophagus, which may lead to other pathophysiological changes and neoplasms of the gastrointestinal tract.

Authors' Contributions

Conception and design, final approval of the article: MBE
Analysis and interpretation of the data: EEJ
Drafting of the article: CCM, EIB
Critical revision of the article: MBE, TB

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Original Article

Association of Mitochondrial DNA 10398 Polymorphism in Invasive Breast Cancer in Malay Population of Peninsular Malaysia

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Abstract

Background: **The mitochondrial DNA (mtDNA) 10398 polymorphism is hypothesised to alter a mitochondrial subunit of the electron transfer chain and is associated with several neurodegenerative disorders and cancers.**

Methods: **In this study, an mtDNA polymorphism at nucleotide position 10398 was screened in 101 Malay female patients with invasive breast cancer and 90 age-matched healthy female controls using minisequencing analysis.**

Results: **The Malay women with the 10398G variant showed a significantly increased risk of invasive breast cancer (OR = 2.29, 95% CI 1.25–4.20, *P* = 0.007). Immunohistochemistry analysis was conducted to investigate the effect of this polymorphism on the levels of apoptosis in breast cancer cells. The level of Bax (a pro-apoptotic protein) expression was significantly higher than that of Bcl-2 (an anti-apoptotic protein) in patients carrying the G allele (*P* = 0.016) but not in those carrying the A allele (*P* = 0.48).**

Conclusion: **Based on these findings, we propose that the mtDNA 10398 polymorphism may be a potential risk marker for breast cancer susceptibility in the Malay population.**

Keywords: *breast cancer, DNA sequencing, genetic marker, mitochondrial DNA, oncology, single nucleotide polymorphism*

Introduction

Mitochondria are a major site and target of intracellular reactive oxygen species (ROS), which are a natural by-product of electron transport chain activity (1,2). Mitochondrial DNA (mtDNA) is vulnerable to the effect of these molecules and has a limited ability to repair itself. Therefore, the excessive formation and continuous accumulation of ROS could lead to a cellular stress response and the inhibition of apoptosis (3). Several findings (4–6) show that both defects and reduction in the apoptosis threshold can extend the life span of the cell, contributing to continuous proliferation that may lead to cancer development. However, the exact role of mtDNA mutations in inhibiting apoptosis, either by suppression of pro-apoptotic genes or by activation of anti-apoptotic genes, has not been defined.

Several mutations, including single nucleotide polymorphisms in certain genes in both the nuclear and mitochondrial genomes, are implicated in breast cancer susceptibility (7,8). An A to G polymorphism at nucleotide position 10398 in the mitochondrial genome causes a non-conservative amino acid substitution from threonine to alanine within the NADH dehydrogenase (ND3) subunit of Complex I (9,10). This particular polymorphism has also been reported to alter both mitochondrial pH and intracellular calcium levels (11,12); these alterations have been associated with the modulation of ATP production and apoptosis (13). The structural alteration and impairment of Complex I may lead to increased production of free radicals and has been associated with increased risk of several mitochondrial disorders, such as Parkinson's disease (14,15) and bipolar disorders (16).

The association of the mtDNA 10398 polymorphism in Complex I with breast cancer was first studied by Canter et al. (17), which showed that the risk of invasive breast cancer was significantly higher in African-American women carrying the 10398A allele compared with non-carriers. This polymorphism is also associated

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with an increased risk of prostate cancer in African-American men (18) as well as an increased risk of breast cancer and oesophageal cancer in individuals of Indian descent (19). However, the opposite was observed in Polish populations and non-Jewish European American populations as the frequency of the 10398G variant was significantly higher percentage in breast cancer patients of these groups compared with the controls (20). Similar results were obtained in a study of a non-Jewish European American population (21). Finally, the 10398G variant was reported at high frequency in several Asian populations (22,23). As different populations have a variable risk of breast cancer susceptibility associated with this polymorphism, in this work, we determined whether this association held in the Malay population of Peninsular Malaysia. The effect of this polymorphism on the levels of apoptosis in breast cancer tissues was studied through the expression levels of the pro- and anti-apoptotic proteins, Bax and Bcl-2, respectively, using immunohistochemical analysis.

Materials and Methods

Sample collection

Ethical clearance for this study was obtained from the human ethics committee, Universiti Sains Malaysia. A total of 101 paraffin-embedded breast cancer tissue samples of Malay females (from 2003 until 2009) were obtained from the Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia. Buccal swabs were collected from 90 age-matched healthy Malay females with no history of breast cancer in their family as controls.

DNA extraction and PCR amplification

Total DNA was extracted from buccal swab samples using a Puregene DNA Extraction Kit (Gentra System, US), while DNA extraction from paraffin-embedded tissues was performed using a QIAamp DNA FFPE Tissue Kit (Qiagen, USA), both according to the manufacturer's protocol. mtDNA fragments bearing the 10398 polymorphism were amplified by the polymerase chain reaction (PCR) technique using the following primers: 10342-F 5' CAT CAT CCT AGC CCT AAG TC 3' and 10518-R 5' GAA GTG AGA TGG TAA ATG CTA G 3'. The final PCR product was 176 bp in length. The following PCR thermal cycle conditions were performed: 94 °C for 10 min, followed by 30 cycles of 94 °C for 30 s, 49 °C for 30 s, 72 °C for 45 s, and a final extension step at 72 °C for 10 min.

Minisequencing

Minisequencing analysis was performed using the ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems, US). The PCR products were purified using 5 µL (1 unit/µL) of shrimp alkaline phosphatase (SAP) and 0.1 µL (20 unit/µL) of *Exo* I. The minisequencing reactions contained 1.0 µL (10 pM/µL) of HPLC purified minisequencing primer (5' CTA CAA AAA GGA TTA GAC TGA 3'), 3 µL of SNaPshot Multiplex Reddy Mix and 1 µL of purified PCR template. Single base extension of 25 cycles were performed on GeneAmp PCR System 9700 (Applied Biosystems, US) using the following conditions: 96 °C for 10 s, at 48 °C for 5 s, and 60 °C for 30 s. SAP (1 unit) was added to the post-extension product and incubated at 37 °C for 1 h. The product was then incubated at 75 °C for 15 min to deactivate the enzyme. The purified product was subjected to electrophoresis in ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, US). Each well contained 9 µL of Hi-Di formamide, 0.5 µL of minisequencing product, and 0.5 µL of GeneScan-120 LIZ size standard. The results were analysed using GeneMapper ID software.

Immunohistochemistry

Paraffin-embedded breast tissues were cut into 4 µm sections and mounted on poly-L-lysine treated microscope slides. After deparaffinisation and hydration, antigen retrieval step was performed using antigen retrieval solution (citrate buffer, pH 7.6) in a stainless steel pressure cooker. Samples were incubated for 30 min with either monoclonal anti-human Bcl-2 (Dako, US) or polyclonal rabbit anti-human Bax (Dako, US) overnight at 1:100 dilutions. The slides were then incubated with the corresponding biotinylated secondary antibodies for 30 min, followed by incubation with streptavidin–horseradish peroxidase solution for 30 min. The samples were subsequently incubated with the substrate, diaminobenzidine, in the presence of H_2O_2. After washing with Tris-buffered saline, the slides were counterstained and mounted. Negative and positive controls (tonsil tissue for Bcl-2 and Hodgkin's lymphoma for Bax) were included routinely.

Protein expression, visualised as a brown staining pattern, was assessed using light microscopy (Nikon, JP). The areas with the highest levels of antibody staining within the tumour sections were assessed at low magnification (40×) according to Van Diest et al.'s method (24) to obtain a general impression

of the overall distribution of tumour cells. The percentage of immunopositive cells (Figure 1) was quantitated at higher magnification (400×) (25,26) independently by 2 investigators without knowledge of the scoring data. The scoring method used was based on Umemura et al., (27) with some modifications: 1 (< 10%), 2 (< 50%), 3 (< 80%), and 4 (> 80%).

Statistical analyses

Statistical analyses were performed using the SPSS version 12.0 for Windows (SPSS Inc., Chicago, Illinois, US). Chi-square analysis was carried out to establish the association of the 10398 polymorphism with invasive breast cancer. The Wilcoxon signed ranks test was used to compare the expression of both Bax and Bcl-2 proteins in patients with 10398A and 10398G variants. A *P* value of less than 0.05 was considered significant.

Results

Statistical analysis revealed a significant correlation between the 10398 polymorphism and invasive breast cancer in the Malay samples (Table 1). A chi-square test indicates a *P* value of 0.007 ($P < 0.05$) with an odds ratio (OR) of 2.29 (95% CI 1.25–4.20). The frequency of the 10398G allele (73%) is much higher than that of the 10398A allele (27%) in breast cancer patients. The frequency of 10398G is also higher in patients (73%) compared with controls (54%).

Paraffinised tissues from 20 patients carrying the 10398A allele and 50 patients carrying 10398G allele were successfully immunostained for both Bax and Bcl-2 antibodies. The level of Bax (pro-apoptotic protein) expression was significantly higher than Bcl-2 (anti-apoptotic protein) expression in patients carrying the G allele ($P = 0.016$), but not in those carrying the A allele ($P = 0.48$) (Figure 2).

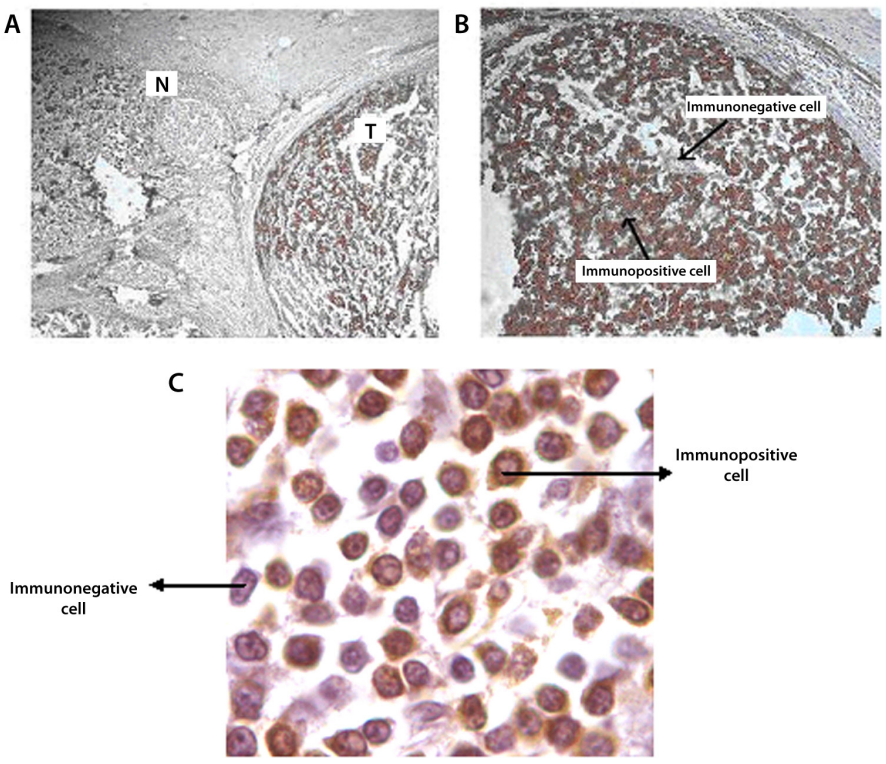

Figure 1: Immunohistochemistry staining of the Bax protein in breast cancer tissue. A: The protein expression levels (stained brown) were assessed in both tumour cells (T) and normal cells (N) (40× magnification). B: Immunopositive and immunonegative cells within the tumour sections (100× magnification). C: Immunopositive and immunonegative cells within the tumour sections (400× magnification).

Table 1: Frequencies of the mtDNA 10398 variant in the Malay population.

Allele	Patients (n = 101)	Controls (n = 90)	OR (95% CI)	P value
A	27 (27%)	41 (46%)	2.29 (1.252–4.200)	0.007
G	74 (73%)	49 (54%)		

Abbreviations: OR = odds ratio, CI = confidence interval.

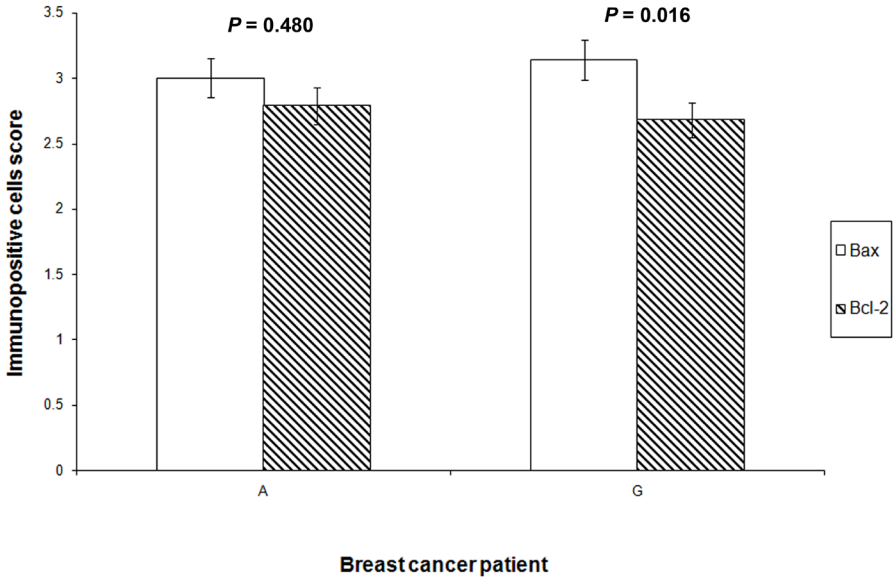

Figure 2: The expression of Bax and Bcl-2 (immunopositive score) in breast cancer cells in relation to the 10398 variant.

Discussion

Alterations or polymorphisms of the mtDNA have emerged as new biomarkers in population studies and for the detection of a variety of diseases and tumours (28,29). Various mutations in both the non-coding and coding regions of the mitochondrial genome are associated with an increased risk of breast cancer (29,30). Despite the established role of both nuclear and mitochondrial genes in breast cancer susceptibility, the list of genetic factors involved is still incomplete. More effort will be required to determine additional genetic alterations that would identify individuals with increased risk, who then would undergo intensive screening, prevention, and treatment programmes (28,29).

The mtDNA 10398 polymorphism has been reported to alter the ND3 subunit of the electron transport chain Complex I and to cause oxidative stress (9–12). Recently, an increasing number of studies have reported the significance of this polymorphism in cancers. However, the literature contains multiple conflicting reports regarding which nucleotide is associated with cancer. Several reports suggested that the 10398A allele is associated with breast cancer susceptibility (17–19), while others suggested the association of the 10398G allele with invasive breast cancer (20,21). The differing results may be due to the variability of risk modifiers that exist in diverse geographical areas (32–34).

