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CONTENTS

Maternal deaths: Think of rare causes when common causes have been eliminated

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Introduction

In Sri Lanka, maternal mortality ratio is among the lowest when compared with other developing counties (1,2). Most deliveries take place in a health facility with the support of a skilled birth attendant. It is documented that during an hour, around 40 maternal deaths occur worldwide, whereas in Sri Lanka 40 women would die over a period of about 105 days (3). Once the common causes of maternal mortality have been controlled or eliminated, the uncommon causes come into picture.

Case 1: A 30-year-old pregnant mother with a period of amenorrhoea (POA) of 32 weeks was transferred from a peripheral hospital with vomiting and icterus for three days. She was suspected of having HELLP syndrome. Investigations showed abnormal liver, renal and clotting profiles. Emergency caesarian section was performed and after the delivery she developed profuse post-partum haemorrhage. Subtotal hysterectomy was done and she was transfused with blood, plasma and platelets. Her liver, renal functions and platelet count continued to deteriorate. A week after delivery she died in spite of ICU care.

At autopsy, yellow discolouration of conjunctiva, nail beds, pleural effusion and ascites was detected. All the organs were yellowish. Lungs were congested and heavy (Figure 1). Heart was flabby and Liver was enlarged. Kidneys were soft and enlarged with congested cortex. Histopathology revealed extensive pulmonary haemorrhages (Figure 2) and focal hepatic necrosis. Kidneys showed evidence of acute renal tubular necrosis.

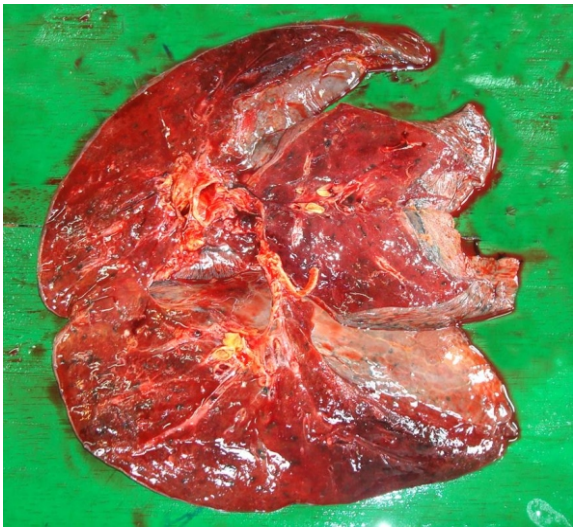

Figure 1: Congested lungs

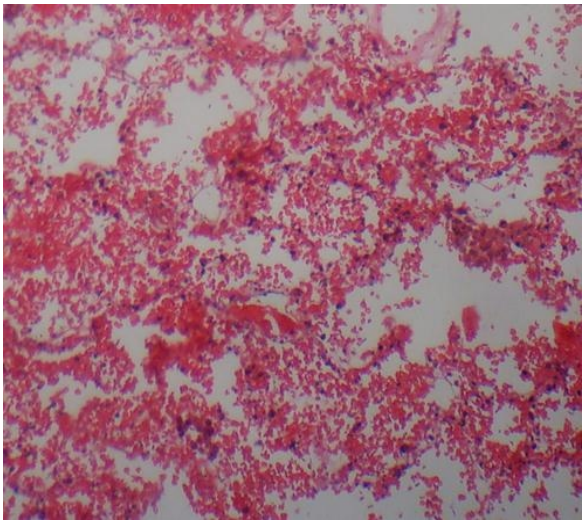

Figure 2: Pulmonary haemorrhages

Though the clinicians treated this mother as complicated HELLP syndrome with multi-organ failure, the possibility of leptospirosis was not considered. Microscopic agglutination of leptospira antibody test was positive with a titer of 1600. Cause of death was leptospirosis.

Although, HELLP syndrome is specific to pregnant state, leptospirosis is rarely described and it may mimic puerperal sepsis or hepatorenal failure (4). Though the most common causes of acute liver failure are viral hepatitis, drugs, and toxins, leptospirosis also can cause hepatitis and acute liver failure (5). Approximately 10% of those infected with leptospira develop jaundice with hepatocellular necrosis, and massive pulmonary haemorrhage (6). Early recognition and treatment would have prevented this death.

Case 2: A 23-year-old mother developed abdominal pain and convulsions two days after the delivery. Later she developed bile stained vomiting. Intestinal obstruction was suspected and emergency laparotomy was performed and necrotic bowel segments were removed. Her condition gradually deteriorated. At autopsy, faecal peritonitis and (Figure 3) infarctions in the spleen (Figure 4) were detected. Histopathology of the bowel revealed a preexisting vascular disease (vasculitis) with thickened vessel walls but the type of vasculitis could not be ascertained (Figure 5). Histopathology of the spleen also showed vasculitis with thickened walls.

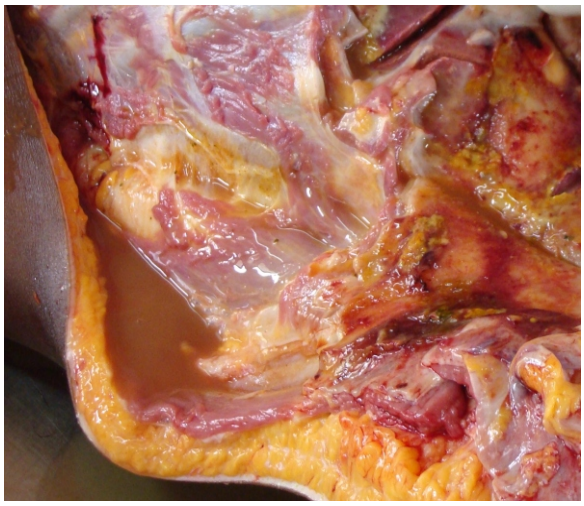

Figure 3: Faecal peritonitis

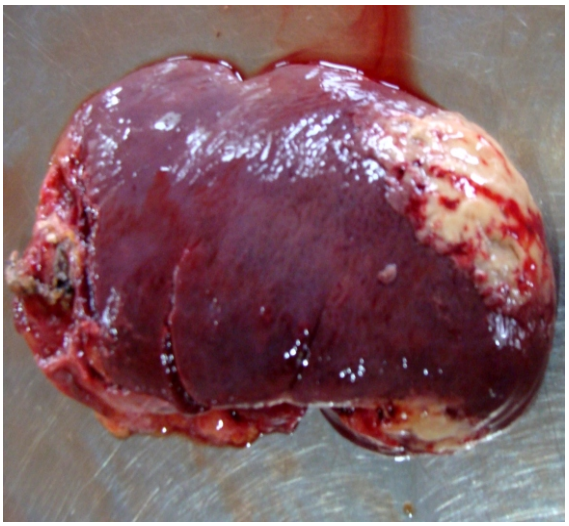

Figure 4: Infarctions in spleen

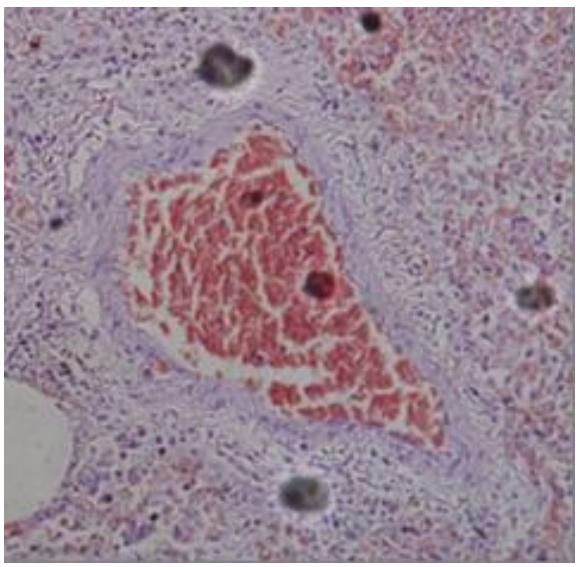

Figure 5: Vasculitis of bowels with thickened vessel walls

This patient had vasculitis and necrosis of the bowel resulting intestinal obstruction. Cause of deaths was septicemia due to generalized peritonitis following spontaneous bowel rupture due to vasculitis of the bowel. At the maternal mortality review, it was revealed that the patient had an episode of polyarthritis and defaulted treatment. Vasculitis can interrupt blood supply to tissues and organs causing tissue damage and even death. Splenic infarctions could be related to vasculitis (7). Some forms of vasculitis are self-limiting but others require treatment (8). In this case, the importance of obtaining a detailed history is highlighted.

Case 3: A 29-year-old mother developed vomiting, fever, abdominal pain, icterus and altered level of consciousness one week after delivery. She was admitted to hospital and managed as HELLP syndrome. In spite of treatment, her condition deteriorated. At autopsy, dissection of the ears showed evidence of chronic otitis media. Brain was congested with purulent discharge in subarachnoid space. Histopathology revealed acute pyogenic meningitis.

Altered level of consciousness was suspected as a complication of HELLP syndrome with multi-organ failure though the cause of death was pyogenic meningitis. Otitis media is a well recognized cause of pyogenic meningitis (9).

In conclusion, detailed history taking, meticulous investigation and multi-disciplinary analysis are important to prevent maternal deaths due to rare causes during post-partum period.

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Immune-haemolytic anaemia: An unusual etiology

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Case report

A previously healthy, 70-year-old woman was admitted to our medical unit with the complaints of gradual onset shortness of breath after moderate exertion and fatigability for one week duration. She had been well with good exercise tolerance until about one week before admission. She did not give a history of blood loss, jaundice or change of urine color. Her dietary history was satisfactory and the systemic inquiry did not reveal any significant findings. She had no past medical history of note and particularly not used tobacco, alcohol, herbal supplements or exposed to medications or toxins. There was no family history of similar diseases.

On admission to ETU she was severely pale and mildly icteric. There was no clubbing or lymphadenopathy. Her temperature was 98.6^0 F, respiratory rate was 8 breaths per minute and oxygen saturation 99% while breathing ambient air. Lungs were clear and the abdomen was soft without organomegaly. Auscultation of the heart was normal without extraneous sounds. The remainder of the examination was normal.

Laboratory tests revealed White cell count of 20,400/mm^3 with 45% lymphocytes, 6% monocytes, and 49% neutrophils. Rest of the blood count showed haemoglobin; 3.4 g/dL, haematocrit; 11%, MCV; 106 fL, MCH; 36pg, RDW-CV; 29% and platelet count of 268,000/mm^3. Smear showed the presence of spherocytes suggestive of either an autoimmune haemolytic anaemia or spherocytic haemolytic anaemia.

The *reticulocyte* count was 13% (Reticulocyte index 2.8%) while in the direct Coombs test IgG was positive and IgM was negative. Liver function tests showed a total serum bilirubin of 69.2 umol/l with an indirect fraction of 56.2 umol/l and normal liver transaminases. Urine urobilinogen was raised and urine hemosiderin was not detected. Donath-Landsteiner test was negative.

ESR was 65 mm 1st hr and CRP was 45 mg/dL. Anti nuclear antibody test and mycoplasma antibody were negative. Clinical findings and laboratory tests were consistent with an acute haemolysis and a working diagnosis of Warm type auto immune haemolytic anaemia was made.

