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Tel/Fax: +94 91 2232560
E-mail : gmj@gma.lk
gmjgalle@gmail.com

Web Site: www.gma.lk
Internet Home Page:
http://www.sljol.info/index.php/GMJ

Editorial

The COVID-19 pandemic: Looking into the future

The pandemic of corona virus disease 2019 (COVID-19) has drastically transformed the lives and almost all aspects of livelihoods of the global population in unprecedented proportions. We have moved away from the containment phase, which is generally of value in the initial stages of an epidemic. Although social distancing and vaccination are generally considered as a panacea for this scourge, it is very likely that we will have to live with it for some time to come. Telenti *et al.,* giving their perspective on the future trajectory of the pandemic in *Nature*, discuss about uncertainties about the type of long-term association that SARS-Co-V2 virus will establish with the human population.[i] With a possibility of COVID-19 becoming an endemic disease with probable seasonal peaks, they detail many mechanisms that may cause endemicity; susceptible individuals and waning immunity after infection or vaccination, viral changes through antigenic drift that diminish protection and re-entries from zoonotic reservoirs. Into the second year of the pandemic, we are facing the threat of antigenic drifts caused by mutations as in the case of the current Delta variant of the virus which is highly infectious. Another major problem into the future is the danger of "long Covid" with variable multisystem involvement (sometimes with progression) in some patients.

The pandemic has highlighted many unfortunate aspects related to social inequity at different levels; globally, between countries and within countries. In the care of the infected, the lack of facilities and infrastructure to cope up with the demands is a major problem throughout the world, requiring the application of principles of distributive justice. At an institutional

level, a rational redistribution of manpower and resources are required to cater for Covid patients, although there is a reluctance to give up resources by some for the benefit of the suffering. This sudden change in the paradigm calls for empathy, benevolence and magnanimity from all levels of health workers, based on equitable principles.

By mid-August 2021, 58% of the population of high-income countries had received at least the first dose of a Covid vaccine while in low income countries as a whole it stood at a dismal 1.3%. This shows gross inequity from a global perspective reflecting disparities in economic, political and other factors. This wide disparity caused the World Health Organization (WHO) to call for a moratorium on a third (booster) dose of the vaccine being planned in high-income countries. It is broadly held that focusing on boosters when more than half the world lacks vaccine doses will only keep the pandemic burning longer.[ii]

However, from a vaccination coverage perspective, Sri Lanka has fared extremely well with its robust health system. By the end of August 2021, 37.2% of the Sri Lankan population have received both doses of the vaccine and 56.9% have received the first dose.[iii] (Assuming a projected total Sri Lankan population of 21,919,000).

With a trend of global travel and wide international interactions, it is abundantly clear that the majority of human inhabitants of our planet ideally should have immunity against the virus. From a global context, the broad immunization of a mere country will not be of much value unless other countries too achieve such a coverage. This calls for a globally planned focused universal vaccination strategy transcending geographical and political boundaries. The vaccination process needs to be well structured, purposeful and organised with minimal interference.

We now live in a world ravaged by a hitherto unknown virus that has changed our lives, health and livelihood in an unimaginable way. This new world order caused by a tiny corona virus calls for equity at all levels transcending boundaries.

Satish K Goonesinghe
Eisha I Waidyarathne

Editors in Chief / GMJ

i. Telenti A, Arvin A, Corey L, Corti D, Diamond MS, Garcia-Sastre A *et al*. After the pandemic: perspectives on the future trajectory of COVID-19. *Nature* 2021;**596**:495-504

ii. The WHO is right to call for delay to vaccine boosters (Editorial). *Nature* 2021;**596**:317

iii. Epidemiology Unit, Ministry of Health, Sri Lanka [Internet] Colombo : Progress of COVID-19 Immunization as of 8.30 pm on 31.08.2021 [Updated 2021 Aug 31; cited 2021 Sep 5]. Available from: https://www.epid.gov.lk/web/images/pdf/corona_vaccination/covid_vaccination_2021-08_31.pdf

The *Journal*

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Editorial Office

Joint Editors, *Galle Medical Journal,* GMA Office,
Teaching Hospital Karapitiya, Galle,
Sri Lanka.
Tel: +94 91 2232560
E-mail: gmj@gma.lk
gmjgalle@gmail.com
Internet home page: https://gmj.sljol.info/

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The *Galle Medical Journal* is published by the Galle Medical Association. The *Journal* is published quarterly in March, June, September and December each year. Submissions are accepted throughout the year. The aims of the journal are to foster co-operation among the medical fraternity and to be a forum to make literary contributions, share experiences encountered in medical practice, update their knowledge and have debates on topics related to all aspects of medicine. Also, we attempt to cater to the educational needs especially of the postgraduate trainees. The *Journal* publishes original articles, reviews, leading articles and case reports. When an article is submitted for publication, we expect that the work it reports has not been published, submitted simultaneously to another journal or accepted for publication elsewhere. All manuscripts will be reviewed anonymously before acceptance.

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Original articles: Should normally be in the format of introduction, methods, results and discussion. Each manuscript must have a structured abstract of 200 words. The text should be limited to 3000 words and maximum of 5 tables/ figures taken together with no more than 15 references. Lengthy manuscripts are likely to be returned for shortening. The discussion in particular should be clear, concise and should be limited to matters arising directly from the results. Avoid discursive speculation.