In this study, we found that the 10398G allele can be considered a potential risk marker for breast cancer susceptibility in the Malay population. To the best of our knowledge, this study represents the first mtDNA polymorphism screening in breast cancer in Malaysia. Understanding the pathological impact of this polymorphism is important, and indeed, several papers have reported on the mtDNA 10398 polymorphism in cancer. Although no apoptosis data associated with this polymorphism are currently available, we hypothesise that the mtDNA

10398 polymorphism increases the production of ROS as a result of altered Complex 1 function, thus inhibiting the apoptotic mechanism. The mtDNA 10398G variant was found to be associated with breast cancer susceptibility in the Malay population of our study, suggesting that cells carrying the 10398G allele may express a high level of anti-apoptotic protein Bcl-2. It was reported that Bcl-2 expression increased in cells with respiratory defects involving Complex I (35,36). However, in the present study, Bax expression levels were found to be significantly higher than Bcl-2 expression levels in patients carrying the G allele, but the expression levels of these 2 proteins showed no significant difference in patients carrying the A allele.

This result is not in agreement with previous studies (37,38), which reported that levels of the pro-apoptotic protein Bax are low and undetectable compared with the significantly higher levels of Bcl-2 in human breast cancer. Other studies showed similar findings in which Bax was expressed along with Bcl-2 (26,39). We suggested that alterations in Complex I of the electron transport chain may cause dysfunction of proteins involved in apoptosis signaling cascades and sensitise normal cells to undergo apoptosis, allowing cancerous cells, which are protected against the induction of cell death, to become the dominant, surviving cells. The presence of anti-apoptotic proteins other than Bcl-2 in breast tumours may also counteract the suppression of cell death, presumably by forming heterodimers with Bax; however, excessive of anti-apoptotic proteins may also inhibit apoptosis by antagonizing the activated conformation of Bax (5,26,40,41). Based on this finding, we suggest that the 10398 polymorphism demonstrates a relationship between the levels of apoptosis and the incidences of breast cancer. Further understanding of this polymorphism at the molecular and biochemical level is necessary to elucidate its role in breast cancer.

Conclusion

From the present study, we can conclude that mtDNA 10398 polymorphism may be useful as a risk marker for breast cancer susceptibility in the Malay population. Intensive screening program for individuals carrying this polymorphism should perhaps prevent late detection and increase the chance of recovery. Significant increase of Bax expression observed in patients carrying the G allele may indicate its role in the apoptosis signaling cascades in breast cancer.

Acknowledgement

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Authors' Contributions

Conception and analysis, obtaining of funding, critical revision and final approval of the article, administrative, technical, or logistic support: ZZ
Provision of study materials: JH
Collection and assembly of the data, drafting of the article: TBN
Analysis and interpretation of the data: JH, TBN

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Original Article

Association of the Cocaine- and Amphetamine-Regulated Transcript Prepropeptide Gene (*CARTPT*) rs2239670 Variant with Obesity among Kampar Health Clinic Patrons, Malaysia

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Abstract

Background: **Cocaine- and amphetamine-regulated transcript (CART) is a hypothalamic anorectic neuropeptide that controls feeding behaviour and body weight. The study objective was to investigate the association of the CART prepropeptide gene (*CARTPT*) rs2239670 variant with obesity and its related anthropometric indicators among patients of a Malaysian health clinic in Kampar, Perak, Malaysia.**

Methods: **A total of 300 Malay/Peninsular Bumiputera, Chinese, and Indian subjects (115 males, 185 females; 163 non-obese, 137 obese) were recruited by convenience sampling, and anthropometric measurements, blood pressures, and pulse rate were taken. Genotyping was performed using *Ava*II polymerase chain reaction–restriction fragment length polymorphism.**

Results: **Genotyping revealed 203 (67.7%), 90 (30.0%), and 7 (2.3%) subjects with the GG, GA, and AA genotypes, respectively, with a minor allele (A) frequency of 0.17. No significant difference in the *CARTPT* rs2239670 genotype and allele distribution was found between obese and non-obese subjects, and logistic regression showed no association between the mutated genotypes (GA, AA) and allele (A) with obesity, even after adjusting for age, gender, and ethnicity. Furthermore, the measurements did not differ significantly between the genotypes and alleles. No significant difference in the genotype and allele distribution was found among genders, but they were significantly different among ethnicities (P = 0.030 and P = 0.019, respectively).**

Conclusion: ***CARTPT*** **rs2239670 is not a predictor for obesity among the Malaysian subjects in this study.**

Keywords: *cocaine- and amphetamine-regulated transcript protein, anthropometry, genetic association study, obesity, single nucleotide polymorphism, Malaysia*

Introduction

Malaysia has experienced a rapid phase of industrialisation and urbanisation throughout the last few decades; thus, the nation's lifestyle has also changed, leading to an increase in the incidence of obesity (1). This increasing trend has become a threat in developing countries including Malaysia, where the prevalence of the overweight and obese population has markedly increased from 16.6% to 29.1% and 4.4% to 14.0%, respectively, as reported by the Malaysian National Health and Morbidity Survey (NHMS) II (2) and NHMS III (3). Because the pathogenesis of obesity is multifactorial, the investigation of obesity-related genes involved in energy metabolism regulation, feeding behaviour, and body homeostasis has become a vital project (4).

The cocaine- and amphetamine-regulated transcript prepropeptide gene (*CARTPT*, Gene ID: 9607) encodes for the cocaine- and amphetamine-regulated transcript (CART) protein, which is one of the neuroendocrine modulators of energy balance present in the anorectic pro-opiomelanocortin (POMC)/CART neurons in the arcuate nucleus of the hypothalamus (5). The *CARTPT* is located at chromosome 5q13-14, and it produces long and short peptide fragments in rats; however, only the short form is found in humans, with 95% amino acid homology (6,7). The short peptide is believed to be a neuropeptide that regulates many physiological processes, especially those controlling feeding behaviour and body weight, and its mRNA expression is known to be induced with increased leptin levels (8). Also, CART is able to overcome the increase

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in appetite and feeding behaviour induced by neuropeptide Y, and vice versa (7).

Genetic variations in *CARTPT* may influence expression and/or function of the peptide and may associate with diseases/disorders. A missense mutation of G729C in *CARTPT* resulting in the substitution of leucine by phenylalanine at codon 34 has been identified in an early-onset obese patient (9), while the -156G/A variant of the 5' flanking region of *CARTPT* has been associated with obesity among Japanese subjects (10). However, 3 polymorphisms in the 3'-untranslated region (UTR), namely +1361 A>G (A1475G), 1457delA (rs5868607), and C1442G (rs1800926) that have been identified in Danish, Pima Indian, British, and Italian subjects showed no associations with variations in body mass index (BMI) (9,11–13). In addition to obesity, 3 previous studies have examined polymorphisms in CARTPT and their association with addictive behaviours. Jung et al. (14) showed association of the rs2239670 SNP (G5514A located in the first intron of *CARTPT*) with alcoholism in Korean subjects, but no association was found with bipolar disorder or schizophrenia. Furthermore, no association of rs35862863 and rs2239670 was found with methamphetamine dependence in Japanese subjects (15) nor was there an association of rs6894758, rs11575893, or rs17358300 with cocaine dependence in African–American subjects (16). Because neurobiological research has shown commonalities in brain reward processes between obesity and substance abuse disorders (17), *CARTPT* variants associated with substance abuse might also be associated with obesity.

Due to the lack of data on the association between the *CARTPT* rs2239670 variant and obesity in the published literature, it is of interest to investigate this association in the Malaysian population. Therefore, the objectives of this study were to determine the prevalence of the *CARTPT* rs2239670 variant among the patients of a health clinic in Kampar, in the state of Perak, Malaysia, and to collect anthropometric measurements, blood pressures, and pulse rate as indicators of obesity in order to determine if there is an association between this gene variant and obesity and its related indicators.

Subjects and Methods

Subject recruitment and anthropometric measurements

A total of 300 subjects were recruited by convenience sampling from April to December 2010. They were visitors of the Kampar Health Clinic who had fasted overnight and were waiting for their blood samples to be collected by nurses for various biochemical tests ordered by the resident physicians. This cross-sectional study was registered under the National Medical Research Registry of Malaysia (NMRR-09-826-4266), and the protocol was approved by the Medical Research and Ethics Committee, Ministry of Health, Malaysia. Informed consent was obtained from all the respondents in this study, and the blood samples were taken in accordance with the World Medical Association Declaration of Helsinki (as revised in Seoul, 2008). Each subject's socio-demographic information including age, gender, ethnicity, marital status, occupation, monthly household income, and educational status were obtained. The ethnicities of the subjects were self-identified in 3 given choices: Malay/Peninsular Bumiputera (Orang Asli), Chinese, or Indian. The systolic blood pressure (SBP), diastolic blood pressures (DBP), and pulse rate were determined by using SEM-1 Model automatic blood pressure monitor (Omron, Japan) after the subjects had rested for 10 min. Height, waist circumference (WC), and hip circumference (HC) of subjects were measured with a measuring tape to the nearest 0.1 cm, and their waist-to-hip ratio (WHR) was calculated by dividing the WC by the HC. A bio-impedence body fat weighing scale, Model HBF-362 KaradaScan body composition monitor with scale (Omron, Japan), was used to determine both weight and body composition such as percentage of skeletal muscle (SM), total body fat (TBF), visceral fat level (VFL), and subcutaneous fat (SF). BMI and resting metabolism (RM) were also obtained with the weighing scale. Subjects with a BMI of 27 kg/m^2 or above were considered obese (18).

Genotyping

Blood samples of approximately 5 mL were collected in EDTA anticoagulant tubes by medical practitioners. Genomic DNA isolation was conducted using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin, US) according to its technical manual and the amount of DNA isolated was quantified with a NanoPhotometer (Implen, Munich, Germany).

Polymerase chain reaction (PCR) was performed using MyCycler thermal cycler system with gradient option (BioRad, California, US) by adapting the conditions and reagent concentrations from the study by Jung et al. (14). The forward and reverse primers were 5'-CCCGAGCCCTGGACATCTACT-3' and

5'-CCGGACCCACACGCACTCT-3', respectively, and PCR was carried out under the following conditions: denaturation at 94 °C for 30 s, annealing at 69 °C for 30 s, and extension at 72 °C for 45 s for 35 cycles. The reaction cycles were hot-started with a single cycle of denaturation at 94 °C for 5 min and ended with a single cycle of extension at 72 °C for 10 min. Final concentrations of reagents for a single reaction was 1× PCR buffer without Mg^{2+} (Fermentas, Lithuania), 1 mM $MgCl_2$, 0.2 mM dNTP (RBC Bioscience, Taiwan), 1 µM of each *CARTPT* forward and reverse primers, 1 U/µL *Taq* DNA Polymerase (Fermentas, Lithuania), and 20 ng of DNA template. The restriction fragment length polymorphism (RFLP) analysis for the rs2239670 variant was performed by incubating the PCR products (approximately 394 bp) with *Ava*II (Fermentas, Lithuania) at 37 °C for at least 2 h. Both PCR and RFLP products were electrophoresed on 3% agarose gels in TBE running buffer and subsequently detected with ethidium bromide under ultraviolet illumination. The *CARTPT* rs2239670 wild-type G allele should be detected as an uncut products at 388 and 6 bp, and the mutant allele should yield bands at 278, 110, and 6 bp. The heterozygous subjects have both the G and A alleles, and should yield 4 bands at 388, 278, 110, and 6 bp. Figure 1 illustrates these 3 genotypes; however, the lowest band (6 bp) was not detected as it might have migrated out of the gel.

Statistical analysis

The data obtained from the questionnaire and *Ava*II PCR-RFLP genotyping were analysed using SPSS version 17.0 (SPSS Inc., Chicago, Illinois, US). Because a majority of the subjects had the *CARTPT* rs2239670 GG genotype and only a few had the AA genotype, the mutated genotypes (GA+AA) were combined with the wild-type genotype (GG) for Pearson's chi-square test. Then, the genotype and allele frequencies of *CARTPT* rs2239670 with respect to BMI status, gender, and ethnicity were assessed for association using Pearson's chi-square test. Associations between the genotype as well as the allele and obesity were examined using logistic regression (enter method; unadjusted and adjusted with age, gender, and ethnicity). Anthropometric measurements, blood pressures and pulse rates for each genotype and allele were checked for normality using the Shapiro–Wilk test and were compared using one way analysis of variance (ANOVA) and Student's *t* test for normally-distributed data (DBP and WHR) or Kruskal–Wallis and Mann–Whitney *U* tests for non–normally distributed data.

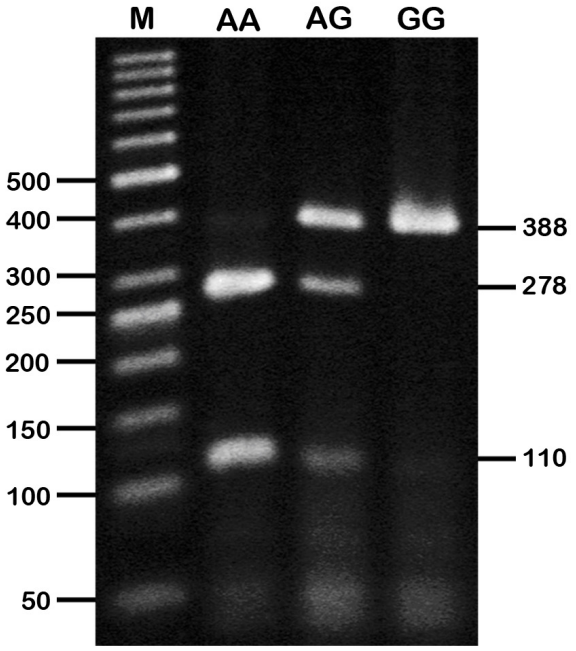

Figure 1: Genotyping of *CARTPT* rs2239670 variant by *AvaII* PCR-RFLP analysis. AA = homozygous mutated (278 and 110 bp), GA = heterozygous mutated (388, 278, and 110 bp), GG = homozygous wild type (388 bp).

A *P* value of less than 0.05 was considered statistically significant.

Results

The socio-demographic information collected from the 300 subjects ranging from 21 to 80 years old is shown in Table 1. The non-obese subjects had a mean age of 53.18 years (SD 15.85), while the obese subjects had a mean age of 51.46 years (SD 12.40). Overall, there were more non-obese than obese subjects, and more females than males. The majority of the subjects were Chinese (47.0%), followed by Malays/Peninsular Bumiputeras (32.7%), and Indians (20.3%), which is reflective of the Kampar population. Additionally, more than half of the subjects were retired or not working (54.7%), or had a monthly household income of lower than RM1000 (54.0%). A third of the subjects had at least a primary-level education. According to this study, the prevalence of obesity was higher among females, Malay/Peninsular Bumiputeras, subjects 51–60 years of age, and those having a monthly household income of less than RM1000.