Further investigations were carried out to identify the etiology of autoimmune haemolytic anaemia. The appearance of bone marrow was compatible with acute haemolysis with no evidence of concomitant lymphoma. Chest radiograph showed a homogeneous opacification in the left lower zone (Figure).

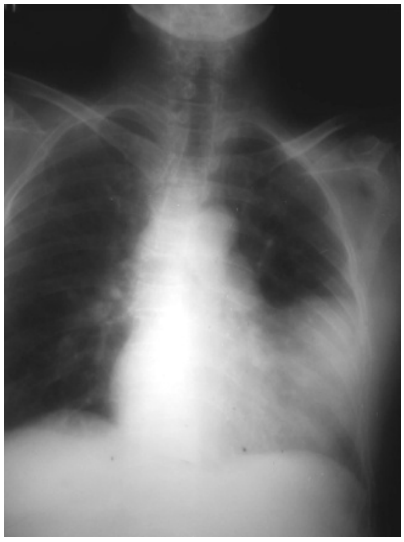

Figure: Chest radiograph showing a homogeneous opacification in the left lower zone

Ultrasonography of thorax revealed a well-defined mass measuring 7x5 cm extending laterally to the chest wall pleura and medially up to the left ventricle. CT scan thorax showed Stage $T_3N_0M_0$ malignant neoplasm in the left lingular lobe. Ultrasound guided biopsy confirmed the diagnosis of small cell carcinoma of the lung. In the absence of other causes, auto-immune haemolytic anaemia in this patient was attributed to the small cell carcinoma of the lung.

Her severe symptomatic anaemia necessitated blood transfusions. In order to eliminate the etiology patient was referred to oncologist for chemotherapy. Oral steroids was started initially for autoimmune haemolytic anaemia but later it was tailed-off while continuing the specific chemotherapy targeted for the malignancy (3). A year later she was found to be in remission of haemolytic anaemia after the successful treatment for small cell carcinoma of the lung. She did not need further blood transfusions.

Discussion

Autoimmune haemolytic anaemia (AIHA) is associated with auto-antibodies**.** The clinical manifestations of AIHA, depend greatly on the type of antibody that is produced by the abnormal immune reaction. Warm AIHA is commonly mediated through IgG auto-antibodies while the Cold AIHA is mediated through C3 component of the complements that binds the red blood cells coated with IgM antibodies.

While AIHA is commonly associated with hematological malignancies, it is an uncommon but well-described complication of solid malignancies (1-3). It is usually a late complication of malignancy and very rarely the presenting feature (2,3). Our patient's presenting features were related to haemolytic anaemia and she had no clinical features that could be attributed to the underlying lung carcinoma at the time of presentation.

Medline search revealed 53 cases of solid organ malignancies associated with AIHA reported between 1945 - 2009 (1). Of those, 9 cases were lung carcinomas and all the 9 cases were non-small cell carcinomas (1,4).

This is an unusual case of small cell carcinoma of lung presenting as haemolytic anaemia. We suggest that the search for underlying malignancies should be extended to the lungs in order to not to miss out lung malignancies. Our patient was referred for chemotherapy and after completing the chemotherapy, in one-year follow up she was found to be Coombs test negative and there was no evidence of ongoing haemolysis. Thus the casual link between the haemolytic anaemia and underlying malignancy was supported by the dramatic resolution of haemolysis after initiation of lung cancer directed treatment.

Therefore when a patient presents with haemolytic anaemia the search for underlying malignancies should be extensive and include solid malignancies as well.

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A postpartum woman with icterus: A case of G6PD deficiency in a female

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Introduction

G6PD deficiency is an X-linked recessive hereditary disease. G6PD is involved in the production of NADPH which is important in the prevention of oxidative damage of red blood cells (1). Since it is an X-linked disorder the clinical manifestations should be seen almost exclusively in males. However, heterozygous females will also be affected if there is an inactivation of normal X chromosome which is an unfavourable lyonization.

We present a case report of a 28-year old woman with G6PD deficiency, most likely due to random inactivation of normal X chromosome.

Case report

A 28-year-old woman in her second twin pregnancy at a period of amenorrhea (POA) of 36 weeks was admitted with three day history of fever, lower abdominal pain and dysuria. She was started on nitrofurantoin100mg six hourly to cover a possible urinary tract infection. During her stay at ward she had labour pains and an emergency caesarean section was done due to the indication of twin in labour. Two days after caesarean section she developed yellowish discolouration of her eyes. There was no change in the colour of urine or stools and she did not complain of pruritus.

Her past medical history was unremarkable other than for previous caesarean section two years back. She gave no history of neonatal jaundice, foreign travel or family history of anaemia or jaundice.

Examination was unremarkable except for mild icterus and conjunctival pallor.

Investigations revealed anaemia with haemoglobin of 7.3 g/dL and indirect hyperbilirubinaemia. Her reticulocyte count was 6% and LDH was 1911 U/L suggestive of an ongoing haemolytic process. Blood picture showed many bite cells, blister cells, fragmented cells and numerous polychromatic cells suggesting the possibility of underlying G6PD deficiency (Figure). In order to confirm the diagnosis sample of blood from patient's father was assessed for G6PD level and it was 0.40 U/g Hb (4.6 - 13.50). G6PD level of the patient was assessed three months later and it was found to be only 2.50 U/gHb. Genetic studies were not done.

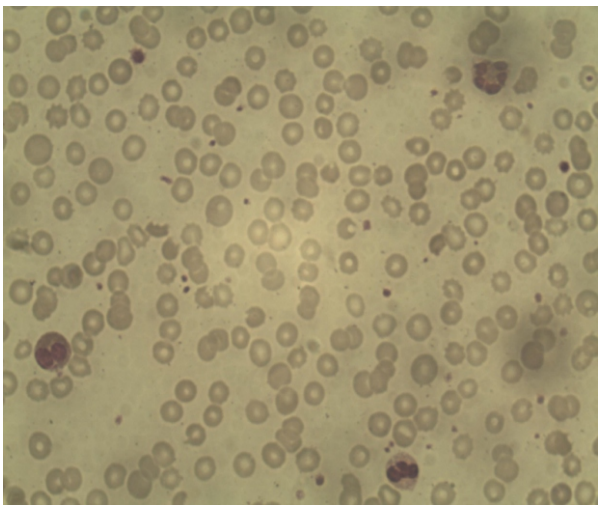

Figure: Blood film of the patient showing bite cells, fragmented cells, numerous polychromatic cells, few blister cells and spherocytes (probably transfused cells)

She was transfused one pint of blood and managed with folic acid 5mg daily. Dietary advice was given to avoid antioxidants.

Haemolysis in our patient was most probably triggered by nitrofurantoin given as a treatment for her urinary tract infection.

Discussion

G6PD deficiency is a X-linked recessive hereditary disease in which abnormally low levels of glucose-6-phosphate dehydrogenase (G6PD) occurs (1, 2). It is the commonest enzyme deficiency seen worldwide (4).

G6PD is an enzyme in the pentose phosphate pathway. It is involved in the production of NADPH which maintains glutathione in reduced state when erythrocytes are subjected to an oxidant stress (1, 2).

The disease is highly prevalent in Africa, southern Europe, the Middle East, South East Asia, and Oceania and in descendants of migrants from these areas (1). Approximately 400 million people are affected worldwide (4). Reduced concentrations of G6PD render red blood cells susceptible to haemolysis under conditions that occur when oxidant drugs are administered, when fava beans are ingested (favism), or during infection (1).

Most individuals who are G6PD deficient remain clinically asymptomatic (2). However, they are at risk of developing acute haemolytic anaemia in response to triggers. In most cases the haemolytic attack is self limiting but in rare instances it can be severe enough needing blood transfusions (4). Depending on the number of red blood cells that have been destroyed, the haemoglobin concentration returns to normal in three to six weeks (2).

Since G6PD deficiency is an X-linked disorder, the main clinical manifestations are seen in hemizygous males (2). The situation in females is complex. Many heterozygote females with G6PD deficiency have red cell G6PD levels which are approximately 50% of normal (3). Human female is a genetic mosaic containing cells with genetically active maternal X-chromosome and cells with genetically active paternal X-chromosome; fixation of the role of each chromosome is determined at an early stage of embryonic development (3). Female patients heterozygous for G6PD deficiency have two cell populations one with a normal enzyme content and one deficient in enzyme (3). Therefore the cell population deficient in enzyme would probably lyse when exposed to an oxidant challenge without affecting the population of cells with a normal enzyme content (2). When there is unfavourable lyonization with the random inactivation of normal X chromosome, the population of G6PD deficient cells is much larger and the total red cell enzyme concentration will be similar to those in hemizygous males (2). This would result in a greater degree of haemolysis upon exposure to an oxidant challenge.

G6PD deficiency is significantly less frequent in females than males (5). Most clinicians believe that G6PD deficiency is exclusively a male disease. For this reason some female patients may go under diagnosed (5).

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From the Editors,

With great pleasure, we present the September 2013 issue of the Galle Medical Journal. Timely publication of the journal has helped to attract the attention of medical writers and the number of submissions has increased substantially during the recent past. Inevitably, this leads to a higher proportion of rejections.

Although the GMJ is freely accessible through the Sri Lankan Journals Online website, efforts are being made to make the contents of the journal more visible and accessible. Despite major advances in the IT front, communication barriers still exist.

The majority of submissions and inquiries we receive are in the fields of original research and case reports. We would encourage readers to submit manuscripts of other types such as pictures, comments, debates and view points. They can enhance the spectrum of the content and improve the outlook of the journal.

Sarath Lekamwasam
Chandrani Liyanage
Editors / GMJ

Epaltes divaricata in paracetamol poisoning: A biochemical and histopathological investigation

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Abstract

***Introduction and Objective**:* In the practice of traditional ayurvedic medicine in Sri Lanka, a number of herbs have been recognized for their potential benefits in the treatment of liver disorders. This study was conducted to investigate the protective effect of *Epaltes divaricata* plant extract in paracetamol induced hepatotoxicity in mice.

Methods: ICR mice (n=20) were treated with acetaminophen at a single dose of 300 mg/kg (after a 16h fast) to induce hepatotoxicity. Drug control group and pre and post-treated groups were administered 0.9 g/kg of *Asparagus falcatus* orally. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatise (ALP) and liver reduced glutathione (GSH) levels were determined. Liver damage was also assessed histopathologically. The effect of the plant extract was compared with *N*-acetyl cysteine.

***Results**:* Acetaminophen produced liver damage, as manifested by a significant rise (P<0.001, one-way ANOVA) in serum ALT, AST, and ALP, and a reduction (P<0.001) in GSH as compared to respective controls. All enzyme activities and liver GSH were significantly improved in *Epaltes* treated mice, with post-treatment providing better results than pre-treatment. Histopathological changes were compatible with the observed biochemical abnormalities.

***Discussion**:* This study shows the ability of the aqueous extract of *Epaltes divaricata* to preserve liver functions in mice treated with high dose of paracetamol.

Introduction

Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Drug discovery from medicinal plants leads to the isolation of early drugs such as cocaine, codeine, digoxin, and quinine, in addition to morphine, of which some are still in use. It continues to provide new and important leads against various pharmacological targets including cancer, HIV/ AIDS, Alzheimer's disease and malaria. Several natural product drugs of plant origin have either recently been introduced to the United States market, including arteether, galantamine, nitisinone, and tiotropium, or are currently involved in late-phase clinical trials (1). There are a number of natural remedies that have claimed to possess curative effects in liver disorders. Recent progress in the study of traditional drugs has resulted in the isolation of numerous active compounds, including antihepatotoxic constituents. Our previous studies provided scientific evidence for the use medicinal plants as hepatoprotective agents against both carbon tetrachloride and acetaminophen-induced hepatotoxicity in mice (2-4).