Table 1: Frequencies of the mtDNA 10398 variant in the Malay population.

Variables	Non-obese (n = 163)		Obese (n = 137)	
Sex				
Male	68	(41.7)	47	(34.3)
Female	95	(58.3)	90	(65.7)
Race				
Malay/Peninsular Bumiputera	37	(22.7)	61	(44.5)
Chinese	93	(57.1)	48	(35.0)
Indian	33	(20.2)	28	(20.4)
Age groups (years)				
21–30	22	(13.5)	9	(6.6)
31–40	8	(4.9)	16	(11.7)
41–50	32	(19.6)	33	(24.1)
51–60	37	(22.7)	47	(34.3)
61–70	44	(27.0)	27	(19.7)
71–80	20	(12.3)	5	(3.6)
Occupation				
Professional	3	(1.8)	6	(4.4)
White-collar	9	(5.5)	8	(5.8)
Blue-collar	23	(14.1)	25	(18.2)
Retired/not working	89	(54.6)	75	(54.7)
Own/others	39	(23.9)	23	(16.8)
Monthly household income (RM)				
Below 1000	91	(55.8)	71	(51.8)
1001–3000	56	(34.4)	55	(40.1)
3001–5000	10	(6.1)	9	(6.6)
Above 5000	6	(3.7)	2	(1.5)
Educational status				
No formal education	27	(16.6)	15	(10.9)
Primary	55	(33.7)	44	(32.1)
Lower secondary	27	(16.6)	30	(21.9)
Upper secondary	27	(16.6)	32	(23.4)
Pre-university	7	(4.3)	5	(3.6)
University	20	(12.3)	11	(8.0)

Numbers in parentheses are percentage of total non-obese or obese.

A significant difference in the distribution between genotypes and alleles among ethnicities but not obesity status groups and gender was found and is reported in Table 2. Anthropometric measurements, blood pressures, and pulse rates were grouped according to genotypes, and alleles to determine the effect of having the mutated genotype or allele on these obesity indicators. However, none of these anthropometric measurements were significantly different between the genotypes and alleles.

To further test for the direct association between *CARTPT* rs2239670 genotypes and alleles with obesity, logistic regression (enter

method) was performed, with and without adjustment for other confounders of obesity such as age, gender, and ethnicity (Table 3). Having a mutant genotype (GA or AA) or a mutant allele (A) did not appear to affect the obesity status of the subjects (all $P > 0.05$), with all odds ratios near 1.000. This result indicates that the subjects having the mutant genotypes or allele had nearly an equal chance of being obese compared with the wild-type genotype or allele.

Discussion

Based on the socio-demographic data reported herein, there were more female obese subjects compared with males. More specifically, the prevalence of obesity in Malaysian women was greater than that in Malaysian men (19), which is in accordance with one of the national studies on obesity among Malaysians that reported a significantly higher prevalence of obesity in

Table 2: Obesity status, ethnicity and gender frequencies, and body measurement values of the Kampar Health Clinic subjects according to their CARTPT rs2239670 genotypes and alleles

Allele	Genotype			*P* value	Allele		*P* value
	GG	GA	AA		G	A	
Obesity status							
Non-obese	111 (68.1)	48 (29.4)	4 (2.5)	0.862[a]	270 (82.8)	56 (17.2)	0.913
Obese	92 (67.2)	42 (30.7)	3 (2.2)		227 (82.8)	47 (17.2)	
Ethnicity							
Malay/Peninsular Bumiputera	71 (72.4)	26 (26.5)	1 (1.1)	0.030[a]	158 (84.9)	28 (15.1)	0.019
Chinese	85 (60.3)	50 (35.5)	6 (4.3)		220 (78.0)	62 (22.0)	
Indian	47 (77.0)	14 (23.0)	0 (0.0)		108 (88.5)	14 (11.5)	
Gender							
Male	77 (67.0)	36 (31.3)	2 (1.7)	0.836[a]	190 (82.6)	40 (17.4)	0.976
Female	126 (68.1)	54 (29.2)	5 (2.7)		306 (82.7)	64 (17.3)	
Measurements							
SBP (mmHg)[b]	138.33 (21.43)	140.43 (22.11)	135.00 (20.07)	0.731	138.71 (21.52)	139.70 (21.75)	0.602
DBP (mmHg)[c]	80.81 (11.13)	82.98 (10.26)	74.86 (8.90)	0.504	81.20 (10.98)	81.88 (10.39)	0.642
Pulse rate (mmHg)[b]	73.51 (14.58)	73.09 (13.38)	74.71 (14.51)	0.923	73.44 (14.34)	73.31 (14.34)	0.905
WC (cm)[b]	90.41 (11.34)	91.05 (11.18)	86.10 (12.79)	0.469	90.52 (11.29)	90.38 (11.40)	0.876
WHR[c]	0.89 (0.08)	0.90 (0.09)	0.87 (0.08)	0.640	0.89 (0.08)	0.89 (0.09)	0.128
Height (cm)[b]	159.50 (8.91)	158.23 (18.02)	156.64 (12.10)	0.590	159.27 (11.11)	158.01 (17.26)	0.755

Allele	Genotype			*P* value	Allele		*P* value
	GG	GA	AA		G	A	
	(58.81)	(13.43)	(15.68)		(53.44)	(13.62)	
BMI (kg/m²)[b]	26.90	27.17	27.04	0.945	26.95	27.15	0.756
	(5.06)	(4.91)	(5.35)		(5.02)	(4.91)	
TBF (%)[b]	33.44	33.44	33.29	0.961	33.44	33.32	0.824
	(7.08)	(6.72)	(6.86)		(7.00)	(6.67)	
SF (%)[b]	27.34	27.23	28.27	0.949	27.32	27.37	0.891
	(8.30)	(8.18)	(9.07)		(8.27)	(8.22)	
VFL (%)[b]	11.82	12.52	11.71	0.603	11.95	12.41	0.418
	(6.15)	(6.22)	(6.75)		(6.15)	(6.23)	
RM (kcal)[b]	1431.58	1451.04	1392.43	0.596	1435.11	1443.15	0.653
	(255.53)	(243.07)	(287.82)		(252.93)	(247.20)	
SM (%)[b]	24.91	24.82	24.80	0.993	24.90	24.82	0.973
	(4.17)	(3.90)	(4.42)		(4.11)	(3.93)	

Values for obesity status, ethnicity, and gender are in number of subjects (percentage). *P* values were determined by Pearson's chi-square test, significant at $P < 0.05$. [a] Pearson's chi-square test was performed for wild-type and combined mutant genotypes (GG and GA+AA) as some AA frequencies had values of less than 5.
Values for measurements are in mean (SD). *P* values were determined by [b] Mann–Whitney *U* or Kruskal–Wallis tests for non-normally distributed data and [c] Student's *t* test or ANOVA for normally distributed data, significant at $P < 0.05$.
Abbreviations: SBP = systolic blood pressure, DBP = diastolic blood pressure, WC = waist circumference, WHR = waist-to-hip ratio, BMI = body mass index, TBF = total body fat, SF = subcutaneous fat, VFL = visceral fat level, RM = resting metabolism, SM = skeletal muscle.

Table 3: Association studies of *CARTPT* rs2239670 genotypes and alleles with obesity in the Kampar Health Clinic subjects

CARTPT rs2239670	Unadjusted		Adjusted[a]	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Genotype				
GG	1.000			
GA	1.105 (0.241, 5.064)	0.898	0.702 (0.136, 3.624)	0.702
AA	1.167 (0.247, 5.514)	0.846	0.898 (0.169, 4.763)	0.898
Allele				
G	1.000			
A	0.977 (0.639, 1.492)	0.913	0.809 (0.511, 1.280)	0.365

[a]Adjusted for age, gender, and ethnicity.

females (13.8%) compared with males (9.6%) (2). This higher prevalence may be due to a majority of the females in the study being housewives, married, or having a lower educational level. Hence, there may be increased susceptibility to a sedentary lifestyle and reduced health literacy (19). Among the ethnic groups, obesity was more prevalent among Malays, as supported by the nationwide study by Rampal et al. (2). There was an increased prevalence of obesity in the 21–30 and the 51–60 age groups and a decreased prevalence in the 61–70 and the 71–80 age groups. These observations may be due to a shift towards a sedentary lifestyle as age increases. Meanwhile, there was a higher prevalence of obesity in subjects that were retired or not working compared with other occupational groups. This higher prevalence may be due to the sedentary lifestyles

and increased accessibility to food for those who were not working. Among the household income categories, the prevalence of obesity was the highest in subjects with an income below RM1000. There was also an increased prevalence of obesity in subjects with only primary-level education. According to Kee et al. (19), there is an inverse relationship between the prevalence of obesity with the level of education possibly because high educational attainment can affect a person's attitude towards body weight control, dietary pattern, and healthier lifestyle.

The *CARTPT* rs2239670 variant has shown a positive association with alcoholism in the Korean population (14), and because substance abuse and obesity share a common neurobiological basis (17), we sought to investigate the association of this common variant with obesity in our study. To the best of our knowledge, this study is the first to examine the prevalence of *CARTPT* rs2239670 and its association with obesity in the Malaysian population. According to our results, the genotype and allele distribution of this gene variant was similar to the study reported in another Asian population in Korea (14) where the GG genotype showed the highest frequency and the AA genotype had the lowest frequency; however, the minor A allele frequency in this study was approximately 2-fold lower (0.17 versus 0.30). The Korean study population of 877 subjects included patients with alcoholism, bipolar disorder, schizophrenia, and the respective controls, and the differences in study population number, demographics, and disease/disorder conditions might explain this discrepancy in the minor allele frequency. Both rs2239670 variant genotypes and alleles were not associated with gender, but their distributions were significantly different among the ethnic groups. Both chi-square analysis and logistic regression analysis (adjusted for age, gender, and ethnicity) showed no association between the *CARTPT* rs2239670 variant genotypes and alleles with obesity. In addition, the anthropometric measurements, blood pressures, and pulse rate were not significantly different among the genotypes and alleles. Taken together, the *CARTPT* rs2239670 variant may not be a predictor for obesity and its related body indicators in the Malaysian population, at least among patients attending the Kampar Health Clinic. This variant falls within the non-coding region, and the potential effects on obesity may be eliminated during mRNA splicing; however, it is not possible to conclude at present whether the observed molecular variant rs2239670 directly affects CART function or whether there is a functional variant that may have a direct effect on obesity and other related metabolic syndromes.

There have been a few association studies between other *CARTPT* polymorphisms with obesity or diabetes. Two variants in the 3'-UTR were first identified in the Caucasian population (1457delA and A1475G); however, these variants were not associated with obesity (11). Also, 1457delA (rs5868607) and another variant in the 3'-UTR, C1442G (rs1800926) were not associated with type 2 diabetes in Chinese (20) and obesity in Pima Indians (12), respectively. However, Yamada et al. (10) showed that the A-156G polymorphism in the promoter region was associated with obesity in the Japanese population. In exon 2 of the *CARTPT* coding region, Challis et al. (13) found that the Ser66Thr (serine to threonine substitution at codon 66) mutation was detected in 1 obese subject, but not in 100 unrelated Caucasian controls, while del Giudice et al. (9) reported that the Leu34Phe (leucine to phenylalanine substitution at codon 34) was associated with reduced resting energy expenditure as an obesity phenotype in a large family. All of these inconsistencies in findings from different populations warrant further association studies in the Malaysian population, not only involving obesity and metabolic syndrome, but also neuropsychiatric disorders (14) and substance abuse or addictive disorders such as alcohol (14,21), methamphetamine (15), cocaine (16), and nicotine (21) dependence.

There were several limitations in present study. First, there was not an equal distribution of subjects across age groups, as most patients of the clinic were elderly. Indeed, age could be one of the predictors of obesity. Moreover, the non-probability convenience sampling could have a risk of bias, where the target population may not be sufficiently generalised to assess the reliability of the data. In addition, the study population was heterogeneous because different ethnic groups were included. A larger number of subjects with investigations based on separate ethnicities would help to define the effects of variants on obesity and allow us to compare the results with other populations.

Conclusion

The rs2239670 variant of the *CARTPT* gene was not associated with obesity and its related indicators among the patients of the Kampar Health Clinic. This variant was neither a risk factor nor a predictor for obesity. Also, there was no significant difference in the genotype and

allele distributions among genders, but they were significantly different among ethnicities. This finding suggests that other genes or lifestyle and dietary factors may contribute to obesity among the Kampar Health Clinic patients.

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Obtaining of funding, critical revision and final approval of the article: YHS
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Analysis and interpretation of the data: LY, SHF, YHS
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Original Article

Comparison of Image Quality Criteria between Digital Storage Phosphor Plate in Mammography and Full-Field Digital Mammography in the Detection of Breast Cancer

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Abstract

Background: **Digital mammography is slowly replacing screen film mammography. In digital mammography, 2 methods are available in acquiring images: digital storage phosphor plate and full-field digital mammography. The aim of this study was to compare the image quality acquired from the 2 methods of digital mammography in the detection of breast cancer.**

Methods: **The study took place at the National Cancer Society, Kuala Lumpur, and followed 150 asymptomatic women for the duration of 1 year. Participating women gave informed consent and were exposed to 4 views from each system. Two radiologists independently evaluated the printed images based on the image quality criteria in mammography. McNemar's test was used to compare the image quality criteria between the systems.**

Results: **The agreement between the radiologists for the digital storage phosphor plate was $\kappa = 0.551$ and for full-field digital mammography was, $\kappa = 0.523$. Full-field digital mammography was significantly better compared with the digital storage phosphor plate in right and left mediolateral oblique views ($P < 0.05$) in the detection of microcalcifications, which are early signs of breast cancer. However, both systems were comparable in all other aspects of image quality.**

Conclusion: **Digital mammography is a useful screening tool for the detection of early breast cancer and ensures better prognosis and quality of life.**

Keywords: *breast cancer, comparative studies, early detection of cancer, mammography, radiology*

Introduction

Breast cancer is a common cancer in women throughout the world and is the leading cause of cancer death among Malaysian women (1). While metastasis to other parts of the body can occur through lymphatic and blood vessels (2) and causes fatalities, early detection can save lives. Therefore, various methods have been used for early detection, such as breast self-examination, mammography, ultrasound, and magnetic resonance imaging.

Digital mammography was developed from screen film mammography (SFM) over 4 decades ago. However, mammography has been used to detect, diagnose, and manage a variety of breast diseases (3). Mammography is a procedure used to produce X-ray images of the breast and is widely used as a screening procedure for the early detection of breast cancer (4). The main objective of a mammography examination is to demonstrate the internal structures of the breast in order to detect abnormalities (5) in both symptomatic and asymptomatic women.

However, no one modality is 100% accurate, and most SFM interpretations are reportedly in the range of 68%–92% accuracy (6). Therefore, cancers may have been missed due to false negative interpretations, resulting in an increase in the mortality rate of breast cancer patients. Thus, there is an urgent need to determine a modality that is more accurate for breast cancer detection

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with the introduction of digital technology. The transition of SFM to digital mammography has gradually shifted to the use of digital storage phosphor plate (DSPM), which indirectly converts X-rays to light and subsequently to digital signals, which may cause degradation of the image. However, full-field digital mammography (FFDM) directly converts X-ray energy to a digital signal without a loss in image resolution. Thus, there is a need to determine which digital mammography system is able to produce superior quality in mammography.

The term "image quality" is described as the ability to visualise the anatomy of the breast sufficiently. Early works reported in the European Guidelines on Quality Criteria for Diagnostic Radiographic Images for conventional mammography were released in 1996 (7) to address the criteria required for image quality, which is of paramount importance in mammography. The following year, the 3 image quality criteria most important in radiography were reported to be sharpness, contrast, and noise, which are also important criteria in mammography (8). With the technological advancement in the field of breast imaging, improvement in image quality criteria was observed.

Thus, the European Commission further redefined the criteria to incorporate the changes in mammographic clinical image quality of FFDM consisting of 12 image quality criteria and 8 physical characteristics of the image (9). The image quality criteria here refer mainly to the depiction of internal structures of the breast, whereas physical attributes consisted of contrast, sharpness, artefacts, and visualisation of microcalcifications and opacities. Similarly, in an article by the United States Food and Drug Administration (10), it is stated that image quality is affected by sharpness, contrast, brightness, artefacts, noise, and anatomical structures such as skin, glandular tissue, retromammary space, and microcalcifications.

Though there are multitudes of definitions on image quality, the ultimate goal of high quality mammograms are to enable "detection of lesions or microcalcifications suggesting of malignancy" (11). Hence for this study, image quality criteria were adopted from the Schueller et al.'s study (12), which consisted of brightness, sharpness, contrast, noise, artefacts, and detection of anatomical structures, such as skin, glandular tissue, retromammary space, and microcalcifications.

Sharpness refers to the outline or edges of structures that are clearly depicted. It has also been defined as the delineation of linear structures, feature margins, and microcalcifications, whereas noise was described as a visually striking mottle pattern (13). Noise causes interference with the appearance of an image that impairs the radiologist from interpreting the mammogram. Noise in SFM due to "quantum mottle" (14) was because of "fluctuation in the X-ray photons that are absorbed in the intensifying screen", but in digital mammography, it appears as graininess on soft copy display. Brightness refers to the clarity of the breast parenchyma that is being demonstrated.

Artefacts are foreign objects that are present in the area of interest (breast and armpit), such as talcum, antiperspirant, or "crimp marks" that are caused during film handling, which should not be present on the mammography image. Clinical presentation of artefacts was divided into the following 5 groups: related to patient, technologist, mammography unit, software and viewing condition, and others (15). The detection of microcalcifications, when present within the breast parenchyma, is suggestive of malignancy (5).

The image quality of mammograms is affected by the 9 criteria mentioned above (12), and when it is lacking in one of the image quality criteria, it affects the overall outcome of the image. It is believed that the shortcomings of SFM have been overcome with digital mammography. Ultimately, the goal of the chosen digital modality is based on its higher performance in detecting and diagnosing breast cancer with the intention of reducing mortality rate and providing various treatment options. Thus, the aim of this study was to compare the image quality of FFDM, which involves direct conversion, with that of DSPM, which involves indirect conversion in acquiring images.

Materials and Methods

A diagnostic comparative study was conducted at the National Cancer Society, Kuala Lumpur, for the duration of 1 year. Prior ethical approval was obtained from the Medical Research Ethics Committee, Ministry of Health, and the Research and Ethics Committee, Universiti Kebangsaan Malaysia. Recruited women were between 40 (16) to 69 years old for this study. The exclusion criteria were having a previous history of cancer, having breast implants, pregnant, or being on hormone replacement therapy.

The present study replicates the study design and methods previously employed in Vienna (12).

For the present study, a sample size calculation was based on the formula for sensitivity and specificity of the mammography system (17). A confidence interval of 95% with a level of accuracy of 10% was considered; the expected sensitivity/specificity was 70%/92%, as obtained from a previous study (18).

The calculated sample size required for sensitivity and specificity were 259 and 41 women, respectively. However, the estimated sample size possible for this study was 150 women due to the limited budget, manpower, and time. The sampling method used for this study was convenience sampling. Women who participated in this study gave informed consent and filled out a demographic form on personal data and risk factors. Two routine projections of each breast using both DSPM and FFDM were performed.

The mammography images were rated by 2 blinded, independent radiologists with 16–20 years of experience in the field of radiology. Image quality was evaluated based on 9 criteria: brightness, contrast, sharpness, noise, artefacts and detailed depiction of anatomical structures, such as skin, retro-mammary space, glandular tissue, and the detection of microcalcifications. Occasionally, a magnifying glass was utilised to verify the visualisation of microcalcifications in the breast.

The radiologists were given a guideline for image quality assessment using a 4-level ordinal scale (0 = not applicable, 1 = inadequate, 2 = moderate, 3 = excellent) to improve understanding and reduce discrepancies in the ratings between the radiologists. Level 1 (inadequate) indicates that the image quality criteria were insufficiently displayed on the mammography images. The differences in image quality assessment for level 2 (moderate) and level 3 (excellent) were moderately shown for the former yet were excellent for the latter in image quality criteria. Besides the 3 levels mentioned above, for the detection of microcalcification, level 0 (not applicable) was an extra score added to indicate absence or presence of microcalcifications, which was important to verify the status of malignancy in a mammography examination.

Statistical analysis

Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, Illinois, US). Descriptive and inferential statistics were performed for the data. For descriptive statistics, the frequency (percentage) of frequently appearing scores was computed, and for the inferential statistics, the McNemar's test for *P* value was performed to evaluate the association between the digital mammography systems and the image quality. A *P* value of less than 0.05 was considered statistically significant. The inter-rater agreement of observations was compared using an unweighted kappa and weighted kappa (19) using κ statistics, and $\kappa \geq 0.8$ was considered perfect, $\kappa = 0.61–0.8$ was considered good, $\kappa = 0.41–0.60$ was considered moderate, $\kappa = 0.21–0.40$ was considered fair, and $\kappa \leq 0.20$ was considered poor. With reference to the score of the radiologists, when both scored (0:0), (1:1), (2:2), or (3:3), the weightage is 100%. If there was a 1-level difference in scoring and the scoring was (0:1), (1:2), or (2:3), the weightage is 75%. If there was a 2-level difference in scoring and the scoring was (0:2) or (1:3), the weightage is 50%. Finally, if there was a 3-level difference in scoring and the scoring was (0:3), the weightage is 25%. The weighted kappa was used because of the ordinal scoring used for the image quality criteria and the detection of anatomical structures (detection of microcalcification).

Results

A total of 1200 mammography images from 150 participants were assessed independently by 2 radiologists. The number of women who participated in the study according to the age groups are shown in Figure 1. The frequencies of the excellent rating (score 3) for each mammographic view and image quality criteria for DSPM and FFDM are presented in Figure 2. DSPM and FFDM did not have excellent scores in noise, retromammary space, glandular tissue, and detection of microcalcifications in all views. However, Figure 3 shows a comparison of the total scores between DSPM and FFDM, in which 6 of the criteria are similar, while FFDM is superior to DSPM in brightness, depiction of anatomical structures and skin line, and detection of microcalcifications (Table 1).

There was moderate agreement with unweighted kappa (inter-rater agreement), $\kappa = 0.551$ and $\kappa = 0.523$ for DSPM and FFDM, respectively, between the 2 radiologists for image quality, but no weighted kappa could be computed. For the detection of microcalcifications, the present study showed a significant difference only in the mediolateral oblique views using McNemar's test ($P < 0.05$) where FFDM showed better detection. There was fair agreement with unweighted kappa for DSPM and FFDM between the radiologists whereby $\kappa = 0.259$ and $\kappa = 0.222$, respectively, for the detection of

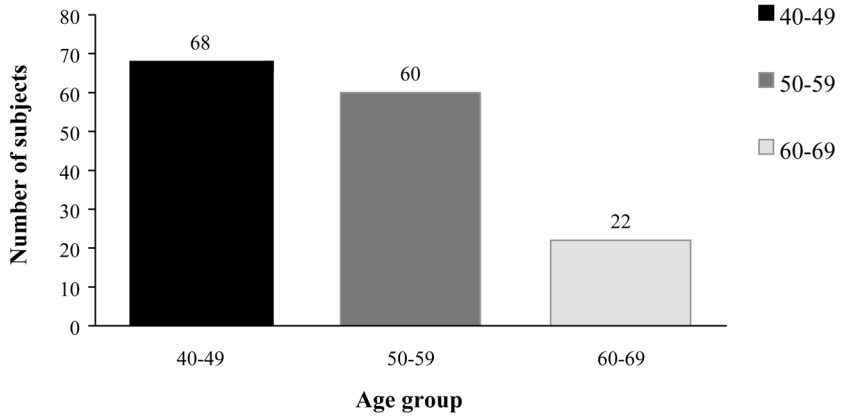

Figure 1: Distribution of subjects according to age group.

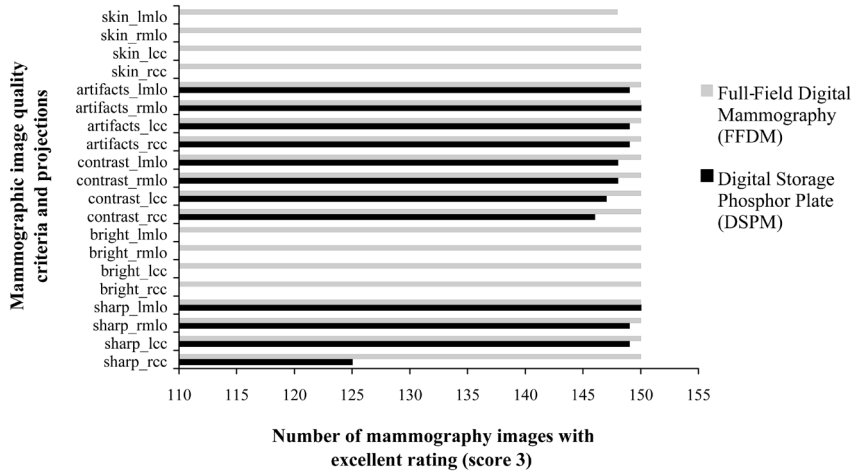

Figure 2: Frequency of the mammography image quality criteria with excellent rating (score–3). Abbreviation: RCC = right craniocaudal, LCC = left craniocaudal, RMLO = right mediolateral oblique, LMLO = left mediolateral oblique.

microcalcifications. Similarly, the weighted kappa also showed fair agreement (κ =0.300) for the detection of microcalcifications. All other image criteria did not show any significant differences ($P > 0.05$).

Discussion

All image quality criteria used in the current study are equally relevant to obtain a diagnostic mammogram. However, the detection of microcalcifications is an important criterion for the early detection of breast cancer. Though the presence of microcalcification itself is not cancerous, if it appears pleomorphic, linear, or fine and branching calcifications are observed, it is highly suggestive of malignancy (5).

Although the study hypothesis suggested that FFDM would be able to demonstrate superior image criteria to DSPM, the present study showed that FFDM presented better only in certain criteria ($P < 0.05$) compared with DSPM. In right and left mediolateral oblique views, FFDM was significantly better than DSPM in detecting microcalcifications ($P < 0.001$).

The number of samples used in the present study and the methodology employed were similar to the Schueller et al.'s study (12). However, the findings of this study did not replicate the findings of the former study. Contributory factors may have been that symptomatic subjects were used as well as a different study design in the former study (12).

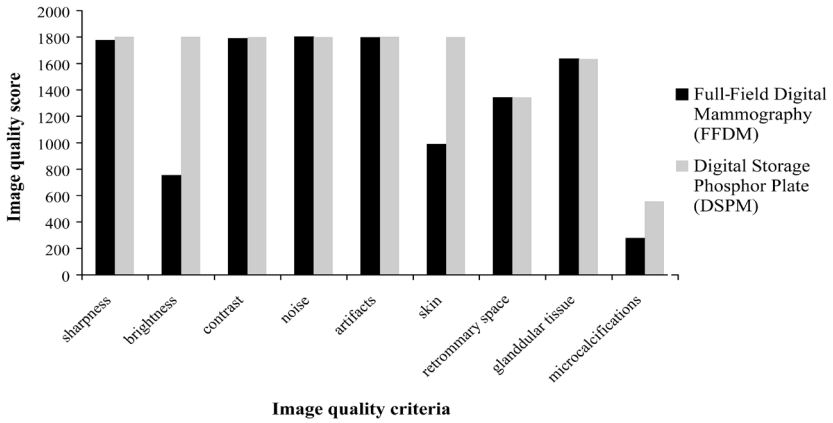

Figure 3: Image quality scores between digital storage phosphor plate and full-field digital mammography.

Table 1: Detection of microcalcifications within the digital mammography systems

Mammography projection	Mammography system	Percentage (%)	Number of subjects
RMLO	DSPM	14. 4	21
	FFDM	26. 0	38
LMLO	DSPM	12. 0	18
	FFDM	23. 3	35

Abbreviations: RMLO = right mediolateral oblique, LMLO = left mediolateral oblique, DSPM = digital storage phosphor plate, FFDM = full-field digital mammography.

In this current study, FFDM was only better in the detection of microcalcifications, whereas in the Schueller et al.'s study (12), FFDM scored significantly better in sharpness, contrast, and the detection of all anatomical structures ($P < 0.05$). They found FFDM to be significantly better in detecting microcalcifications, as detected in 85 women using FFDM compared with in 75 women using DSPM (12). Similarly, the present study also detected microcalcifications in 73 asymptomatic women using FFDM and 39 women with DSPM.

The American College of Radiology developed a landmark screening trial (18), which showed no significant difference between FFDM and SFM. However, FFDM was beneficial for women below 50 years of age, peri- or pre-menopausal women, and women with dense or extremely dense breasts. FFDM was significantly better in the depiction of microcalcifications, nipple, and skin, which is consistent with the current study (20).