Figure 1: *Epaltes divaricata* (Sinhala: Heen Mudamahana) Family: Compositae

Acetaminophen (paracetamol) is one of the most widely used non-narcotic analgesic and antipyretic agents available over the counter (5). Although considered safe at therapeutic doses, in overdose, acetaminophen produces centrilobular hepatic necrosis which can be fatal. Acetaminophen is frequently misused and its indiscriminate ingestion can lead to poisoning and life-threatening hepatotoxicity (6). Every year in the United States, overdoses of acetaminophen causes acute liver failure in as many as 800 people, one third of whom die (7). The incidence of self-induced poisoning with acetaminophen is not a significant problem in the developing world as compared to the developed countries (8). However, the number of admissions due to paracetamol poisoning has increased significantly over the last decade. According to a study conducted at the National Poisons Information Centre, of the 992 reported cases of poisoning to the National Hospital Sri Lanka due to medicines, 845 cases were due to ingestion of paracetamol (85%) either alone or with another drug or poison from 2003-2005 (abstract in www.asiatox.org). N-Acetyl cysteine (NAC) is the standard therapy for the treatment of acetaminophen overdose in patients. The primary role of NAC in the treatment of acetaminophen toxicity is in the replacement of intracellular stores of hepatic GSH which allows for detoxification of the electrophile N-acetyl-p-benzoquinone imine (NAPQI), the reactive metabolite formed during metabolism of acetaminophen (9). NAC was used as the reference drug in the present study.

Epaltes divaricata (Family- Compositae, Figure 1), a divaricately branched annual herb, is found in Sri Lanka, India, Myanmar, Java and China. It is commonly known as "Heen Mudamahana" in Sinhala. *Epaltes* is used in traditional ayurvedic medicine to alleviate jaundice, urethral discharges and acute dyspepsia. It is also regarded as a diaphoretic and a diuretic. *Epaltes divaricata* is widely used in Sri Lanka not only as an ayurvedic medicine but also as a delicacy in villages. The objective of the present study was to evaluate the hepatoprotective and antioxidative effects of *Epaltes divaricata* against acetaminophen (paracetamol) induced hepatotoxicity in mice.

Methods

Experimental animals

Healthy male ICR mice, 6- 8 weeks old and weighing 30 - 35 g, were allowed free access to water and pelleted food *ad libitum*. All animals were fasted for 16 h before administration of the hepatotoxin. All protocols used in this study were approved by the Ethics Review Committee of the Faulty of Medicine, University of Ruhuna, Sri Lanka, guided by the CIOMS international guiding principles of biomedical research involving animals.

Chemicals

Diagnostic kits for serum alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1) and alkaline phosphatase (ALP, EC 3.1.3.1) were purchased from Randox (UK). Acetaminophen was a gift from the Sri Lanka Pharmaceutical Manufacturing Corporation. 5,5'-Dithiobis (2-nitrobenzoic acid) was purchased from Sigma (St Louis, MO). N-acetyl cysteine (NAC) was obtained from the Teaching Hospital, Karapitiya, Galle, Sri Lanka. All other chemicals were commercially available and of reagent grade.

Preparation of the plant extract

Epaltes divaricata plants were collected from the Galle district in the Southern Province. The sample was authenticated by comparison with the herbarium specimen preserved at the National Herbarium in the botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen was deposited at the Department of Biochemistry, University of Ruhuna, Sri Lanka.

Epaltes plants were cut into small pieces and dried at 40^{0}C for two days. The normal therapeutic dose of humans extrapolated to mouse was used (10). The dried plant material weighing 2.625 g was refluxed in 30 mL of distilled water for 1h and concentrated to 20 mL. Each mouse was administered a dose of 0.9 g/kg orally by gavage. The extract was prepared daily from the dried plant material.

Treatment of animals

Control groups

Mice were divided into two groups of 20 animals in each. The first group (Group 1) served as the normal control group and received distilled water orally. The second group (Group 2) was treated with the *Epaltes* extract for 7 days. Animals were killed 7 days after the administration of the plant extract.

Paracetamol induced hepatotoxicity

Mice were randomly divided into four groups (Groups 3-6) of 20 animals in each. Paracetamol 300 mg/kg (dissolved in saline and heated at 60 ^{0}C) was administered orally after a 16 h fast. Group 3 was given paracetamol alone and was killed 4h later. Group 4 received the same dose of paracetamol and half an hour later NAC was given orally at 500 mg/kg. The mice were killed 4 h later. In group 5, *Epaltes* extract was administered instead of NAC (Post-treatment). *Epaltes* extract was administered for seven days in group 6 and on the seventh day paracetamol was administered orally half an hour after the administration of the plant extract (Pre-treatment). Animals were killed 4h after the administration of paracetamol in each group.

Assessment of liver damage

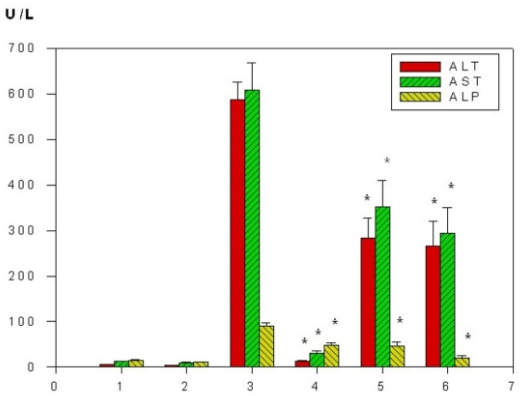
Blood was drawn by cardiac puncture under ether anaesthesia to determine ALT, AST and ALP activity. Liver tissues were excised, weighed and a section of the liver was fixed in 10% buffered formalin for histopathological assessment of liver damage. A liver section was homogenized and used for the determination of reduced glutathione (GSH) level in the liver. Serum ALT, AST and ALP activities were measured using an assay kit from Randox, UK (11). The liver GSH level was estimated by the method of Jollow *et al* (12). Histological sections of the formalin fixed liver tissue were stained with haematoxylin and eosin.

Statistical analysis

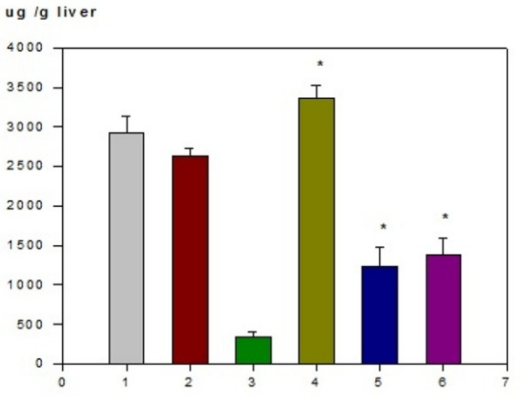
The results were analysed by one-way ANOVA and Tukey's multiple comparison test. A probability (P) value of less than 0.05 was considered significant.

Results

The mice examined in this study showed significantly high serum enzyme activities of ALT and AST, 588.12 and 609.37 U/L, respectively (P<0.001, Figure 2), 4 h following the administration of 300 mg/kg of acetaminophen. All parameters significantly improved in *Epaltes* treated mice as compared to the acetaminophen control, post-treatment being better than pre-treatment. Percentage improvement of serum ALT, AST, and ALP were 51.82, 42.10, 48.20 and 54.76, 51.63, 77.81 in the pre- and post-treated groups, respectively (P<0.05). In the present study, the liver GSH concentration decreased significantly to 346.0 µg/g liver in the acetaminophen control group compared to 2916.04 µg/g liver in the control group (P<0.001, Figure 2). Both pre and post treatments with *Epaltes* increased GSH activity significantly (P<0.05) from 346.16 µg/g liver in the acetaminophen treated group to 1232.17 and 1388.44 µg/g liver in groups pre and post treated with *Epaltes*. In the NAC-treated group, there was a marked reduction in the serum enzyme concentrations and a significant increase in the GSH concentration. The percent reductions in serum ALT, AST, and ALP were 97.8, 94.9, and 46.98, respectively, whereas the percent increase in GSH concentration was 871.6 (P<0.05).



Effect of Epaltes and Paracetamol on serum enzyme levels of ALT, AST and ALP



Effect of Epaltes and Paracetamol on liver reduced glutathione level

Figure 2: Group 1: Normal control group, treated with distilled water; Group 2: Plant extract (0.9 g/kg, p.o) for 7 days; Group 3: a single dose of paracetamol (300 mg/kg in saline) and sacrificed 4h later; Group 4: Paracetamol + a single dose of N-acetyl cysteine (500 mg/kg) and sacrificed 4h later; Group 5: Post-treatment, sacrificed 4h later; Group 6: Pre-treatment, sacrificed 4h later. Results given as mean S.E.M. ALT-alanine aminotransferase, AST-aspartate aminotransferase, ALP-alkaline phosphatase. n=20 mice in each group.

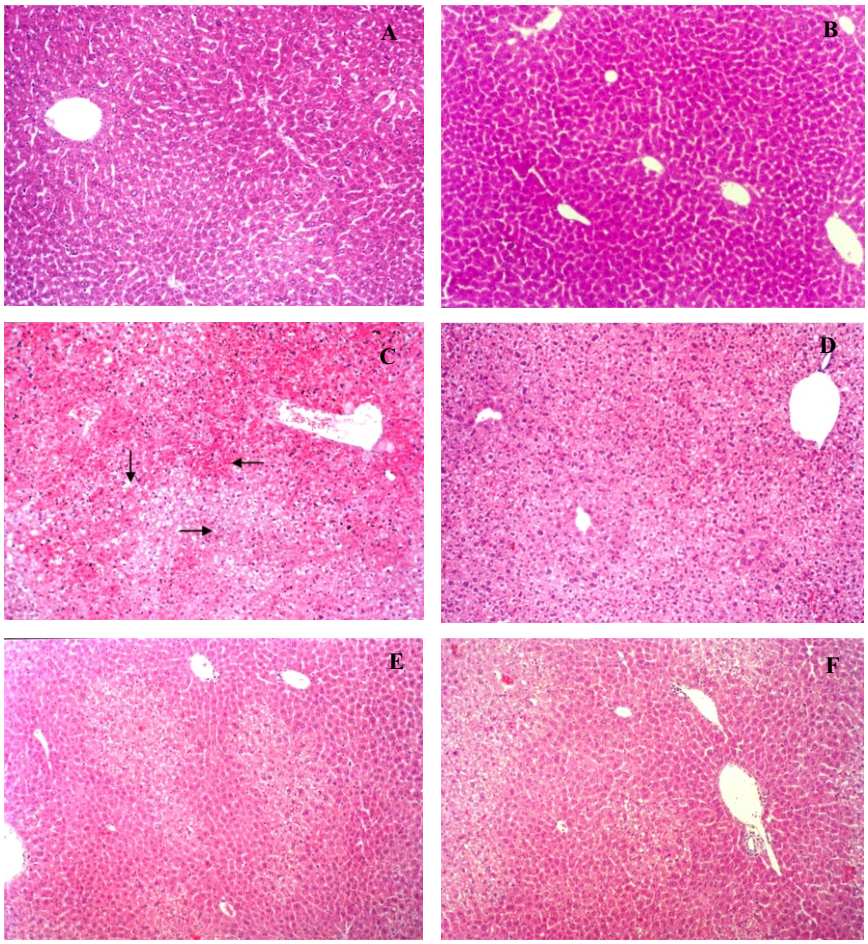


Figure 3: Assessment of acetaminophen induced hepatotoxicity by histopathology.

Haematoxylin and eosin stained liver sections. A: untreated; B: *Epaltes* (0.9 g/kg, p.o) for 7 days. C: acetaminophen control group shows severe confluent necrosis 4h after the administration of acetaminophen D: acetaminophen + N acetylcysteine 4h after the administration of acetaminophen does not show necrosis but vacuolar degeneration is visible. E: Post-treatment with *Epaltes* and sacrificed 4h later show necrosis (⟶), congestion (⟵), ballooning degeneration and vacuolation (⇓) F: Pre-treatment with *Epaltes* for 7 days and sacrificed 4h after the administration of acetaminophen shows no necrosis but diffused vacuolar degeneration is visible. 100 x magnification.

Histopathological Assessment of Liver Damage

Histopathological examination provided supportive evidence for the results obtained from the enzyme analysis. Microscopically, liver sections from control animals stained with haematoxylin and eosin showed normal parenchymal architecture with cords of hepatocytes, portal tracts, and centrilobular venules without noticeable alterations. Macroscopically, the liver appeared dark and congested in acetaminophen-treated mice. Histologically, the liver showed confluent necrosis with vacuolation and ballooning degeneration in the surviving hepatocytes (Figure 3C). NAC treated liver tissue did not show necrosis but vacuolar degeneration was visible. Compared to the acetaminophen control, extent of damage was less in the *Epaltes* post-treated group (Figure 3E), whereas in the pre-treated group, no significant change was noted (Figure 3F). No deviation from the normal parenchymal architecture was noted in the *Epaltes* control group (Figure 3B).

Histopathological observations clearly complement--ed results from biochemical analysis and overall results indicated that post-treatment of *Epaltes* is superior to pre-treatment in the model of acetaminophen induced hepatotoxicity.

Discussion

Acetaminophen is mainly metabolized by sulfation and glucuronidation at therapeutic doses. A small proportion is metabolized through cytochromes P450 2E1, 1A2, 3A4, and 2A6 to form a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) which binds covalently to proteins. Following therapeutic doses NAPQI is efficiently detoxified by conjugation with reduced glutathione (GSH). However, overdose causes depletion of GSH by as much as 90%, leading to the formation of acetaminophen protein adducts such as acetaminophen-cysteine adducts (13). The acetaminophen toxicity following NAPQI generation is chiefly due to oxidative stress and can effectively be ameliorated by antioxidants.

There are a number of natural drugs that have been claimed to have curative effects of liver disorders in traditional medicine. However, scientific information regarding the efficacy of many of the plants with reputed hepatoprotective activity is far from adequate. The purpose of this study was to evaluate the hepatoprotective effect of *Epaltes divaricata* compared to the known antidote NAC and to investigate its antioxidative properties as a mechanism of action. Initially, the hepatoprotective effect of the plant extract was identified by the determination of serum enzyme activities of ALT, AST, ALP, and histopathology. Significant increase in serum enzyme activity after the administration of acetaminophen in this study is consistent with previous data published by Ali, Bashir, and Rasheed (14) where increase in serum ALT and AST activities to 500.0 and 381.10 U/l were published in mice intoxicated with acetaminophen. All serum enzyme concentrations were significantly decreased in both *Epaltes* pre-treated and post-treated mice. The histopathological observations showing a faster regeneration of hepatic cells in mice suggest the possibility that the plant extract may possess the ability to condition the hepatic cells to a state of accelerated regeneration, thus decreasing the leakage of ALT, AST, and ALP into systemic circulation. This is consistent with previous findings by Singh and Handa (15) where the plants extract treated groups showed significant improvements in the liver histopathology as compared to the acetaminophen control group. Thus, from the results of the preliminary investigation it was concluded that the plant extract possesses a hepatoprotective effect against acetaminophen-induced hepatotoxicity in mice. Oxidative stress is a mechanism that has been postulated to be important in the development of acetaminophen toxicity. Accumulation of harmful oxidants in the cell as a consequence of oxidative stress is prevented through the action of small antioxidant molecules such as GSH, vitamins, and antioxidant enzymes (16). GSH is a critical determinant of tissue susceptibility to oxidative damage, and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals including acetaminophen and carbon tetrachloride (CCl_4) (17). Cell injury induced by xenobiotics occurs only if mitochondrial GSH is depleted. In the present study, the liver GSH level was decreased 88.1% in the acetaminophen treated group as compared to the control group ($P<0.001$). The results of the present study are in agreement with results reported by Lin et al. (18) where 130% decrease in liver GSH activity was observed as compared to the control group. Since the toxicity is enhanced by factors that cause GSH depletion,

enhanced NAPQI formation or reduction in the antioxidative capacity of the liver, it could be suggested that the partial hepatoprotection afforded by *Epaltes* may be ascribed to the opposing action on one or more of these factors. Increased GSH level in mice post-treated with plant extract may result from the enhancement of either de novo GSH synthesis or GSH regeneration or both. As a consequence of the action of plant extracts in GSH metabolism, hepatic GSH level can be sufficiently maintained to counteract the increased formation of free radicals as in the case of carbon tetrachloride toxicity.

A comparison of the hepatoprotective activity of the plant extract with NAC, the widely used antidote for acetaminophen poisoning, showed that under the experimental conditions used, plant extract was not as effective as NAC. Serum enzyme activities of ALT, AST, and ALP in NAC-treated mice were reduced by 97.8, 94.9, and 46.9%, respectively as compared to the acetaminophen-treated group. But percentage reductions of the same in *Epaltes*-treated mice were 54.76, 51.63, and 77.81%, respectively. There are limitations to the use of NAC. The effect of the treatment is extremely limited if it is not given within 16 hr of the overdose. Further- more, NAC is associated with a number of adverse reactions (19) such as nausea, diarrhoea, vomiting, rashes, and anaphylaxis (20).

Although the mechanism of chemical-induced liver injury is different from that of viral hepatitis, the pathological changes of parenchymal cell necrosis are a common phenomenon. Therefore, compounds that can either decrease the necrotic damage to hepatocytes via enhanced defense mechanisms against toxic insult or increase repair of damaged hepatocytes are considered potentially useful in the treatment of human hepatitis (21). Further studies are required to determine the active components of *Epaltes* and the exact mechanism that underlie its protective effect against liver damage. Based on the biochemical and histopathological evidence, it can be concluded that *Epaltes* has hepatoprotective and antioxidant activity against acetaminophen-induced hepatocellular damage in mice.

The observed protective effect of *Epaltes* against the hepatotoxin, acetaminophen, may be attributed to the presence of different phytochemicals. The flavonoids are known to be antioxidants, free radical scavengers and antiperoxidants leading to hepatoprotection. While the present investigation has scientifically confirmed the ability of the aqueous extract of *Epaltes divaricata* as an effective hepatoprotectant in mice model, further studies are needed to examine its effects on patients. Also its exact mechanism of action needs to be established.

Acknowledgements

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Haemostasis in laparoscopic splenectomy

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Abstract

Laparoscopic splenectomy reduces the morbidity associated with open access surgery. Obtaining haemostasis in laparoscopic splenectomy is a challenge. In cases described in this series haemostasis was achieved with titanium clips, bipolar diathermy and ultrasonic dissector. It is less costly to use of vascular staplers and quicker than intra corporeal suture ligation.

Key words: *Laparoscopy, Splenectomy, Haemostasis*

Introduction

Splenectomy by open surgical access involves a midline or left sub costal incision which can cause a substantial morbidity. Post operative pain which can affect mobilization and breathing, wound infection and incisional hernia are a few to mention.

These complications can be reduced by laparoscopic approach which requires smaller incisions (1-3). In addition it provides a clear vision by magnification and zooming which enable a better surgical dissection (4). Exposure of peritoneal cavity to exterior is less and there is minimal tissue handling resulting a lower risk of post operative infections. Early mobilization, feeding and early discharge from hospital are other potential advantages.

Achieving haemostasis in laparoscopic surgery, however, is a challenge to be met. The vessels which need to be controlled are short gastric and splenic arteries and veins.

Vascular staplers are effective in controlling the splenic vessels but are expensive. They may be controlled by clips where cost is considerably less but should be reliable to hold a large vessel well. Bipolar diathermy and ultrasonic dissector too obtains haemostasis (5). After the initial investment maintenance costs of these are less. The alternative is to use intra-corporeal ligation. However suture ligation increases the operating time, as it requires extra skill.

In order to prove the safety and efficacy of clips and energy sources which are less costly we present our experience using a series of patients who underwent the procedure.

Methods

A retrospective analysis of laparoscopic splenectomies performed from 2008 was carried out. In all surgeries informed consent has been obtained. The surgeries were performed under general anaesthesia. Patients were placed in right lateral decubitus position with a 45degrees inclination. The head end of the table was elevated by 30 degrees. A pneumoperitoneum was established with veress needle with an insufflation pressure of 14mmHg. A 10mm camera port was inserted 5cm from the left costal margin. Additional ports for retraction and two hand dissection were inserted as follows (Table 1).

Table 1: Site, size and functions of the ports created

Port site	Port size	Port function
Epigastric	5mm	Retraction of stomach/spleen
Midway of xiphoid and umbilicus	5mm	Left hand working
Mid clavicu lar, umbilical level	10mm	Left hand working
Anterior axillary	5mm	Retraction of colon/ pancreas

Gastrocolic, gastrosplenic, splenocoloic, splenoph--renic ligaments were mobilized. Mobilization using ultrasonic dissector and bipolar diathermy allowed a quick and bloodless procedure. With entry into the lesser sac, at an early stage of the surgery, the splenic artery was defined and clipped. The splenic hilum was dissected exposing splenic artery and vein once the spleen was completely mobilized. The vessels were controlled by bipolar diathermy, clips and divided with ultrasonic dissector. The spleen was placed in a pouch made by fashioning an uri bag. The pouch together with spleen was retrieved via a mini incision of about 5cm. The following parameters were recorded; duration of surgery, blood loss, conversions to open surgery

Results

A total number of 18 patients had undergone the procedure (females = 13). The age at surgery ranged from 10 to 72 years. Two patients were between 10-19 yrs, 13 between 20-50 yrs and three between 51 and 70 yrs.

The duration of surgery ranged from 60 to 270 minutes, the operating time improving in the latter patients. Average blood loss was 100 ml and no one required blood transfusion. There were no conversions to open surgery.

Discussion

Haemostasis during laparoscopic splenectomy is an important step. The short gastric vessels can be easily controlled with energy sources as they are small in diameter. The splenic artery and vein may be too large to control with energy sources per-se.

In our patients the splenic artery was dissected early as soon as the gastro-splenic ligament was divided. The splenic artery was located at the upper border of the pancreas and controlled with clips. Subsequent to complete mobilization of the spleen, the splenic artery and vein were dissected out at the hilum. The splenic artery could be easily controlled with clips and divided with ultrasonic dissector adding to sealing of the vessel. The splenic vein size was suitable to control with clips. Using bipolar quatery prior to clip application tends to shrink the vessel allowing easy application of clips. Division with ultrasonic dissector adds to sealing of the vessel. In our series all cases had successful hilar control with clips and energy sources. It did not increase the operating time as it is an easy technique. There were no conversions to arrest bleeding or any other failure.