Several studies comparing FFDM with SFM were conducted to assess the diagnostic efficacy within a screening population. To date, 4 landmark studies comparing the SFM with FFDM have been performed, and the cancer detection rate was not statistically significant (21–23). Furthermore, digital mammography was found to be equivalent to SFM (24,25) in breast cancer detection rate.

Breast cancer in dense tissue is difficult to detect especially with SFM. However, digital mammography with post-processing features easily overcomes this problem. A study performed in Japan (26) revealed that the detection of microcalcifications was better with SFM compared with DSPM, and later in 2003, improvement to the imaging plates resulted in comparable detection of microcalcifications between the 2 modalities. Continuous advancement has been ongoing to improve the capabilities of DSPM. The Healthcare's DX-S was introduced by Agfa, which provides better detail and improved image quality, reduction in patient dose, increased productivity, and shorter examination time for the patient (27).

The mammography imaging system alone will not be sufficient for acquiring high quality mammograms. Radiographers play a role in producing mammography images of good diagnostic value, which influences

the interpretation of the radiologist. Thus, radiographers involved in the imaging chain must be proactive to learn, unlearn, and re-learn their skill in performing mammography examinations. As for the radiologist, reader training is important to keep abreast with the dynamic changes in the field of imaging to ensure that standards are maintained, as they influence the outcome of the mammography examination (12).

The clinical importance of the findings of the present study revealed that both DSPM and FFDM were capable of depicting nearly all the image quality criteria specified, thus making it a suitable system for screening mammography. Furthermore, the capability of performing post-processing to manipulate the density and contrast with digital mammography makes it an exceptional system for women with dense breast tissue, which was previously a challenge for SFM.

The determination of whether these 2 digital mammography technologies are comparable or whether one is superior over another was a challenge. Based on the findings of the present study, DSPM and FFDM were comparable in image quality. Thus, the decision to select a system depends on the affordability, workload, and the future plans of the mammography facility. A screening centre would benefit from a digital mammography system because many women with dense breast tissue (below the age of 50 years) would be screened for early detection of breast cancer.

The major impediment in acquiring the FFDM is its exorbitant cost, which is approximately 3 to 5 times more than the SFM (28); however, the cost effectiveness of the equipment for the future and the continually evolving technology makes it a good investment. To join the trend towards using digital mammography at an affordable cost, DSPM is an option that should be considered. The results from various randomised clinical trials have suggested that the quality of mammograms affects cancer detection rates, the stage of detection and interval cancer rates, and FFDM has been shown to be beneficial for certain women, especially those with dense breast tissue. The benefits of FFDM was noted in the Digital Mammographic Imaging Screening Trial (29).

The limitation of this study is that there was no radiologist workstation for the DSPM system, and it was not compatible according to the Digital Imaging and Communications in Medicine standard to be networked to the FFDM workstation. To overcome this challenge, the DSPM images were compressed and saved into JPEG formats, but the resolution of the images were affected, and the interpretation of mammography images were performed on printed copies.

Depiction of fine microcalcifications and subtle soft-tissue masses on mammograms is the key to the detection of early breast cancer (23). Because breast cancer is the leading cause of death among women aged 40 to 50 years (2), an imaging tool that is accurate and reliable would definitely assist in the early detection of breast cancer. For this study to be more powerful, more time, a greater budget, and a larger sample size may have given a true reflection of the performance of these units among Malaysian women.

Conclusion

Digital mammography is rapidly replacing SFM because it is able to overcome the challenges of the SFM, the gold standard in breast imaging. Based on the image quality criteria of this study, both DSPM and FFDM systems were similar in most image quality criteria except for in the early detection of microcalcifications. In conclusion, both digital mammography systems were capable of producing mammography images of comparable quality due to their digital capabilities. Because mammography is a diagnostic tool to screen for the presence of abnormalities, further investigations, such as biopsy, should be performed on subjects with microcalcifications to confirm the status of true positives or the presence of cancer.

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Authors' Contributions

Conception and design, critical revision of the article: TRP, KCK
Analysis and interpretation of the data: TRP, KV, TS, KCK
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Original Article

Assessment of Prospective Physician Characteristics by SWOT Analysis

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Abstract

Background: **Thailand is one of the developing countries encountering medical workforce shortage. From the national registry in 2006, there were 33 166 physicians: 41.5% worked in the government sector, 21.6% worked in the private sector, and the remaining worked in non-medical fields. There is no current data to confirm the effectiveness of the national policy to increase physician production. We demonstrate our findings from the strength, weakness, opportunity, and threat (SWOT) analysis in medical students and the potential impact on national workforce planning.**

Methods: **We introduced SWOT analysis to 568 medical students during the 2008–2010 academic years, with the objective of becoming "a good physician in the future".**

Results: **Pertinent issues were grouped into 4 categories: not wanting to be a doctor, having inadequate medical professional skills, not wanting to work in rural or community areas, and planning to pursue training in specialties with high salary/low workload/low risk for lawsuit. The percentages of medical students who described themselves as "do not want to be a doctor" and "do not want to work in rural or community areas" increased from 7.07% and 25.00% in 2008 to 12.56% and 29.65% in 2010, respectively.**

Conclusion: **Further intervention should be considered in order to change the medical students' attitudes on the profession and their impact on Thai health system.**

Keywords: *health planning, health systems plans, medical students, manpower*

Introduction

Thailand is one of the developing countries encountering medical workforce shortage. According to the Thai Medical Council's latest registry in 2006, there were 33 166 physicians in the Thai health system. Of the total population, 41.5% worked in the government sector (12.6% at regional medical centres, 12.4% at general hospitals, and 16.5% at community hospitals), whereas 21.6% worked in the private sector; the remaining worked in non-medical fields (1,2).

Currently, there have been several measures implemented in order to produce more medical professionals in response to societal needs. For instance, the number of medical schools in Thailand rose from only a few to 18 medical schools in 2011. However, there is no data to confirm that increasing the doctors' population can efficiently serve the demand as expected.

The Faculty of Medicine, Chulalongkorn University, is one of the leading medical schools in Thailand, producing approximately 200 medical graduates per year, which is the highest among all medical institutions. Normally, the medical curriculum in Thailand requires a 6-year training, with an additional 1-year internship for every medical undergraduate and a 2-year government work according to the contract. From the faculty point of view, it has been noted that quite a high percentage of medical graduates were not present in the health system due to resignation from the hospitals before the end of their contracts or selecting further training in specialised areas that might not be needed by society. However, definite and up-to-date data are still lacking, and many agencies are searching for ways to obtain estimated data to verify the characteristics of medical workforce in the country.

An analysis of strengths, weaknesses, opportunities, and threats (SWOT) is one of the most popular analytical tools used by intelligence analysts. It can be used to analyse either individuals or agencies, and for strategic planning in either biomedical or public health fields (3–13). We have adopted this technique to assess the characteristics of 5th-year medical students with regards to their prospective physician status.

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Subjects and Methods

We retrospectively analysed the data retrieved from the SWOT assignments of 5th-year medical students from 2008–2010 academic years at Chulalongkorn University. The SWOT assignment process begins with an introduction of SWOT techniques to analyse the status of individual and organisation levels with pre-defined statements or objectives. In this case, we set the objective/desired state for medical students as becoming "a good physician in the future". The SWOT analytical matrix is shown in Table 1.

All medical students were also advised to assess themselves using the following questions: in what way can the strengths be used to achieve the objective, how can the weaknesses be shored up, what is the best way to take advantage of each opportunity to achieve the objective, and what needs to be done to mitigate each threat.

In a tactical way, the students were instructed to formulate strategies in order to achieve the objective/desired state by matching the factors, as follows:

1. Strengths/Opportunities: formulate the ways that will use strengths so that opportunities can be realised
2. Weaknesses/Opportunities: formulate the ways to address weaknesses in order to provide relief so that opportunities can be followed
3. Strengths/Threats: formulate the ways that use strengths "offensively" to moderate threats
4. Weaknesses/Threats: formulate defensive ways that will protect against threats

Content analysis was done by focusing on pertinent characteristics in strength and weakness assessments that are potentially related with the national health workforce planning system. In order to test if the SWOT analysis could be used as a situation assessment tool to demonstrate the medical students' attitudes toward their prospective careers, we set the hypothesis that the attitude of "do not want to be a doctor" should be less than 5%. The sample size was calculated by using the following formula:

$$\text{Sample size} = \frac{Z^2 \times (p) \times (1-p)}{d^2}$$

where Z = Z value (1.96 for alpha = 0.05), p = proportion of the attitude of "do not want to be a doctor" (0.05), and d = acceptable margin of error for proportion (estimated at 0.05).

For a finite population, the sample size was corrected using the following formula:

$$\text{New sample size} = n/[1+(n-1/\text{pop})]$$

where pop = population.

From the sample size calculation, 53 subjects were needed per class year. STATA version 10.0 (StataCorp LP, Texas, US) was used to analyse the data. This study was approved by the Chulalongkorn University review board.

Results

A total of 568 medical students undertook the SWOT analysis assignments during the 2008–2010 academic years: 184 (male:female = 85:99), 185 (male:female = 94:91), and 199 (male:female = 78:121) medical students in each year, respectively.

From the content analysis, pertinent issues with potential impact on the national workforce planning from SWOT analysis were grouped into 4 categories:

1. Do not want to be a doctor
2. Have inadequate medical professional skills
3. Do not want to work in rural or community areas
4. Plan to pursue training in specialties with high salary/low workload/low risk for lawsuit

The summary of the results is presented in Table 2.

Although there was no significant differences in the number of times these 4 categories were expressed by medical students over the 3-year period, the percentages of medical students who described themselves as "do not want to be a doctor" and "do not want to work in rural or community areas" were continuously increasing from 7.07% and 25.00% in 2008 to 12.56% and 29.65% in 2010. Additionally, no statistically significant difference was detected between males and females students in all 4 categories during the 3-year period.

Table 1: Analysis of strengths, weaknesses, opportunities, and threats (SWOT)

Environment	Supportive	Detrimental
Internal	Strengths are the attributes associated with him/her that are conductive to achieving desired state, i.e., a good physician in the future.	Weaknesses are the attributes associated with him/her that are detrimental or may prevent achieving the desired state.
External	Opportunities are the conditions (political/economic/social/ technological/environment/legal) that would assist achieving the desired state.	Threats are the conditions (political/ economic/social/ technological/ environment/legal) that might be detrimental to the way he/she carries out to achieve the desired state.

Table 2: Comparative figures of pertinent issues and 3-year statistics

Issue	Number of expression						χ^2 (df)	*P* value*
	2008		2009		2010			
	N/Total (%)		N/Total (%)		N/Total (%)			
	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)		
Do not want to be a doctor	13/184 (7.07%)		19/185 (10.27%)		25/199 (12.56%)		3.217 (2)	0.200
	8/85 (9.41%)	5/99 (5.05%)	10/94 (10.64%)	9/91 (9.89%)	11/78 (14.10%)	14/121 (11.57%)	4.546 (5)	0.474
Have inadequate medical professional skills	17/184 (9.24%)		22/185 (11.89%)		20/199 (10.05%)		0.735 (2)	0.693
	9/85 (10.59%)	8/99 (8.08%)	11/94 (11.70%)	11/85 (12.94 %)	12/99 (12.12%)	8/94 (8.51 %)	1.948 (5)	0.856
Do not want to work in rural or community areas	46/184 (25%)		48/185 (25.95%)		59/199 (29.65%)		1.187 (2)	0.553
	20/85 (23.53%)	26/99 (26.26%)	20/94 (21.28%)	28/85 (32.94%)	24/99 (24.24%)	35/94 (37.23%)	8.827 (5)	0.116
Plan to pursue training in specialties with high salary/low workload/low risk for lawsuit	37/184 (20.11%)		46/185 (24.86%)		47/199 (23.62%)		1.275 (2)	0.529
	14/85 (16.47%)	23/99 (23.23%)	22/94 (23.40%)	24/85 (28.24%)	20/99 (20.20%)	27/94 (28.72%)	5.441 (5)	0.364

* Pearson chi-square test

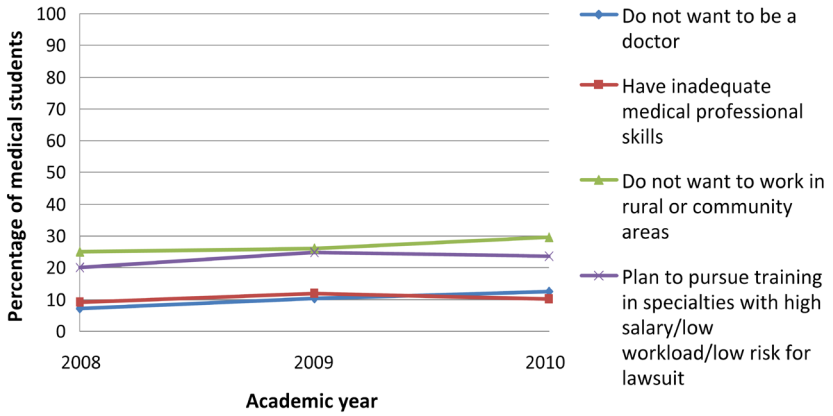

Figure 1: Three-year trend in 5th-year medical students

In terms of the total numbers of annual production, the results also showed increasing numbers of respondents expressing these responses in all 4 categories between 2008 and 2010. Comparative trends of all 4 issues from 2008–2010 academic years are shown in Figure 1.

Discussion

According to the latest national statistics in 2010, Thai medical schools have produced 1303 physicians to work in the government sector after graduation; 602 physicians (46%) resigned from the system after a 1-year period of internship. Although it has been estimated that the government sector currently needs at least 20 885 physicians, actual supplies are less than 8000(2).

Our study demonstrated the important issues that may affect the medical workforce planning of the national health system, such as a potential unwillingness to be a doctor, a feeling of inadequate medical professional skills, an unwillingness to work in the rural or community levels, as well as the desire to become specialists in high-salary/low-workload/low-risk-for-lawsuit environments.

It is possible for the results to be underestimated, since SWOT analysis was assigned to the medical students to complete with the provision of general guidelines and a common goal to be a good physician in the future, and some of them might not have wanted to reveal their attitudes toward the above issues. However, those pertinent expressions have been described by themselves, which, in turn, may indicate actual feelings or intentions at that point. Therefore, it should be of value to take those statistics into account for monitoring how much these attitudes will impact on prospective workforce planning.

At Chulalongkorn University, which presently produces the highest number of medical graduates in the country (200 per year), at least US$30 000 has been spent for each doctor during the 6-year training period (2). If this trend of unwillingness continues after graduation, theoretically, the government will have spent more than US$1.7 million on educating doctors who will finally work outside the medical field. Moreover, the availability of doctors in the rural areas will be threatened by a 30% loss of the number produced each year. The solution to these problems requires a more careful and up-to-date medical workforce planning.