Conclusions

Clips, bipolar diathermy and ultrasonic dissector provide safe and effective control of splenic artery and vein at the hilum and other vessels in the splenic attachments. The use of above is more economical than the use of vascular staplers and quicker than ligation.

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Knowledge and practices of iodized salt consumption among pregnant women in Galle district

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Abstract

Introduction: Sri Lanka introduced national salt iodization programme in 1995 to control iodine deficiency disorders (IDD) as iodized salt is the main source of iodine in the population. Although Sri Lanka achieved a satisfactory control of IDD in 2005, a recent study showed that iodine nutrition in pregnant women in Sri Lanka is far below the WHO recommendation. Lack of knowledge on iodized salt and improper practices of its usage may cause iodine deficiency during pregnancy.

Methods: Study was conducted in Bope-Poddala health division of Galle District in the Southern province. Pregnant women (n=425) with gestational age ≤12 weeks were studied. An interviewer administered questionnaire was used to collect data.

Results: Forty percent of pregnant women in the sample had poor knowledge on iodized salt and the importance of iodine in the diet. Less than 50% of subjects were aware of bad consequences of maternal iodine deficiency on their babies and about 50% of them had improper practices on iodized salt usage. A poor correlation between the good knowledge and proper practices, were observed.

Conclusion: Overall knowledge about iodine and iodized salt is not satisfactory and the health educational programmes expressing the importance of eliminating IDD at various levels is important to prevent iodine deficiency during pregnancy.

Introduction

Sri Lanka introduced the national salt iodization programme in 1995 as a main strategy to control iodine deficiency disorders (IDD). Further, by legislation it was made compulsory to iodize the salt made for human consumption and the level of iodine in salt at the consumer level was recommended as 15 - 30 ppm. In 2005, it was declared that Sri Lanka achieved a satisfactory control of IDD (1).

Iodized salt is the main source of iodine in Sri Lankan population. It was reported that the iodized salt coverage with adequate iodine (>15ppm) at household level was 90.1% (by titration method) according to the survey on Iodine Nutrition Status in Sri Lanka in 2005 (2). Contrary to this, another study in 2010 reported that only 69.4% of salt at household level contained an adequate iodine concentration in Sri Lanka (3). Even though Sri Lanka was categorized as a country with adequate iodine intake and having iodine nutrition as optimal (4), those estimations were based on the iodine status of the school aged children. This may not reveal the true situation of iodine nutrition in the most vulnerable groups such as pregnant women. A recent study showed that iodine nutrition in pregnant women in Sri Lanka was far below the WHO recommendation of 150µg/L of urinary iodine (5).

With this background it is important to find out possible causes for the poor iodine nutrition in our population especially among pregnant women. A recent study showed that, only 64% of the salt products available in the market contained iodine within the recommended range of 15 - 30 ppm (6). In addition to the unsatisfactory iodine content, lack of knowledge about the iodized salt and improper practices regarding its usage may contribute and therefore worth to be investigated. As such, this study was conducted in the Bope-Poddala health division of the Galle District with an aim to assess the knowledge and practices on consumption of iodized salt among pregnant women.

Methods

This study was conducted in Bope-Poddala health division of the Galle District in the Southern province during the period of July 2012 to September 2013. Maternal and child health services in the health division are provided through five antenatal clinics belonging to17 public health midwife (PHM) areas.

All the pregnant women visiting antenatal field clinics during the above period were considered as the study population and all with gestational age: ≤ 12weeks (as judged by the date of last menstrual period) were included in the study. The number of pregnant women to be included in this study was calculated based on WHO, 1991 (7).

Minimum sample size for the study was taken as 385 assuming that 50% of pregnant women were having poor knowledge on iodized salt. This was inflated by 10% to cover up possible termination of pregnancies during the period and the dropouts. Therefore, it was decided to enroll 425 pregnant women for the study.

The data collection tool used was an interviewer administered questionnaire. It was pre-tested among ten pregnant women to check the feasibility of the questions and the necessary modifications and corrections were made on the questionnaire before it was finally administered. Ethical approval for the study was obtained from the Ethical Review Committee of the Faculty of Medicine University of Ruhuna.

The questionnaire consisted of questions related to knowledge on iodized salt, sources of knowledge and questions related to practices of iodized salt usage. In the analysis, scores were given to each question related to knowledge and practices separately. The participants who scored above the mean for knowledge questions were considered as having good knowledge while others (below the mean level) were considered as having poor knowledge. Similarly, subjects who scored above the mean for questions related to practices were considered as having proper practice about the usage of iodized salt while others were considered as having improper practices. The questions related to sources of knowledge were analyzed separately. The answer sheets were checked for completeness and the data were entered to Statistical Package for the Social Sciences (SPSS version 19, SPSS Inc., Chicago, Illinois, USA) for analysis.

Results

Altogether 425 pregnant women responded to the questionnaire. The answers given by them are summarized in **Table 1**. Out of 425 subjects 397 (93.4%) were aware of iodized salt and 304 (71.5%) identified that consumption of iodized salt is essential. Majority (83.5%) were aware of the fact that iodine deficiency had a significant influence on the body and 90.1% knew that iodine deficiency causes the goiter. However, only 50.8% had the knowledge about the connection between iodine and thyroid hormones. Nearly 52% of pregnant women knew that iodized salt is especially important during pregnancy and worthwhile to note that 48.2% were not aware of the importance of iodized salt during pregnancy.

It was observed that the subjects received information on the importance of consuming iodized salt during pregnancy, through the media (36.5%) such as radio, television, newspapers, etc., from PHMs (35.5%) and from both sources (14.2%). It was interesting to note that only 2.4% of study subjects revealed that they were educated about the importance of iodized salt during pregnancy by medical personnel (i.e., medical officer of health (MOH), family doctor, Consultant Obstetricians).

In the study sample, 259 (61%) of pregnant women were in their second pregnancy or more. Of those only 142 (55%) reported that they were educated about the importance of iodine during the previous pregnancies at the clinics while others (45%) have not received such information. Of those who were

educated during past pregnancies, 96.5% received information through the public health midwives either at the clinics or while doing field visits. Table 1 illustrates their responses on the questions related to knowledge and the subjects were with a satisfactory awareness about the iodized salt and the availability.

When considering their knowledge about the correct usage of salt, 206 (48.5%) mothers were of the view that adding salt after cooking is the correct way. Some (28.7%) did not have any idea on the correct usage where as 22.8% of them felt that salt should be added before cooking.

The overall analysis revealed that 60% of pregnant women in this study sample had a knowledge score of above the mean and they were considered as having good knowledge on iodized salt, iodine and its importance while the remainder was otherwise. However, less than 50% of subjects were aware of the bad consequences of maternal iodine deficiency on their babies.

The data on the practices of iodized salt usage are given in Table 2. Even though the pregnant women had adequate knowledge on iodized salt only 6.8% of them were following the correct practice of adding salt after cooking while majority (66.8%) used to add salt before cooking. The rest (26.4%) were following both practices.

When buying salt from the market, majority of the subjects (81.9%) had the practice of buying any brand of salt available at the market and only few of them (18.1%) were used to purchase the same brand always. Majority (64.9%) of subjects purchased both powdered and crystal salt and 19.3% and 15.8% of them consumed only powdered salt and crystal salt respectively.

In summary 50.8% of pregnant women were found to have scores given for proper practices above the mean score while 49.2% had improper practices on iodized salt usage.

Table1: Responses to the questions on knowledge

Knowledge regarding iodized salt	Correct answer (n=425)	
	n	%
Awareness of iodized salt	397	93.4
Necessity of iodized salt intake	304	71.5
Availability of iodized salt in the local market	389	91.5
Legality of selling of uniodized salt in Sri Lanka?	44	10.3
Knowledge on the conditions that occur due to deficiency of iodine		
Significant influence on the body	70	16.5
Causes goiters	383	90.1
Impact on hormone production by the thyroid gland	216	50.8
Effects of iodine deficiency in pregnancy		
Special importance of iodized salt during pregnancy	218	51.8
Effect on physical development of the child	199	46.8
Direct effect on the brain development of the child	200	47.1
Effect on future education of the child	204	48.0
Salt in food preparation		
Add salt before cooking	97	22.8
Add salt after cooking	206	48.5
No idea	122	28.7

Table 2: Practices of using iodized salt (n=425)

Household practices	n	%
Adding salt to food		
Before cooking	284	66.8
After cooking	29	6.8
Both	112	26.4
Purchase the same brands		
Yes	77	18.1
No	348	81.9
Type of salt consumed		
Powdered	82	19.3
Rock salt	67	15.8
Both	276	64.9

Effect of socio-demographic characteristics of pregnant women on their level of knowledge and practices on the usage of iodized salt

The level of knowledge was compared with socio-demographic status of study subjects and is presented in Table 3. Majority of the subjects belonged to the age category of 26 - 35 years. Forty two percent of the sample had education above Advanced Level. The level of knowledge showed significant associations with age ($p<0.001$), level of education ($p<0.001$) and social class ($p=0.007$).

A similar analysis was done comparing the practices on iodized salt usage, with different socio-demographic characteristics of pregnant women (Table 4). There were no significant associations between age groups ($p=0.43$); levels of education ($p=0.78$) and social classes of the subject ($p=0.35$) with the practices of salt usage among pregnant women.

Table 3: Socio-demographic characteristics on the level of knowledge

Characteristics	Knowledge level				χ^2	p-value
	Good		Poor			
	n	%	n	%		
Age group in year s						
16 – 25	52	12.3	86	20.3		
26 – 35	163	38.4	79	18.6	48.35	p<0.001
= 36	39	9.2	5	1.2		
Total*	**254**	**59.9**	**170**	**40.1**		
Level of education						
Primary & sec.	30	7.1	70	16.6		
Passed O/L	87	20.6	58	13.7	54.94	p<0.001
Passed A/L & above	135	32.0	42	10.0		
Total* *	**252**	**59.7**	**170**	**40.3**		
Social class						
Class – I & II	48	11.3	20	4.7		
Class – III & IV	132	31.1	81	19.1	7.25	p=0.007
Class – V&VI	75	17.6	69	16.2		
Total	**255**	**60.0**	**170**	**40.0**		

*One subject n ot responded, **Three subjects not respon ded, chi-square test for trend was applied

Table 4: Association of the practices of pregnant women on salt usage with their Socio-demographic characteristics

Characteristics	Practice of adding salt during cooking				χ^2	p-value
	Proper		Inadequate			
	n	%	n	%		
Age group in years						
16 – 25	66	15.6	72	17.0		
26 – 35	123	29.0	119	28.1	1.69	0.43
≥ 36	26	6.1	18	4.2		
Total*	**215**	**50.7**	**209**	**49.3**		
Level of education						
Primary & sec.	54	12.8	46	10.9		
Passed O/L	72	17.1	73	17.3	0.50	0.78
Passed A/L & above	89	21.0	88	29.9		
Total**	**215**	**50.9**	**207**	**49.1**		
Social class						
Class – I & II[1]	38	8.9	30	7.1		
Class – III & IV[2]	101	23.8	112	26.3	2.08	0.35
Class – V[3]	75	17.6	65	15.3		
Class – VI**[3]	2	0.5	2	0.5		
Total	**216**	**50.8**	**209**	**49.2**		

*One subject not responded, **Three subjects not responded, chi-square test for trend was applied

Discussion

Two decades have passed since the implementation of the national salt iodization programme in Sri Lanka but there have not been any studies done among pregnant women to assess the knowledge and practices related to iodized salt and its usage. Even in countries with very good iodine nutrition, there is evidence that pregnant women are susceptible to iodine deficiency (8,9).

Iodized salt is the main source of dietary iodine in Sri Lankan population and it has been the main strategy to control the iodine deficiency disorders. In this study sample most of the pregnant women (>90%) were aware of iodized salt and about 90% knew the connection between iodine deficiency and goiter. Yet, only about half of the subjects (51.8%) were aware of the importance of consumption of iodized salt especially during pregnancy. This shows that the public awareness about iodized salt is not satisfactory. Similar results have been reported in studies done in Ghana a developing country in sub-Saharan Africa, where >90% of subjects heard about iodized salt and about 33% knew iodine deficiency causes goiter (10) and in Iraq, Ebrahim and Muhammed (11) reported that >92% of subjects heard about iodized salt and only 27.1% knew about the health benefits.

Of the pregnant women who were in their second pregnancy or more (n=259), 45% have not received information on the importance of iodine during

previous pregnancies from health workers at antenatal clinics. Further, more than half the numbers of pregnant women were not aware of possible harmful effects of iodine deficiency on the foetus during pregnancy and later, on the newborn. Jooste *et al.,* (12) in 2004 reported a very low level of knowledge in their study (only 3.9% had considered brain damage, as the most important health consequence of iodine deficiency) on iodine nutrition among the mothers in African region. However, in the present study 60% of subjects had a good knowledge about iodized salt and iodine. When considering the good literacy rate in Sri Lanka (13), the level of knowledge in our population can expected to be better. These results showed that the overall knowledge about importance of iodine, especially during pregnancy is not satisfactory in this study sample.

The area selected for the study is close to the main city of the Southern province and almost all pregnant women attended the antenatal clinics at main tertiary care unit in the province where good antenatal care facilities are available. Although 3/4 of the study subjects, generally had educational qualifications above the basic (passed O/L or above) in this setting, the knowledge on iodine was not satisfactory. Therefore situation may even be worse in other under developed areas of the country.

It is important to know, how the pregnant women receive knowledge about the importance of iodine during pregnancy. Majority of study subjects (86.2%) received the knowledge through public media and PHM's. This is probably due to the health education programmes frequently conducted through television and radios and the health talks given by PHM at the antenatal field clinics. The lack of involvement of medical personnel in health education at the clinics may be due to their busy schedules and heavy work load and it's a drawback in the present health care delivery system.

It has also been observed in the present study that the association of the age of mothers, their level of education and social class are associated with the level of knowledge in them on iodized salt and iodine. With advancing age their level of knowledge has increased and this may probably be due to the fact that they acquire more knowledge and experiences with the maturity. The results showed that higher the educational level, their level of knowledge regarding iodine nutrition is also higher. Apart from that the study subjects who belonged to higher social class had a better knowledge on iodine

There were many brands of iodized salt available in the market. A previous study revealed that forty two brands of salt with varying degrees of iodine levels were available in the study area (6). Majority (81.9%) of pregnant women have indicated that they used to buy any brand of salt available in the market. This can be considered as a good practice in one hand because they might be getting the salt products with high, low and average iodine contents and ultimately they might be getting the average amount of iodine which is required. On the other hand if they used to buy the same brand of salt probably with low iodine content for a longer period, they might have iodine deficiency.

Even though some of the selected factors such as age, level of education and social class showed a highly significant association with the level of knowledge, when it came to practice of the study subjects, regarding the usage of iodized salt, it did not show any significant associations with above factors. This shows that, though they have a good knowledge on iodine and iodized salt they did not put it in to practice. The reason for that may be their lack of understanding of the gravity of IDD as a significant health problem.

In conclusion, overall knowledge about iodine and iodized salt in this study population is not satisfactory and about half of the subjects had improper practices related to the iodized salt usage, even after two decades of the implementation of national salt iodization programme. Public media and PHM's appear to play a major role in health education but the lack of involvement of medical personnel in imparting the necessary information seems to be a critical issue. The unsatisfactory level of knowledge on iodine and iodized salt may result in iodine deficiency during pregnancy and more over poor correlation between the good knowledge and proper practices is an issue to be addressed. To resolve this public health issue it is important to intensify health educational programmes at various levels including schools etc. expressing the importance of eliminating IDD. Health teams who are responsible for the antenatal care must be educated about conveying the message regarding the importance of iodine nutrition during pregnancy

in a more meaningful way to the public. It may also be helpful if educational leaflets that carry precise information regarding importance of iodine in pregnancy are made available though antenatal clinics at booking visit.

Acknowledgements

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Prevalence of anxiety among patients with irritable bowel syndrome: A pilot study

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Abstract

Introduction: Aetiological factors of irritable bowel syndrome [IBS] among Sri Lankans are not well known. This study was carried out to find the prevalence of anxiety among patients with IBS and its association with symptoms of the disease.

Methods*:* A group of patients numbering 104 with IBS, presented to Medical and Gastroenterology Units and diagnosed according to the ROME III criteria were assessed with a combination of Generalised Anxiety Disorder-7 questionnaire (GAD) and Public Health Questionnaire-4 (PHQ). This data were compared with 104 age and sex matched controls.

Results: Majority (55.8%) of IBS patients were females. Mean age of them was 46.9 years with a SD of 15.1 yrs (range: 20-76). Majority (53.8%) of IBS patients belonged to Social Class 5. Features of anxiety were found in 26% of IBS patients according to the PHQ and 43.3% according to the GAD. Only 3 controls (2.88%) were detected to have anxiety according to the PHQ. Anxiety among controls according to the GAD was 4.8%.

Conclusions*:* There is a higher occurrence of anxiety among patients with IBS when compared with age and sex matched controls. Whether this is a primary etiological factor or a secondary phenomenon due to the chronicity of the disease is unknown to us.

Keywords: Anxiety, Irritable Bowel Syndrome, Sri Lanka

Introduction

Irritable bowel syndrome (IBS) is a gastrointestinal syndrome characterized by chronic abdominal pain and altered bowel habits in the absence of any organic cause. In Sri Lanka no data are available on the prevalence of irritable bowel syndrome. However, it is not an uncommon condition in clinical practice. The prevalence of IBS in North America estimated from population-based studies is approximately 10 to 15% (1-6). A similar prevalence (11.5%) has been reported from Europe as well. In subsequent studies, however, the prevalence varied widely among countries (7).

IBS affects men and women, young and the elderly. However, younger patients and women are more likely to suffer from IBS (3). A systematic review estimated that there is an overall 2:1 female predominance in North America (3).

IBS accounts for a significant number of visits to primary care physicians and is the second highest cause of work absenteeism after the common cold (8). IBS is associated with increased health care costs

with some studies suggesting annual direct and indirect costs of up to $30 billion (9).

Aetiology of IBS is not clearly understood. Several factors are thought to be responsible for the disease. Gastrointestinal motility, visceral hypersensitivity, intestinal inflammation, infections, alterations in faecal microflora, food allergy, genetics factors, and psychosocial dysfunctions are some of them. Psychosocial factors may influence the expression of IBS (10). In a study of patients with IBS or non-ulcer dyspepsia, patients with GI symptoms reported more lifetime and daily stressful events than control groups (11). Another study found that compared with controls, patients with IBS exhibit increased anxiety, depression, phobias, and somatization (12). In a prospective study, psychosocial factors (anxiety, sleep problems, somatic symptoms) were shown to be independent risk factors for the development of IBS in a population not previously diagnosed with the condition (13).

Finding the aetiology of a disease will help to treat patients effectively. Because of this reason we decided to study the relationship between anxiety disorders and IBS. This study was carried out to find the prevalence of anxiety among patients with IBS and its association with symptoms of the disease.

Methods

Written permission to carry out the study was obtained from the Director of Teaching Hospital, Karapitiya. Ethical clearance for the study was obtained from the Ethics Committee of the Faculty of Medicine, University of Ruhuna, Galle, prior to data collection. Data collection was carried out after obtaining informed consent from the study subjects. The data obtained was treated confidentially. Comparative descriptive cross-sectional study design was used.

A group of 104 patients with IBS presented to Medical and Gastroenterology Units and diagnosed according to ROME III criteria were interviewed. We included only adult patients aged between 18-50 years and patients with the history of disease for more than 1year period. Patients on treatment for psychiatric disorder or on drugs that can potentially change the symptoms of anxiety and patients practicing non-pharmacological measures targeting anxiety reduction (Yoga, Meditation etc) were excluded. Hospital-based 104 age and sex-matched healthy controls were recruited for the study. They were selected among patients visiting the same hospital for reasons other than health issues.

Interviewer administered questionnaire was used as the study instrument. It included socio-demographic data, data on occupation related factors and Generalized Anxiety disorder 7 questionnaire and Public Health Questionnaire-4. Principal investigator collected data to ensure the completeness and accuracy of data.

All data were coded and entered into a database and cleaning and checking were done. Where appropriate, data were expressed as means and standard deviations. Differences between proportions of groups were tested for statistical significance using the Chi-square test. Probability values less than 0.05 were considered as statistically significant.

Results

We interviewed 104 subjects with the disease and 104 age and sex matched controls. Majority (55.8%) of IBS patients were females. Mean age of them was 46.9 years with a Standard Deviation of 15.1 (range: 20 to 76 years) (Table 1). Majority (53.8%) of patients belonged to the Social Class 5 (Table 1). Distribution of duration of disease among patients is shown in Table 2.

PHQ identified 26% of patients with IBS to have anxiety while according to the GAD 43.3% had features of anxiety (Table 3). Only 3 controls (2.88%) were detected to have anxiety according to the PHQ. Anxiety among healthy controls according to GAD was 4.8% (Table 3). There was a higher prevalence of anxiety detected according to PHQ among those with IBS when compared with healthy controls (Table 4). Similarly, there was a higher prevalence of anxiety detected according to GAD among those with IBS when compared with study controls (Table 5). There was no statistically significant association of gender, age, social class or duration of disease with anxiety according to the PHQ or GAD ($p>0.05$).

Table 1: Age and social class distribution among study participants with the disease (n=104).

Socio demographic variable	Number	Percentage
Age		
40 years or less	38	36.5%
41 to 60 years	41	39.5%
61 years and above	25	24.0%
Social class		
Social class 1	4	3.8
Social class 1	3	2.9%
Social class 1	26	25.0%
Social class 1	15	14.4%
Social class 1	56	53.9%

Table 2: Distribution of the duration of disease among patients with IBS

Duration of disease in months	Number	Percentage
12 months or less	28	26.9%
13 to 36 months	37	35.6%
More than 36 months	39	37.5%
Total	**104**	**100%**

Table 3: Anxiety among cases and controls according to GAD.