Not only do critical workforce shortage problems exist, our results also showed the potential overflow of specialties with high salary, low workloads, and low risk for lawsuit such as dermatology, radiology, ophthalmology, and otorhinolaryngology. At present, ongoing surveys from the National Health Insurance Office reveals some evidence of oversupply for those aforementioned specialties in most of the urban areas in Thailand (these results will be available in a forthcoming publication). Planning for medical workforce value chain management with an appropriate strategy to balance demand and supply should be considered a priority at the national level.

Normally, the 5th year of medical training is the last year before clerkship in the 6th year. Necessary knowledge and medical professional skills should be gained before completing the 5th year. However, the national policy to increase medical workforce production has resulted in an increasing trend of having inadequate medical

professional skills among medical students. This situation might be caused by the lack of resources within medical schools that disproportionately impact an increasing number of medical students. This finding may reflect the necessity for medical schools and related authorities to be aware of the quality problem in new graduates and to develop a suitable strategy to deal with the impact of this problem on the health system in the future.

This study has some limitations, such as the necessity of the instructor to introduce the concept of SWOT analysis with simplistic examples to the medical students, and, although this is a simple technique to implement, the data (content) analysis is quite labour-intensive if used as a tool to assess a situation, such as in this study. We suggest that the SWOT analysis should be further adopted and tested in multi-centred settings, as well as compared with existing workforce statistics within interested countries.

Conclusion

Increasing trends in unwillingness to be a doctor and to work in rural areas were demonstrated in this study using SWOT analysis. Further interventions should be considered to deal with the changes in medical students' attitudes and their potential impacts on health systems.

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Conception and design, collection and assembly of the data, administrative, technical, or logistic support: TW
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Case Report

Pheochromocytoma and Pregnancy: A Difficult and Dangerous Ordeal

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Abstract

Pheochromocytoma during pregnancy is potentially disastrous to the mother and fetus. Its ambiguous presentation is often mistaken for pre-eclampsia, although it may imitate other problems during pregnancy. Early diagnosis and timely, appropriate management reduces possible maternal and fetal complications. We identified a case of pheochromocytoma during pregnancy; the condition was initially diagnosed as pre-eclampsia complicated with gestational diabetes. Surgical intervention via left adrenalectomy was successfully performed in the second trimester. After surgery, all of the patient's medical problems nearly subsided and she did not require further treatment. However, her fetus displayed restricted intrauterine growth, and the patient eventually had premature delivery via a caesarean section. A multidisciplinary team to identify and treat pheochromocytoma is mandatory to ensure optimal conditions for tumour removal and to anticipate any possible catastrophic events.

Keywords: *gestational diabetes, gynaecologic oncology, hypertension, pheochromocytoma, pregnancy, proteinuria*

Introduction

Hypertension in pregnancy is a common condition, and pre-eclampsia is the most common problem in primigravidae. Severe cases of pre-eclampsia may be symptomatic and cause significant maternal and fetal complications that require delivery of the fetus and placenta as an ultimate treatment.

Pheochromocytoma is a catecholamine-secreting tumour that is rare during pregnancy, with the prevalence of 1 in 54 000 pregnancies (1). Recognising pheochromocytoma antenatally is difficult because it may mimic pre-eclampsia and other problems during pregnancy. Therefore, the management of pheochromocytoma is a great challenge to healthcare providers. Failure to diagnose the disease and delays in providing necessary treatments may cause fatality and life-threatening situations for the mother and the fetus (2). However, pheochromocytoma is curable by removing the tumour. The ability to accurately identify pheochromocytoma during the antenatal period with timely and appropriate management reduces maternal mortality and fetal loss (1,3,4). In the current report, we discussed a case of pheochromocytoma that was diagnosed during pregnancy and the complexity of its management.

Case Report

A 29-year-old Malay woman (gravida 2, para 0 + 1) presented at 21 weeks of amenorrhea (POA) with uncontrolled hypertension and proteinuria. She was newly diagnosed with gestational diabetes mellitus with no prior medical illnesses. She remained asymptomatic but had episodes of palpitations. Her blood pressure (BP) was labile with episodes of tachycardia during admission. The first impression of this case was a pregnancy with pre-eclampsia. However, the differential diagnosis of pheochromocytoma was suspected based on the evidence of labile BP and difficult-to-control hypertension that was associated with tachycardia.

Further physical examinations revealed a pale woman with BP ranging 100–203 mmHg (systolic BP) and 73–120 mmHg (diastolic BP) with

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episodes of tachycardia. Her weight and height were 37 kg and 161 cm, respectively. No goitre was detected. Her pregnancy was grossly normal, and ultrasound scan parameters corresponded to the estimated dates (approximately 20 weeks of gestation).

She developed acute renal failure with worsening complications that were associated with gestational diabetes requiring insulin treatment. The highest serum creatinine level was 148 µmol/L, and the renal failure was eventually resolved with rehydration. Although her haemoglobin level was 7 g/dL, there was no evidence of occult bleeding. Left ventricular hypertrophy with diastolic dysfunction was evident from echocardiogram. Her thyroid function tests were normal. The 24-hour urine catecholamine test revealed elevations of noradrenaline at 1075 µg (normal range 12.1–85.5 µg) and adrenaline at 196 µg (normal range 1.7–22.4 µg). The abdominal MRI confirmed the presence of a left adrenal tumour that was 4.9 × 5.7 × 7.5 cm (Figure 1).

A multidisciplinary team consisting of obstetricians, endocrinologists, endocrine surgeons, and anaesthetists was involved in treatment management. Controlling the patient's BP was difficult because the BP remained labile. The treatment consisted of numerous antihypertensives, such as prazosin (20 mg once daily), labetalol (300 mg thrice daily), metoprolol (200 mg twice daily), bisoprolol (10 mg twice daily), and felodipine (10 mg once daily). Magnesium sulphate and phenoxybenzamine at 40 mg once daily were used to prepare for surgery. Fluid replacement and blood transfusions were provided during the management of the patient's labile BP and resulted in recurrent pulmonary oedema that required admission to the intensive care unit.

The patient's condition was acceptable at 24 weeks POA to perform an open left adrenalectomy. While anaesthesia was provided, her BP surged to 340/230 mmHg, which was successfully controlled by infusing glyceryl trinitrate and sodium nitroprusside. After the removal of the tumour (Figure 2), the lowest recorded BP was 70/40 mmHg. The patient was normotensive after 2 hours of noradrenaline infusion.

Post-operatively, the patient's BP was controlled with low doses of an antihypertensive agent, with normalisation of urine catecholamine levels. The patient and her fetus were in good health, although the fetus experienced intrauterine restricted growth, which was most likely attributed to all of the patient's complications.

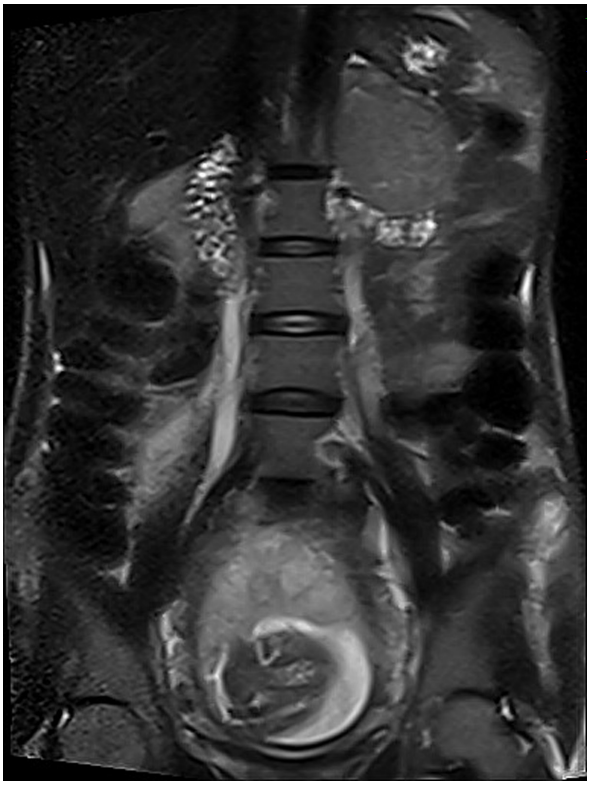

Figure 1: Sagittal section of T2-weighted magnetic resonance image showing the left adrenal pheochromocytoma and a fetus.

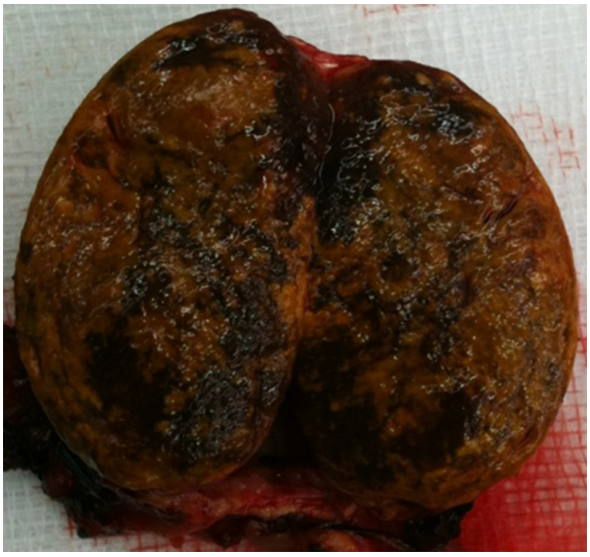

Figure 2: A section of the dissected left pheochromocytoma (70 × 40 × 20 mm).

Her pregnancy was monitored through outpatient antenatal visits. After the surgery, she gained 11 kg. Her hypertension and diabetes had improved and required no medication. In the 27 weeks POA, the growth of her fetus was below the 5th centile. The Doppler study of the umbilical artery flow was normal until 37 weeks POA. Then, an emergency caesarean section due to fetal distress was performed .

The surgery was successful and a baby boy was delivered, weighing 1.86 kg. The neonatology review was satisfactory. Her post-partum period was uneventful, with normal results for the modified glucose tolerance test.

Discussion

The classical presentation of pheochromocytoma with paroxysmal hypertension, headaches, sweating, and palpitation (5,6) may not be simultaneously present, especially during pregnancy. Therefore, the initial diagnosis of hypertension during pregnancy is frequently attributed to pre-eclampsia rather than pheochromocytoma (5,7). Our patient presented with hypertension and proteinuria at 21 weeks POA. At this time, we made the most appropriate diagnosis, especially for a pseudo-primigravida. Excess weight and increased weight gain during pregnancy predisposed women to pre-eclampsia, although sudden weight gain is rarely seen in pheochromocytoma (5). This patient was underweight at the start of her pregnancy. However, her weight increased after the adrenalectomy. Weight loss in pheochromocytoma is due to catecholamine-induced hypermetabolism, which might be the reason for the patient's low weight before surgery and subsequent weight gain after surgery with normalisation of catecholamine levels (6).

In addition to hypertension, altered renal function and proteinuria are caused by catecholamine-mediated renovascular abnormalities, which is common in pheochromocytoma (7). Some of the reported cases may display hyperglycaemia and had been treated as gestational diabetes (5,7,8), which was evident in this case. Episodic hyperglycaemia is most likely due to the effect of adrenaline-secreting pheochromocytoma. These data explained the initial misdiagnosis of this patient. Pheochromocytoma was suspected when the BP became labile and difficult to control, with episodic tachycardia. Pheochromocytoma-associated syndromes, such as medullary thyroid carcinoma, are currently being investigated in this patient, although she did not have any goitre. Further investigation for medullary thyroid carcinoma was not undertaken when she was initially admitted because her condition was not stable and she was pregnant.

Pregnancy itself would reveal the underlying pheochromocytoma through the enlarging uterus, changes of intra-abdominal pressure during fetal movements, compression of the growing tumour by pregnancy, stressful conditions related to delivery and anaesthesia that could have resulted in the release of catecholamines. The suspicion of pheochromocytoma in pregnancy, which was indicated by labile BP and difficulty in controlling hypertension, can direct the appropriate investigation to lead to an early diagnosis (7,8). Meanwhile, pulmonary oedema (6) may further complicate the management of this delicate hypertensive problem. The detection of high 24-hour urine catecholamine levels (2,9) and the imaging of the adrenal mass using magnetic resonance imaging (MRI), which is safe for the fetus as it requires no radiation, were used to diagnose pheochromocytoma in this patient and were the tools that were consistent with previous reports (2,5). Conversely, the ultrasound scan was reported to have poor sensitivity, especially during the 3rd trimester (2).

Because the disease was diagnosed at 22 weeks of gestation in this patient, the fetus was still developing. The fetal growth was restricted to lower than the 5th centile until 37 weeks. Similar cases of intrauterine restriction (8,10) have been reported and may be explained by the excessive production of catecholamines. Although catecholamines do not cross the placenta, the utero-placental insufficiency can occur through maternal vessels with the paroxysmal reduction and increment in blood pressure that may worsen intrauterine hypoxia.

The timing of the surgery is controversial and requires consideration on an individual basis. Surgery is less preferred during the 1st trimester due to the higher incidence of miscarriages. Adrenalectomy is recommended for 2nd-trimester cases. In the 3rd trimester, the surgery is delayed or often performed during caesarean section (6). General anaesthesia is preferred and recommended because most of the anaesthetic gases are safe for fetuses, except for halothane and desflurane (5). Although this is a definitive treatment, medical preparation is essential and can be achieved by using alpha-blockers (prazosin and phenoxybenzamine), beta-blockers, and other drugs, such as magnesium sulphate. Magnesium

sulphate is beneficial in the management of pre-eclampsia and pheochromocytoma. In pre-eclampsia, magnesium sulphate reduces the BP and acts to prevent eclampsia. In pheochromocytoma, magnesium sulphate inhibits catecholamine release from the tumour, blocks peripheral catecholamine receptors, and is a direct vasodilator (7). Almost every report of pheochromocytoma in pregnancy has diligently stressed the importance of correctly balancing vasodilatation and vasoconstriction to optimise the mother's condition and prevent fetal demise (5). Methyldopa, which is frequently used for hypertension during pregnancy, may worsen the symptoms of pheochromocytoma (5).

Diabetes, hypertension, and proteinuria in our patient were almost completely resolved after the adrenalectomy and are similar to the results that have been previously described (5,7). The ultimate aim was to prevent a hypertensive crisis (7) that is predictably disastrous to the mother and fetus. Utero-placental insufficiency affected the growth of fetus in this case. However, the fetus continued growing at the 5th centile. A caesarean section was performed due to fetal distress.

In conclusion, the commitment of a multidisciplinary team is of utmost importance, and individual patient consideration is essential during the management of this dangerous condition.

Authors' Contribution

Conception and design, collection, assembly, analysis, and interpretation of the data, drafting of the article: MINA
Provision of patient, critical revision and final approval of the article: MINA, ARR, AWN, MR, NAK

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Case Report

Two Different Surgical Approaches for Strangulated Obturator Hernias

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Abstract

Obturator hernia is a rare condition that may present in an acute or subacute setting in correlation with the degree of small-bowel obstruction. Pre-operative diagnosis is difficult, as symptoms are often non-specific. A high index of suspicion should be maintained for emaciated elderly women with small-bowel obstruction without a previous abdominal operation and a positive Howship–Romberg sign. When diagnosis is in doubt, computed tomography scan of the abdomen and the pelvis (if available) or laparotomy should be performed immediately, as high mortality rate is related to the perforation of gangrenous bowels. We present 2 cases of strangulated obturator hernia, managed differently with both open and laparoscopic approaches. The diagnostic accuracy of computed tomography scan is highlighted followed by a brief literature review with an emphasis placed on surgical management.

Keywords: *digestive system surgical procedures, gut, intestinal obstruction, laparoscopy, obturator hernia, X-ray computed tomography scanners*

Introduction

Obturator hernia is a rare type of hernia but is an important cause of intestinal obstruction in elderly, thin woman with concurrent medical illnesses. A high index of suspicion is important, as symptoms are often non-specific. A delay in the diagnosis will lead to a high mortality rate due to acute strangulation of the small bowel. Computed tomography (CT) has a good accuracy in diagnosing this disease and should be performed as soon as possible. We present 2 cases of obstructed obturator hernia, managed differently with both open and laparoscopic approaches. We also highlight the difficulty with clinical diagnosis and the use of CT for accurate diagnosis.

Case Report

Case 1

A 72-year-old woman presented with a 3-day history of colicky abdominal pain associated with bilious vomiting. Further history showed that she had had no bowel opening for 4 days but was passing flatus. For the past 2 to 3 years, she had severe intermittent colicky lower abdominal pain that usually lasted several hours but resolved spontaneously. She had no other co-morbidities.

Clinical examination revealed a dehydrated, relatively thin afebrile patient. The abdomen was soft, non-tender, and not distended. There was no palpable mass, and bowel sounds were present. Digital rectal examination revealed brownish stool with no mass felt. Chest and abdominal radiographs were normal with no free air seen under the diaphragm and no dilated bowels. Urgent upper endoscopy was normal, and urgent abdominal ultrasonographic assessment showed only minimal free fluids at Rutherford Morrison's pouch.

Routine blood investigations showed hyponatraemia with impaired renal function, which became normalised after adequate fluid resuscitation.

When attempts to initiate oral feeding resulted in repeated vomiting, a nasogastric tube was inserted for free flow, which drained more than 500 mL of brownish fluid within 12 hours. At that point, the abdomen was still soft and slightly tender towards the right lower quadrant on deep palpation. Bowel sounds were sluggish. An urgent CT scan of the abdomen showed bilateral obstructed obturator hernia (Figure 1). A decision for surgery was made.

At laparotomy, bilateral obturator hernia was identified, with the right side containing omentum and the left side containing a loop of strangulated small bowel entering the defect. The peritoneal cavity was contaminated with pus and slough. Gentle reduction revealed a segment

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of gangrenous small bowel with circumferential perforation, 80 cm from the duodenojejunal flexure. Small-bowel resection of the gangrenous segment was performed, and an end-to-end anastomosis was created. The obturator foramen was repaired bilaterally with nylon 1/0 in a purse-string fashion. The patient recovered uneventfully and was well during follow-up review.

Case 2

A 52-year-old woman with a background medical illness of end-stage renal failure on regular haemodialysis presented with 5 days' history of generalised colicky abdominal pain associated with vomiting clear fluid. She had had no bowel opening for 2 days and no flatus for 1 day. In addition, she also complained of right hip pain. Her past surgical history included a history of Mayo repair for obstructed paraumbilical hernia 1 year earlier.

On examination, the patient appeared to be well, with no signs of dehydration. The abdomen was distended but soft with no peritonitis. There was a supraumbilical midline scar with no cough impulse noted. Inguinal hernia orifices were normal. Rectal and right hip-joint examinations were unremarkable.

The abdominal radiograph showed dilated loops of small bowel. An initial diagnosis of small-bowel obstruction secondary to adhesion was made. When the patient did not improve after 2 days of drip and suck, a decision was made to proceed with CT scan of the abdomen. CT confirmed the findings of right obturator hernia with intestinal obstruction.

Upon laparoscopic assessment, a strangulated loop of small bowel was identified, 20 cm from the ileocaecal valve, with proximal dilation and distal collapse entering the right obturator foramen, which appeared congested and ischaemic (Figure 2). Laparoscopic reduction resulted in perforation of the ischaemic segment and spillage of bowel contents into the pelvis. Laparoscopic intracorporeal suturing of the right obturator foramen in the shape of a figure-8 was performed using polypropylene 1/0. A left obturator foramen was noted to be patent and was repaired similarly. A mini-Pfanenstiel incision was made to retrieve the ischaemic small bowel segment followed by extracorporeal resection and primary anastomosis.

The patient recovered uneventfully and was well during follow-up review.

Discussion

Obturator hernia is rare, accounting for 0.07% to 1.00% of all hernias (1,2) and 0.40% of bowel obstructions. A high index of clinical suspicion is required when small-bowel obstruction of unknown origin is encountered in emaciated elderly women, as delayed diagnosis and delayed surgical intervention are associated with high morbidity and mortality rates. The condition occurs most commonly in thin, elderly multiparous women, hence the nickname "little old lady hernia" (1). It is 9 times more common in females due to their wider pelvis, more triangular obturator canal opening, and greater transverse diameter (3).

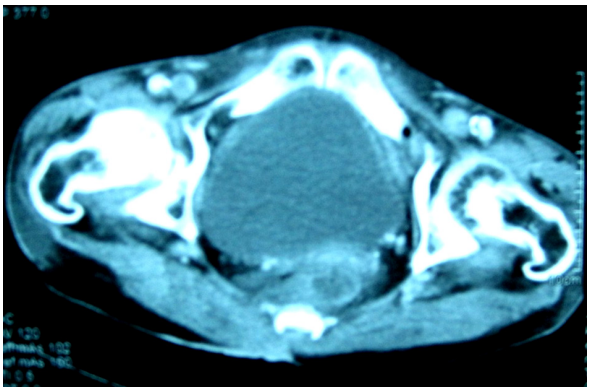

Figure 1: Computed tomography image of the pelvis showing bilateral obturator hernia.

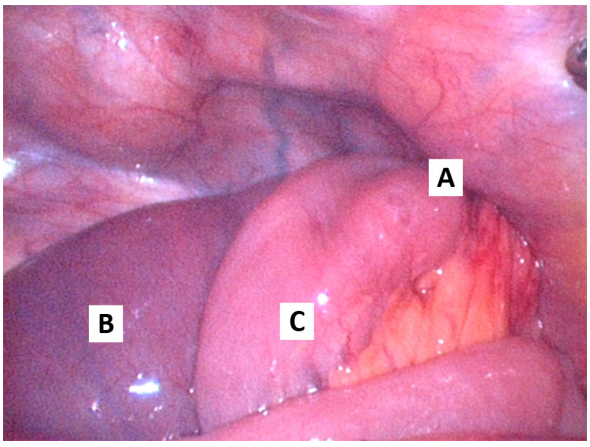

Figure 2: Laparoscopic view of strangulated obturator hernia. A: obturator canal, B: proximal dilated congested small bowel, C: distal collapsed small bowel.

Obturator hernia is a diagnostic challenge because the signs and symptoms are usually non-specific. The clinical course is usually manifested by one of the followings (4):

1. Acute small-bowel obstruction is the most common presentation, as seen in both our cases. The diagnosis is often made upon exploratory laparotomy for small-bowel obstruction of unknown aetiology, unless CT imaging of the abdomen and pelvis is performed before the surgery. The high frequency (41%–100%) of Richter's herniation of the small bowel into the obturator canal (3) causes partial obstruction without overt abdominal distension, as demonstrated in both our cases.
2. The Howship–Romberg sign, which is present in 15%–50% of cases (5), is characterised by pain in the medial thigh, less often in the hip, to the knee along the distribution of the obturator nerve. It is due to compression of the obturator nerve by the hernia sac and its contents. Typically, pain is exacerbated by extension, adduction, and medial rotation of the thigh and is relieved by flexion. It is present in our second patient but was overlooked as hip arthritis, as we lack familiarity with the condition.
3. Past history of recurrent attacks of intestinal obstruction that relieve spontaneously, as demonstrated in our first patient.
4. A palpable mass in the groin or during rectal and vaginal examination.

Various imaging modalities have been used in the diagnosis of obturator hernia. Herniography, plain radiography of the abdomen, ultrasonography of the inguinal region and inner aspect of the thigh, small bowel follow-through study, barium enema, and magnetic resonance imaging were reported in the literature, but CT of the abdomen and pelvis is the gold standard, with a 78%–100% diagnostic rate demonstrating the hernia and its contents in the obturator canal (2,6), and should be recommended for elderly patients with a non-specific small-bowel obstruction. In our case, CT scan confirmed the diagnosis prior to the surgery. Nevertheless, no single imaging modality should compromise operative intervention should patients require emergency laparotomy for clinical signs paramount to intestinal obstruction or peritonitis.

There are several open operative approaches described for the repair of obturator hernia. These include the abdominal, retropubic, obturator, and inguinal approaches (7). In the emergency setting, the abdominal approach via a low midline incision is most commonly favoured, as it allows adequate exposure of the obturator ring as well as the identification and resection of any ischaemic bowel. Closure of the defect by synthetic mesh is not to be encouraged in the setting of perforation and gangrenous bowel. In such cases, the sac is left in situ and the neck closed by non-absorbable purse-string sutures (7) or one or more interrupted sutures (3). Simple closure has an acceptable recurrence rate of less than 10% (3) and is being utilised in both our cases.

Laparoscopic surgery has a role in the management of obstructed obturator hernia, provided the necessary surgical expertise is available, as it is technically more demanding to perform laparoscopic surgery in an obstructed bowel due to limited intraabdominal space. Both transabdominal and extraperitoneal approaches have been described. The laparoscopic transabdominal approach is appropriate for the emergency setting, as it allows exploration of the abdominal cavity, diagnosis of the cause of the bowel obstruction, reduction of the hernia, thorough inspection and identification of ischaemic bowel, and resection of bowel if required (8). The laparoscopic total extraperitoneal (TEP) approach is more feasible if the diagnosis is established before surgery in symptomatic patients. More often than not, obturator hernia is detected during TEP repair for inguinal hernias. This reflects the importance of inspecting all the myopectineal orifices during the TEP approach to allow for the diagnosis and repair of asymptomatic obturator hernias (9).

Whatever the approach, the emphasis should be on rapid evaluation, adequate resuscitation, and early operative intervention to reduce morbidity and mortality (3). The high mortality rate, ranging 11%–50% (2,10), is directly related to the rupture of the gangrenous bowel in elderly patients with multiple comorbid illnesses. In our case, CT of the abdomen and pelvis confirmed the diagnosis, and we were able to intervene early, thus avoiding any morbidity and mortality.

In conclusion, obturator hernia is rare, and a high index of clinical suspicion allied with CT imaging is required for early diagnosis. The role of clinical examination is limited, as the signs are often non-specific. Once diagnosed, the transabdominal approach (either open or laparoscopic) allows the direct repair of hernia defects and identification and resection of any ischaemic bowel.

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Case Report

Management of Spontaneous Perforation of the Bile Duct in an Infant in a Semi-Urban Setup: A Case Report

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Abstract

Spontaneous perforation of the extrahepatic bile duct leading to biliary peritonitis is a rare occurrence once other causes of biliary peritonitis, such as trauma, choledochal cyst, stone diseases, and distal atresia of the bile duct, are ruled out. A 7-month-old male infant was brought to the hospital in critical condition with distension of the abdomen. He had a history of vomiting and diarrhoea, low-grade fever, and refusal to feed for 2 days. Signs of peritonitis were found upon examination. Due to the poor general condition of the patient, the case was taken up for laparotomy, and a diagnosis of spontaneous extrahepatic bile duct perforation was made intra-operatively. In the present case, the cause was idiopathic. An external drain was placed near the site of the leak for 2 weeks. The patient recovered well and was discharged on post-operative day 16. Disease awareness for correct pre-operative diagnosis and interventional planning is required to reduce mortality, morbidity, and complications in spontaneous perforation of the common bile duct.

Keywords: *bile duct diseases, extrahepatic bile duct, gut, perforation, peritonitis, spontaneous rupture*

Introduction

Biliary peritonitis is a serious intra-abdominal emergency. Spontaneous idiopathic perforation of the non-dilated extrahepatic bile duct is a rare finding in infants. The condition, first described by Caulfield in 1936 (1), may present acutely without any sign of previous biliary tract disease. The aetiology of perforation of the common bile duct (CBD) leading to biliary peritonitis, although unclear, is thought to include increased intraductal pressure, calculus erosion, necrosis of the duct wall secondary to the thrombosis, stenosis of the duodenal papilla, and abdominal trauma (2). Several techniques have been applied for pre-operative diagnosis, yet most cases are diagnosed intra-operatively (3).

Case Report

A 7-month-old male infant was brought to the Aakash hospital with complaints of distension of the abdomen, vomiting, intermittent diarrhoea, refusal to feed, and low-grade fever for the past 2 days, for which he was treated by a paediatric physician before coming to the hospital. There was no history of direct or indirect trauma to the abdomen. Upon examination, the patient was found to be lethargic, irritable, and pale, with mild icterus but without any signs of dehydration. Breathing was laboured with a respiratory rate of 50 breaths per minute, and pulse rate was 170 per minute. The abdomen was distended and tense, with shiny skin displaying superficial veins. Upon palpation, the abdomen was tender and rigid; rebound tenderness and guarding were also present. The lab findings revealed anaemia (haemoglobin level of 9 g/dL), leucocytes (total leucocyte count of 12.3×10^9/L), and mild jaundice (serum bilirubin level of 1.5 mg/dL, serum alkaline phosphatase level of 321.0 IU/L, serum glucose phosphatase level of 31.4 IU/L). A plain X-ray of the abdomen, with the patient in an erect posture, revealed the absence of pneumoperitoneum. A paracentesis was performed, and the aspirated fluid was found to be bilious. Due to a lack of modern diagnostic techniques and the poor general condition of the patient, the case was undertaken for an emergency surgery. Approximately 600 mL of bilious fluid was aspirated, and the peritoneal cavity was mopped dry. Aspirated fluid was sent for laboratory examination and was found to be sterile. After exploration, a small biliary leak was found at the junction of the cystic duct and the CBD (supraduodenal part). Thorough peritoneal lavage was performed with a large volume of warm saline. An external drain was placed near

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the site of the leak in the subhepatic space. Post-operatively, the patient was managed on adequate intravenous fluids and parenteral antibiotics (ceftriaxome sodium 100 mg/kg body weight, given intraperitoneally in 2 divided doses). On post-operative day 13, the drain ran nearly dry. The drain was removed on post-operative day 16. The patient was discharged with satisfactory recovery.