Anxiety according to GAD	Among cases		Among controls	
	Frequency	Percent	Frequency	Percent
No Anxiety	59	56.7%	99	95.2%
Mild Anxiety	37	35.6%	5	4.8%
Moderate Anxiety	6	5.8%	0	0%
Severe Anxiety	2	1.9%	0	0%
Total	**104**	**100.0%**	**104**	**100.0%**

Table 4: Anxiety detected by PHQ among those with and without IBS

Disease Status	Anxiety according to PHQ	
	Anxiety positive	Anxiety negative
With the disease	27	77
Without the disease	3	101

Chi square value (Yates corrected) 20.61 $p<0.001$

Table 5: Anxiety detected by GAD among those with and without IBS

Disease Status	Anxiety according to GAD	
	Anxiety positive	Anxiety negative
With the disease	45	59
Without the disease	5	99

Chi square value (Yates corrected) 40.05 $p<0.001$

Discussion

IBS is commonly regarded as a functional disorder and is hypothesised to be associated with anxiety. In current clinical practice we see a reasonable number of patients with IBS. Most of them are in younger age. But majority of these patients are not satisfied with the available treatment options. If we can find the aetiology it will be easy to control this condition.

The aetiology of IBS is not clearly understood. Several factors are thought to be responsible for the disease. Many aspects including genetic and environmental factors have been studied to find out the exact aetiology of the diseases but none have been proven.

Only few studies have addressed both anxiety and depression together. To measure the anxiety level we used GAD-7 and PHQ-4 questioners. All patients recruited were diagnosed according to Rome III criteria. All the patients who fulfilled the criteria presented to medical and Gastroenterology clinics were recruited during the data collection period of six months. Almost all the patients had negative colonoscopy.

According to a systematic review and meta-analysis by Guillaume Fond et al, patients with IBS have significantly higher levels of anxiety (14). This finding is in keeping with our results. Study published by Mariette Bengtsson et al, also reported a higher prevalence of anxiety among patient with IBS (15). According to Kabra N, et al, the prevalence of anxiety among patients with IBS was 31.4% (16). However this was a clinic based study and they have used Hamilton Anxiety rating scale instead of PHQ-4 or GAD. In an Iranian study by Modabbemia MJ et al, including 256 patients with IBS, 74.2% prevalence of Anxiety / Depressive problems was reported (17). But this study included depression too and this probably contributed to the relatively higher value. Study conducted by Lee S et al, from the Department of psychiatry in the Hong Kong University found that generalized anxiety disorder is five times more common in IBS patients than non-IBS respondents (18).

In conclusion we have found that there is a significant association between IBS and anxiety. Whether this is an aetiological factor or a consequence of the chronic nature of the disease is not clear to us. We suggest assessing anxiety level in IBS patients and taking appropriate actions to control anxiety level in these patients.

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Prediction of bone loss in elderly women using bone turnover markers

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Abstract

Osteoporosis related fractures are common in old age and over 40 % of the women above 50 years of age are at a risk of developing a fragility fracture. Biochemical markers of bone turnover (BTMs) have proven to be of some value in fracture predictability. There is also a correlation between rate of decrease of areal bone mineral density (aBMD) and incident fractures.

In this series of studies, the correlation between BTMs and rate of bone loss (change of aBMD and ultrasound variables) over 5 years was investigated in a cohort of 75 year-old Swedish women. In addition, correlation of BTMs and bone metabolism, as assessed by scintigraphy, was tested in postmenopausal women. Finally, the effect of precision error on the longitudinal monitoring of change in aBMD was assessed in elderly women and in elderly men.

There was a strong correlation between all bone turnover markers and the results of scintigraphy (total skeletal uptake of 99mTc-labelled methylene diphosphonate), with no significant difference between bone formation markers and bone resorption markers. BTMs correlated to the 5-year rate of change of aBMD, especially in the legs and the total body, and 5 year change in speed of ultrasound. When serial measurements of BTMs were analysed, the mean value of measurements correlated more strongly to aBMD change than single measurements, and women with constantly high levels of BTMs had higher rates of bone loss. Precision error of aBMD measurement by dual-energy X-ray absorptiometry has an influence on the detection of individuals with aBMD change exceeding the least significant level. The calculated follow-up interval for detection of aBMD change beyond the least significant level in more than 50% of elderly individuals ranged from 332 years, and was dependent on the equipment used and the skeletal site tested.

These results indicate that BTMs are associated with future bone loss although the correlations may not be strong enough to predict bone loss at individual level. DXA also has some limitations when used in longitudinal follow up of elderly individuals. DXA is therefore of limited use in the longitudinal monitoring of bone loss. Further studies with novel bone turnover markers may improve the ability of BTMs to predict bone loss.

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Introduction

This oration is based on the following original publications, which are referred to in the text by Roman numbers (I-V):

I. Lenora J, Norrgren K, Thorsson O, Wollmer P, Obrant KJ, Ivaska KK. Bone turnover markers are correlated with total skeletal uptake of 99mTc-methylene diphosphonate (99mTc-MDP). *BMC Medical Physics.* 2009 Mar 30;**9**:3**. (12)**

II. Lenora J, Ivaska KK, Obrant KJ, Gerdhem P. Prediction of bone loss using biochemical markers of bone turnover. *Osteoporosis International.* 2007 Sep;**18**(9):1297-305. (9)

III. Lenora J, Gerdhem P, Obrant KJ, Ivaska KK. Bone turnover markers are correlated with quantitative ultrasound of the calcaneus: 5-year longitudinal data. *Osteoporosis International*. 2009 Jul;**20**(7):1225-32. (10)

IV. Ivaska KK, Lenora J, Gerdhem P, Akesson K, Väänänen HK, Obrant KJ. Serial assessment of serum bone metabolism markers identifies women with the highest rate of bone loss and osteoporosis risk. *J Clin Endocrinol Metab*. 2008 Jul;**93**(7):2622-32. (11)

V. Lenora J, Åkesson K, GerdhemPEffect of precision on longitudinal follow-up of bone mineral density measurements in elderly women and men. *Journal of Clinical Densitometry* 2010 Oct-Dec;13(4):407-12. (13)

Bone is a living tissue that is continuously subjected to resorption and formation by coordinated action of osteoclasts and osteoblasts on the surface of trabecular bone and in the Haversian canals. In a health individual about 10 % of the skeleton is remodeled each year (1), allowing the skeleton to adjust its strength to mechanical stress and to repair any microdamage (2). Bone remodeling is also necessary for maintaining the metabolic function of the skeleton and calcium homeostasis (3). During the growth period in childhood and in adolescence bone formation predominates; increasing the bone size and strength until the maximum bone mass (peak bone mass) is reached in the 2nd or the 3rd decade of life (4). After reaching the peak bone mass, there is a state of equilibrium, when the rate of bone formation equals the rate of bone resorption. After the age of 40 years the bone resorption starts to predominate over formation. In women, bone resorption is accelerated in the first few years after the menopause due to estrogen deficiency (5). Postmenopausal women decrease the BMD or lose bone at a rate of 2-5% per year (5). Individuals who lose bone at a fast rate can develop osteoporosis and get fragility fractures at early ages.

Osteoporosis is a systemic skeletal disease characterized by low bone mass, micro architectural deterioration of bone tissue leading to increased risk of fragility fracture, most commonly affecting postmenopausal women and elderly men. After 50 years of age more than 40 % of women and 13% of

men in western countries are at a risk of developing a fragility fractures at any site during the rest of their life time (6). Osteoporosis is diagnosed by measuring bone mineral density (BMD) using dual energy X-ray absorptiometry (DXA) and defined as BMD value 2.5 standard deviations or more below the mean of young female adult population. It is important to identify individuals with osteoporosis and individuals with fast bone loss to take preventive measures to avoid fractures. Fast bone losers are detected using DXA, measuring BMD at least one to two years apart. It is expensive and consumes time during which the women lose bone further.

Bone turnover markers, (BTMs) or biochemical markers of bone turnover, are bone tissue proteins or their fragments, or enzymes released from bone cells during bone turnover. Proteins can be by-products of collagen formation or products of collagen degradation, or non-collagenous proteins such as osteocalcin or bone sialoprotein. Enzymes, such as bone-specific alkaline phosphatase and tartrate-resistant acid phosphatase 5b, can also be used as bone turnover markers. Bone turnover markers can be detected in serum or urine. Ideally, they should reflect only the activity of osteoblasts or osteoclasts. Bone turnover markers that are released predominantly during bone formation or resorption are known as bone formation or resorption markers, respectively (7). Formation and resorption are usually tightly coupled in time and space and therefore, any marker reflects the overall rate of bone turnover (8). Certain bone turnover markers may reflect different stages of formation and resorption but they cannot reflect disease-specific processes or for instance distinguish between the activities at cortical or trabecular bone (8).

When we consider countries like Sri Lanka, DXA is too expensive and are available only in few centers. Compared to DXA, assays of BTMs are less expensive and affordable to perform in laboratories with ELIZA and RIA facilities.

This study was conducted in Malmö University Hospital, Lund University, Swedenas a part of the Malmo Osteoporosis Prospective Risk Assessment (OPRA) study and the main objective was to study the possibility of predication of bone loss over five years using baseline levels of BTMs as well as a serial measurement of BTMs. The Malmo OPRA cohort included a population based sample 1604, 75 year old women who were randomly selected using the population register of the Malmo city of south Sweden (9-11). For the baseline investigation, 1,604 women were invited and 1,044 (65%) participated at baseline. *The women were invited for prospective follow-up visits after 1, 3 and 5 years. These women were assessed with DXA and QUS of calcaneus at baseline and after 5 year.* Eleven BTMs were measured at baseline, after 1 year, 3 years and 5 years using protocols published earlier (9-11). BTMs measured were Bone-specific alkaline phosphatase (S-Bone ALP) and four serum osteocalcin assays (serum intact osteocalcin (S-OC[1-49]), serum N-mid osteocalcin (S-Total OC (N-Mid®)), serum total osteocalcin (S-Total OC), serum total carboxylated osteocalcin (S-cOC) as bone formation markers; as bone resorption markers serum C-terminal cross-linking telopeptides of type I collagen (S-CTX-I), serum tartrate-resistant acid phosphatase 5b (S-TRACP5b) and Urinary deoxypyridinoline (U-DPD). In addition, three urinary osteocalcin assays were also performed (U-LongOC, U-TotalOC, U-MidOC). The women included into this analysis had not taken hormone replacement therapy or bisphosphonates during the study period and within two year prior to the study.
The effect of precision error of DXA measurements on the assessment of repeated bone densitometry in elderly women and men was also studied.

Paper I: Bone turnover markers are correlated with total skeletal uptake of ^{99m}Tc-methylene diphosphonate (99mTc-MDP) (12).

This study it was aimed to study whether bone turnover, as assessed by total skeletal uptake of Technetium 99-labelled methylene diphosphonate, correlate more to bone formation markers or to resorption markers.

For this study 22 healthy post-menopausal women (aged 5280 years) were recruited (12). Bone scintigraphy procedure) was performed after injecting intravenous dose of 520 (517 15) MBq of ^{99m}Tc-MDP (Medronate®, Amersham International) at 09.00 h. Whole body imaging was performed directly (3 minutes) after injection and 5 hours after injection using a double-headed gamma camera system (Siemens Multispect 2) Total skeletal uptake (TSU) of ^{99m}Tc-MDP was calculated using 3 minutes images 5 hour images, excluding the urinary bladder and the soft tissue uptake as described by Brenner *et*

al 14). Blood and urine samples were collected at the same time for assessment of nine bone turnover markers.