Discussion

Spontaneous perforation of the bile duct (SPBD) is a rare occurrence and most often seen in early infancy (2 to 20 weeks) with an almost equal sex ratio. However, a few cases have been reported in late infancy (4). The disease typically presents itself in previously healthy infants with unremarkable pre-natal and post-natal histories (5). Perforation commonly occurs in the anterior wall of the CBD near its junction with the cystic duct (4), as in our case, and is believed to occur due to congenital bile duct weakness, possibly due to a mural malformation during early embryogenesis (6). Eighty percent of cases present subacutely with fluctuating mild jaundice, normal to acholic stool, slowly progressive ascites, and abdominal distension, often associated with anoraexia, failure to thrive, fever, dark-coloured urine, and the development of umbilical, inguinal, or scrotal hernia. A history of biliary tract disease was absent; this absence of prodrome is notable and helps to distinguish SPBD from other causes of biliary peritonitis (7). Less commonly (20%), the disease presents acutely with abdominal distension of sudden onset, fever, vomiting, and signs of severe peritonitis (4).

In a number of cases, the diagnosis is made upon laparotomy, as in the present case, but can be easily substantiated pre-operatively (8).

Bilious ascitis, which is indicative of rupture of extrahepatic bile duct, can easily be diagnosed with the help of paracentesis (higher bilirubin level in the ascetic fluid than in the serum). Various investigations such as cholangiography, endoscopic retrograde cholangiopancreaticography (ERCP), magnetic retrograde cholangiopancreaticography, and biliary scintigraphy can reveal the site of the leak. ERCP has an additional interventional advantage, as stenting can be performed, and pancreatitis can simultaneously be ruled out (9). Early surgical intervention reduces mortality and morbidity (6). The aim of the treatment is to rule out distal obstruction and to establish adequate bile drainage. Various treatment modalities such as laparotomy with open drainage, percutaneous drainage, laparoscope guided drainage, and ERCP can be used, depending on the availability of the facilities, the presence of bile duct obstruction distal to the site of perforation, and the condition of the case. If the bile duct is obstructed distal to the site of perforation, T-tube insertion into the bile duct and open drainage can be performed with or without the help of a laparoscope. Alternately, ERCP with bile duct stenting may be performed (10). If the bile duct is patent distal to the site of perforation, any of the said modalities can be chosen, depending on the facility and the expertise available. Simple cholecystectomy can be performed if there is perforation in the cystic duct. In patients with biliary stricture, biliary-enteric bypass can be performed to avoid delayed sequelae, such as biliary cirrhosis and portal hypertension. Similar work has been reported in Northern region of India but in an urban, well-equipped setup (6,7,10). In rural or semi-urban setups, as in our case, where limited facilities are available, laparotomy with open drainage can safely be performed to save the patient's life. If the volume of drained fluid does not show a decreasing trend within 10 days, the patient may be shifted to a tertiary medical care centre, where ERCP and other modern facilities are available.

SPBD should be suspected in a patient presenting signs of peritonitis or bilious ascites, those without pneumoperitoneum (as determined by a plain X-ray of the abdomen taken with the patient in the erect position), and those without a history of biliary disease.

To conclude, disease awareness for correct pre-operative diagnosis and surgical treatment is central in reducing mortality, morbidity, and complications in SPBD patients.

Authors' Contribution

Conception and design: MJ, LS
Provision of patient, drafting of the article: SJ, MJ, DK
Collection and assembly of data: MJ, DK
Analysis and interpretation of the data: SJ, MJ, LS
Critical revision of the article: MJ, DK, LS
Final approval of the article: LS
Administrative, technical, or logistic support: SJ, LS

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Case Report

Gastric Duplication Cyst in an Infant Presenting with Non-Bilious Vomiting

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Submitted: 25 May 2011
Accepted: 18 Jul 2011

Abstract

In an infant presenting with a mass in the abdomen and non-bilious vomiting, duplication cyst needs to be considered in the list of differential diagnoses. Gastric duplication cyst is an uncommon occurrence in children. Diagnosis is based on clinical findings and imaging features. Surgical excision is safe and offers a complete cure. The literature recommends excision even in asymptomatic cases due to isolated reports of malignancy arising in the duplication cyst in later life.

Keywords: *abdominal neoplasms, cyst, differential diagnosis, gastrointestinal tract, paediatrics, vomiting*

Introduction

Vomiting in a child can occur due to various conditions. In the setting of an abdominal mass combined with vomiting, the aetiology is likely a surgical problem. Duplication cysts of the alimentary tract can present with the above features. The gastric duplication (GD) cyst in particular is an uncommon lesion, accounting for 4% of gastrointestinal tract duplications (1). To qualify as a duplication cyst, the following criteria need to be satisfied: lining with the gastrointestinal mucosa, attachment to the gastrointestinal tract, and the presence of a smooth muscle coat (2).

This case report outlines the presentation and management of a GD cyst.

Case Series

A 10-month-old baby girl presented with non-bilious vomiting and an abdominal mass that had been present for 2 weeks. The child was otherwise well. On examination, there was a large, firm, non-tender mass, which moved with respiration, on the right side of the abdomen. The child was well hydrated and was unremarkable upon systemic examination.

A cystic mass was identified on an ultrasound scan. Computed tomography contrast showed a cystic swelling associated closely to bowel mesentery (Figure 1). A possible diagnosis of mesenteric cyst was made. A cystic mass that was 8 × 6 cm in size, dumbbell-shaped, and arising from the greater curvature of the stomach, consistent with a cystic duplication (Figure 2), was excised extramucosally without gastric resection. A gastric lining in which all of the layers of the gastro-intestinal tract had a typical appearance was identified on histology, confirming GD cyst (Figure 3).

The child was discharged on post-operative day 4 and was well at the 6-month follow-up.

Discussion

The stomach ranks next to the small bowel and the oesophagus in the order of occurrence of gastrointestinal tract duplications. Depending on the location, the presentation varies from gastric outlet obstruction to asymptomatic occurrence (3). Pancreatitis has been reported to occur in the uncommon event of communication of the lesion with the pancreas (4). Of note is the acute presentation that can result from bleeding or perforation (5).

Most GD cysts present in infancy and infrequently in age extremes—in utero and among the elderly (6,7). It is supposedly more common in females. The baby girl in our case fits the epidemiology (8).

Contrast study may reveal indentation on the gastric wall, making identification possible (9). Computed tomography or magnetic resonance imaging can help to localise the cyst to its origin, but may not always, as in our case. Plain radiography of the abdomen may sometimes present findings suggestive of GD cyst, including soft-tissue interposition between the gastric shadow and transverse colon (10). Uncommon associations of GD cyst include lung sequestration and multicystic kidney (11,12). Extramucosal excision with preservation of the adjacent gastric wall is recommended, as was performed in our case. Surgical options include laparoscopic

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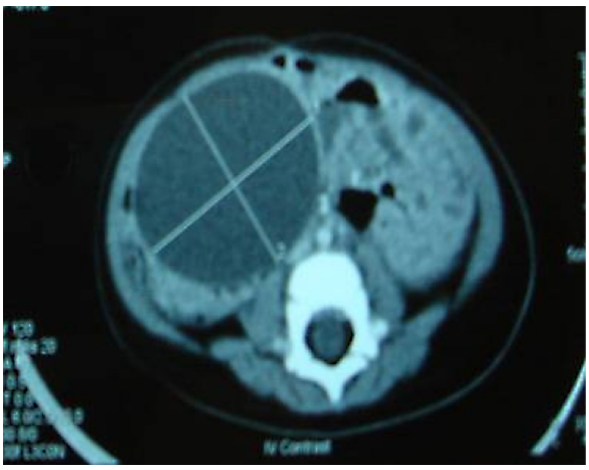

Figure 1: Abdominal computed tomography showing cystic mass occurring close to the bowel mesentery.

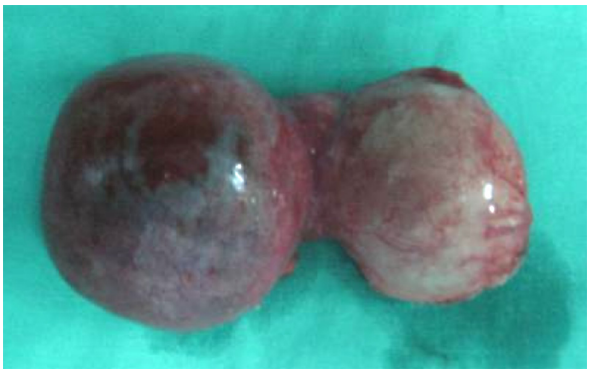

Figure 2: Dumbbell-shaped gastric duplication cyst.

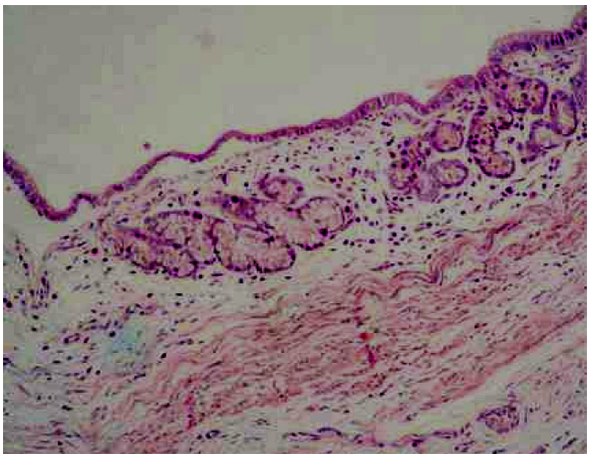

Figure 3: Single-layer columnar epithelium with underlying gastric mucosal glands (haematoxylin and eosin staining, 200× magnification).

excision and the endoscopic approach, depending on the expertise of the surgeons and location of the lesion (13,14). The dumbbell appearance of the gastric duplication in our case was unique and had not been described in the literature. In asymptomatic cases, excision is recommended due to the possible development of tumours such as adenocarcinoma or carcinoid in the GD cyst (15).

Antenatal diagnosis is sometimes possible, enabling expectant management in the post-natal period after appropriate evaluation. Occurrence in the thoracic region has been documented as a rare finding. The embryological aetiology is proposed to be due to faulty separation of the notochord from the endoderm resulting in enteric duplications (16,17).

The diagnosis of GD cyst is to be kept in mind during evaluation of an infant with an abdominal mass and vomiting. Imaging may be helpful but is not confirmatory. Excision with gastric preservation is usually possible, offering a complete cure.

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Letter To The Editor

Evaluation of Glucose and Energy Expenditure in the Acute Care of Severe Head Injury Patients: Indirect Calorimeter versus Harris Benedict Formula

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Submitted: 6 Dec 2011
Accepted: 11 Dec 2011

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Dear Editor,

I read with interest the paper by Rahmat Harun @ Haron titled "An Observational Study of Blood Glucose Levels during Admission and 24 Hours Post-Operation in a Sample of Patients with Traumatic Injury in a Hospital in Kuala Lumpur" published in the *Malaysian Journal of Medical Sciences*, Volume 18, Issue 4, 2011. The study reported associations between mild, moderate, and severe traumatic brain injuries and increased blood glucose levels during admission, and that the increases were based on the severity of the injuries. The blood glucose levels were not significantly changed after surgical intervention. I would like to highlight the value of glucose level and calorimetric measurements in monitoring traumatic head injury.

We recently did a prospective observational study in which severe head injury patients admitted to the Neuro Intensive Care Unit, Hospital Universiti Sains Malaysia, were selected for the measurement of energy expenditure by indirect calorimetry in an acute setting. A total of 31 severe head injury patients in Kelantan, Malaysia, who fulfilled the inclusion criteria were selected for this study from January 2009 to March 2010. The indirect calorimeter (Deltatrac II, Datex Division Instrumentarium Corp., Helsinki, FI) was connected to each patient's ventilator and the patient's energy expenditure was measured for 24 hours (2). The value of the measured energy expenditure (MEE) was compared with the value predicted from the Harris Benedict equation (3).

The patients were categorised into 4 groups according to the severity of the injury, as determined by Marshall's computed tomography grading (grades 1–4). The MEE of the patients in each groups were analysed and compared to see whether there were differences among them (4). In addition, the MEE of operated (major or minor surgery) and non-operated (conservative) patients were documented and analysed using specific statistical tests.

The means (SDs) MEE in the Marshall's grades 1, 2, 3, and 4 groups were 1440 (42), 1484 (349), 1358 (308), and 1595 (277) kcal/day, respectively. By using the Kruskal–Wallis test, there was no significant difference in the MEE among the severity groups in the acute setting ($P = 0.343$). The mean (SD) energy expenditure in the major operation group was 1535 (265) kcal/day, whereas the values in the minor operation and the conservative groups were 1113 (365) and 1565 (305) kcal/day, respectively. By using the one-way analysis of variance test, there was no significant difference in the MEE among the treatment groups in the acute setting ($P = 0.055$).

In this study, the lowest blood glucose level was 3.6 mmol/L and the highest was 9.2 mmol/L. The mean (SD) blood glucose level was 6.4 (1.4) mmol/L. Pearson correlation showed no association between blood glucose level and MEE ($r = 0.013$, $P = 0.943$). The Kruskal–Wallis and the one-way analysis of variance tests showed no significant difference in blood glucose level among the severity groups ($P = 0.432$) and among the treatment groups ($P = 0.830$).

In the absence of fever and sepsis, the MEEs in severe head injury patients who were fully sedated and immobilised were brought down to the basal levels equivalent to the basal energy expenditures calculated using the Harris Benedict equation (6,7). With respect to our specific objectives, we found no significant difference in the MEE among patients with Marshall's grading of 1, 2, 3, or 4. Similarly, there was no significant difference in the MEE among patients who underwent major operation, minor operation, or conservative treatment. Through this findings, we understood that the patients with severe head injury (Glasgow Coma Scale ≤ 8) were already in a homogenous group in whom the metabolic rate has reached its plateau, despite being subdivided into groups based

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on severity of injury as demonstrated by brain computed tomography (Marshall's grading). As for the blood glucose level, there was no significant association between the blood glucose level within 24 hours post-injury and the MEE, and there was also no significant difference in the blood glucose level among the Marshall's grading groups and among the operative/conservative groups.

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