There was a significant correlation between all bone turnover markers, with r-values from 0.52 ($p = 0.013$) to 0.90 ($p < 0.001$). The two bone resorption markers had numerically higher correlations (S-TRACP5b: r = 0.90; and S-CTX-I: r = 0.80) than the bone formation markers (S-Total OC: r = 0.72; and S-Bone ALP: r = 0.66), but the differences were not statistically significant. There was no correlation between the TSU of ^{99m}Tc-MDP and age, weight, body mass index or total body BMD (12).

Prediction of bone loss using bone turnover markers. Papers II, III and IV were aimed to study this objective.

Paper II, *Prediction of bone loss using biochemical markers of bone turnover.*601 women who had attended both the baseline and the 5-year DXA measurements were included for this analysis (9).

Significant associations ($p < 0.01$) in the aBMD change of the leg region (derived from the total body measurement) were found for four different S-OCs (standardized regression coefficient -$ß_{std}$ = 0.20 to -0.22), U-DPD ($ß_{std}$ = -0.19), S-TRACP5b ($ß_{std}$ = -0.19), S-CTX-I ($ß_{std}$ = -0.21), two of the three U-OC/crea ($ß_{std}$=-0.16).

After adjustment for baseline total body BMC, associations were found for all S-OC:s ($ß_{std}$=-0.11 to -0.15), two of the three U-OC:s ($ß_{std}$ = -0.14 to -0.16) and aBMD change at the total hip, and for three of the four S-OC:s ($ß_{std}$= -0.14 to -0.15), S-TRACP5b ($ß_{std}$ = -0.11), two of the three U-OC:s ($ß_{std}$ = -0.14 to -0.15) and aBMD rate of change at the femoral neck.

Paper III, *Bone turnover markers are correlated with quantitative ultrasound of the calcaneus: 5-year longitudinal data.*506 women who had attended both the baseline and the 5-year QUS measurements were included for this analysis (10). When the correlations between the baseline bone markers and 5-year prospective changes in QUS were evaluated, bone turnover markers (S-OCs, S-CTX-I, S-TRACP 5b) showed statistically significant but week correlations with SoS ($ß_{std}$ = -0.09 [$p < 0.05$] to -0.17 [$p < 0.001$]). BUA did not show a significant correlation with BTMs (10).

Paper IV, *Serial assessment of serum bone turnover markers identifies women with the highest rate of bone loss and osteoporosis risk* (11). 573 women were included from OPRA cohort.They attended both the baseline and the 5-year DXA measurements, and had given serum and/or urine samples at baseline and at the 1-, 3- and 5-year follow-ups.

Baseline BTMs showed a weak correlation with change in total body aBMD, but the association was more pronounced when we used the average of two measurements of each marker (standardised regression coefficient from ($ß_{std}$ = 0.12 to 0.23, $p < 0.01$). Adding a third and a fourth measurement further strengthened the correlation ($ß_{std}$ of up to -0.30, $p < 0.001$). Changes in BTMs did not correlate to bone loss as strongly as the average values. Women with constantly high turnover lost significantly more bone at total body (-2.6%) than women with intermediate (-1.6%) or low turnover (-0.2%, p for trend < 0.001). They also had greater bone loss at the hip (-8.3%, -6.0% and -5.1%, respectively; $p = 0.01$). Results were similar in the subgroup of women with osteopenia (11).

Paper V, *Effect of precision on longitudinal follow-up of bone mineral density measurements in elderly women and men* (13).

For this analysis, 691 women were included (13). These women had a baseline and 5-year follow-up DXA measurements available. In addition, 211 men from the Malmö part of the MrOs study who attended DXA measurements at baseline and at the 5 year follow-up were included. The MrOs study is (An international multi-centre study on risk factors for osteoporosis and fracture in elderly men).Precision error (in g/cm^2) for Lunar DPX-L in women ranged from 0.010 at TB to 0.028 at TH. Precision error using Lunar Prodigy for women ranged from 0.009 at TB and TH to 0.039 at LS). Precision error using Lunar Prodigy for men ranged from 0.007 at TB to 0.031 at LS.

Mean change in aBMD (in g/cm^2) per year in women was ranged from -0.003 (0.007) at TB to -0.011 (0.016) at TH. Corresponding results in men were -0.003 (0.006) at TB, to -0.006 (0.009) at TH.

The number of individuals with 5-year aBMD change at TB that exceeded the LSC was 244 women (38.6%) and 73 men (35.6%). The corresponding

results at TH were 265 women (41.4%) and 78 men (38.6%); at LS the numbers were 303 women (45.0%) and 51 men (24.6%).

Monitoring time interval (i.e. LSC/median rate of change in aBMD) for both populations was 8 years (for TH aBMD) and 13 years (for LS aBMD). Based on Prodigy precision data, the monitoring time intervals for women were 3 and 32 years for TH and LS, respectively (13).

Discussion

To the best of my knowledge, this study is the largest study in elderly women to assess the ability to predict bone loss over several years. The design of the OPRA study has several advantages: it has a well-defined population with a high attendance rate, a long follow-up, and the use of novel and established bone turnover markers.

The overall aim of the work described in this study was to improve the prevention of fragility fractures in the future. There are numerous risk factors for fragility fracture. Bone mineral density is one of the most important risk factors that are potentially modifiable. For diagnostic purposes, a diagnostic threshold is used for bone density test results, below which the term osteoporosis is used. However, a large proportion of individuals who sustain a fragility fracture are not osteoporotic (4, 23, 24). Bone density test results only reveal the current situation and do not show the ongoing bone turnover; thus, they do not provide information on future changes in bone density.

There are several reasons for the development and use of bone turnover markers. The work in this study illustrates efforts to find ways of predicting future bone loss by the measurement of bone turnover markers (**Paper II and III**), of how to improve this assessment (**Paper IV**), and to investigate whether some markers are more specific than others (**Paper IIV**). Since the time required to assess bone density changes with bone density equipment is very long (**Paper V**), it seems unreasonable to follow up compliance and effect of anti-osteoporotic medication by repeated bone density measurements.

Currently, bone turnover markers are being used extensively in research applications and also being tested as tools for the management of metabolic bone diseases such as osteoporosis and Paget's disease in clinical practice, because these markers are non-invasive and relatively inexpensive. Monitoring of the efficacy of bone-active drugs is currently the most promising clinical application of bone turnover markers, because of the possibility of detecting a change in the levels of bone turnover markers within a few weeks of treatment (15-18). Some markers, particularly resorption markers such as S-TRACP5b, S-CTX-I, U-CTX-I, U-NTX-I and U-DPD, and some bone formation markers such as S-bone ALP and S-OC, have shown some degree of fracture predictability in different populations(7), but the prediction is not strong enough to use in individual patients. The fracture predictability afforded by bone turnover markers is weaker than the predictability afforded by DXA (19), but it is somewhat inconsistent between studies (20-22).

A high rate of bone turnover is associated with a high rate of bone loss and osteoporosis (23-24). Early detection of individuals who are at high risk of developing osteoporosis could be important for clinical decision-making. In particular, individuals with osteopenia and individuals with a high rate of bone loss may need more careful follow-up.

In **Paper II** and **III**, baseline bone turnover markers, in particular S-OCs, U-DPD/crea, S-TRACP5b, S-CTX-I, U-LongOC/cea and U-MidOC/crea could be correlated to rate of change of aBMD in the legs. To some degree, there were correlations with rate of change of aBMD in the arms, in the total body, in part of the body, in the total hip and in the femoral neck. None of the markers were found to be correlated to rate of change of aBMD at the lumbar spine; nor did S-Bone ALP and U-TotalOC/crea show any correlation with rate of change of aBMD. When the correlation between bone turnover markers and 5-year change of QUS variables was examined, all markers except S-Bone ALP showed correlations with changes in SoS, while none of the markers showed any correlation with changes in BUA **(Paper III)**. When the mean of serial measurement of bone turnover markers was used instead of baseline measurement, the correlations became stronger as the number of samples used increased, and the women with constantly elevated levels of bone turnover markers had a significantly higher rate of bone loss **(Paper IV)**.

In general, the correlation between bone turnover markers and the change in aBMD was not strong.

The strongest correlation coefficients were 0.22 when the baseline levels were used, and they were 0.32 when the mean of four serial measurements was used. None of the markers proved to be superior to the others. Bone formation and resorption markers had almost similar magnitudes of correlations. This could be due to the tight coupling of bone formation and resorption. This idea is supported by the results of **Paper I**, in which no difference between bone formation markers and resorption markers in TSU of ^{99m}Tc-MDP was found. Bone turnover markers are released from the whole skeleton. This may be the reason for higher correlations with bone turnover markers at large skeletal sites including the total body, the partial body and the legs, than smaller sites such as the femoral neck and the lumbar spine (**Paper II and IV**).

Many other factors also affect the clinical usefulness of bone turnover markers. Pre-analytical conditions affecting bone turnover markers such as age, gender, menopausal state, ethnicity and recent fracture are not controllable, whereas other factors such as the effect of food intake, physical activity and circadian rhythm can be controlled (25). The OPRA study was designed to control for factors such as age, gender, ethnicity and menstrual status. Samples were taken in the morning in the non-fasting state, which could have affected the results, mainly the S-CTX-I levels(26). Many other factors such as time of the day, recent fracture and level of physical activity may have an effect on bone turnover markers. The study design was deliberately not changed during the study period, and all samples were collected in the same manner to make comparisons possible within the cohort.

Bone density has a smaller annual change or response to anti-resorptive and anabolic treatment compared to the response of bone turnover markers. Precision has an effect on the shortest follow-up interval between repeated scans. In the population-based cohorts in **Paper V**, several years were needed to detect a significant change between measurements. The estimated monitoring time intervals (i.e. least significant change/median rate of change in aBMD) were between 3 and 32 years, depending on the site of measurement and the equipment used. Only when a high degree of bone loss is expected may a shorter follow-up time be useful. Thus, DXA has shortcomings in detecting rapid losers and individuals with a high risk of developing osteoporosis.

Single measurements of bone turnover markers and follow-up measurements of DXA both have limitations in their ability to detect individuals with rapid bone loss. Serial assessments of bone turnover markers can substantially improve the ability to find individuals with increased loss of bone density. Whether or not intervals shorter than one year could be used to improve the predictive ability of bone turnover markers remains to be evaluated.

Conclusions

There is a correlation between levels of bone turnover markers and the rate of bone loss in elderly women, with varying degrees of correlation coefficients at different skeletal regions. In general, bone turnover markers correlate better with change in aBMD particularly at large skeletal sites, such as the total body, and weight-bearing sites such as the legs, than with aBMD change at specific clinically important relatively smaller regions such as the femoral neck and the total hip. Correlations between bone turnover markers and rate of bone loss become stronger when serial measurements of bone turnover markers are used. The individuals with constantly high levels of bone turnover markers have higher change in aBMD. However, these correlations may not be strong enough to be predictive of bone loss at the level of the individual patients. DXA is used to monitor change in aBMD to aid in treatment decisions. However, long durations of follow-up are needed to detect aBMD changes in elderly women and men that exceed the least significant change. DXA is therefore of limited use in the longitudinal monitoring of bone loss. Therefore there is a need of further studies to develop new bone turnover markers with higher predictive value.

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