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References:
These should conform to the Vancouver style. The reference in the text should be numbered consecutively in Arabic numerals in parentheses in the same line of the text in the order in which they appear. The first five authors should be listed and if there are more than five, then the first three should be listed followed by *et al.* Examples are given below:

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2. Calenoff L, Rogers L. Esophageal complication of surgery and lifesaving procedures. In: Meyers M, Ghahremani G, eds. Iatrogenic Gastrointestinal Complications. New York: Springer, 1981: 23-63.

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Iron deficiency anaemia in pregnancy and its prevention; paradigm changes over three decades

Malik Goonewardene

Emeritus Professor of Obstetrics and Gynaecology, University of Ruhuna, Galle, Sri Lanka.

Correspondence: Prof. Malik Goonewardene
e-mail: *malikg.obsgyn@med.sab.ac.lk / malikg@eureka.lk*
https://orcid.org/0000-0002-7899-7117

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ABSTRACT

In Sri Lanka, the prevalence of anaemia in pregnancy was estimated to be approximately 29% in 2001 but is estimated to have significantly reduced to be < 20%, at least in certain areas, during the last decade. Among its many causes, nutritional iron deficiency anaemia (IDA) is the commonest, but establishing a definitive diagnosis is difficult. Strategies adopted globally, as well as in Sri Lanka, to prevent IDA in pregnancy, have changed significantly during the last three decades.

A comprehensive literature review was carried out and *inter alia* three relevant World Health Organization Guidelines, in which the author was a member of the guideline development group and six other publications of the author, on the topic of iron deficiency (ID) and IDA in pregnancy are included in this review. Ten studies on the detection and prevention of ID and IDA in pregnancy, carried out from 1990 to 2017 at the Academic Obstetrics and Gynaecology Unit (AOGU) of the Teaching Hospital Mahamodera, Galle (THMG) are described. The results and conclusions of these studies were: poor compliance and unsatisfactory methods of ingestion of the antenatal oral iron supplement could have contributed to the high rates of ID and IDA at term during the period 1990 to 1992; haematological indices during pregnancy need to be interpreted with caution; although the agreement between haematological indices obtained from different laboratories in Galle in 2000 was unsatisfactory, better agreement was observed in 2015; the rates of ID and IDA in pregnant women presenting for antenatal care to the AOGU of the THMG have significantly decreased from approximately 69% and 44% respectively in 1990 to approximately 37% and 17% respectively in 2015. Therefore, weekly antenatal oral iron supplements should be adequate to improve birth outcomes in the non-anaemic women attending this clinic.

Key words: *Anaemia, antenatal oral iron supplementation, iron deficiency, pregnancy*

Introduction

Anaemia in pregnancy, defined as a haemoglobin concentration (Hb) < 110 g /L, is a significant public health problem globally (1). Its prevalence in Sri Lanka was estimated to be approximately 29% in 2001(2) and 34% in 2007 (3). However in 2009, using a small sample of 228 pregnant women, the prevalence of anaemia during pregnancy was estimated to be approximately 17% in Sri Lanka, ranging from approximately 7% in Kurunegala to 29% in the Colombo Municipality (4). Furthermore, regional studies on the prevalence of anaemia in pregnant women have reported rates of 8.2% to 14.4%, during the period 1999 to 2012 (5-7), suggesting that the national rate of anaemia in pregnancy could be less than 20%. Several factors affecting Hb and iron status in pregnancy lead to

difficulties in establishing a definitive diagnosis (8-12). Furthermore, a healthy woman could progress to a state of latent iron deficiency (ID), then to ID with no anaemia and finally to a clinical iron deficiency anaemia (IDA). Therefore, the prevalence of ID is always greater and could be 2 - 2.5 times the prevalence of IDA (8, 11, 12). Anaemia in pregnancy, which could be due to multiple factors of which nutritional IDA is the commonest, is associated with increased maternal and perinatal morbidity and mortality, and long-term adverse effects in the new born (8, 9, 11-13). Although routine daily antenatal oral iron supplementation programmes have been implemented in low income countries for several decades, their effectiveness in preventing IDA at term has been shown to be suboptimal, and its safety, especially in areas where Malaria is prevalent, has also been questioned (14). Although Sri Lanka has been declared "Malaria Free" by the World Health Organization (WHO) in 2016 (15), routine daily antenatal oral iron supplementation would probably be beneficial only in pregnant women with ID (16, 17). During the last three decades, as a result of a better understanding of the mechanisms of absorption of oral iron supplements from the gut, there has been a great interest in the role of intermittent iron supplements rather than daily supplements (18-20). The WHO has recommended that iron and folate supplements should be given to all menstruating adult women and adolescents, adjusting the dose and frequency of administration according to the prevalence of anaemia in the particular community (21, 22). Consequent to this, a few years ago, a weekly oral iron supplementation programme for four weeks, has been commenced in Lankan schools for grade seven and grade 10 students. It has also been recommended that, in communities where the prevalence of anaemia in pregnancy is < 20%, intermittent (*e.g.* weekly) antenatal oral iron supplementation should be administered for non anaemic pregnant women (23). Although such a programme could be appropriate at least in certain areas of Sri Lanka, no such programme has been implemented in any area in Sri Lanka up to date.

The aim of this review is to describe the difficulties in establishing a diagnosis of IDA and ID in pregnancy, and the significant changes in the rates of IDA and ID in pregnancy as well as the strategies adopted to prevent IDA and ID in pregnancy, during the period from 1990 to 2017, among pregnant women presenting for antenatal care to the Academic Obstetrics and Gynaecology Unit (AOGU) of the Teaching Hospital Mahamodera, Galle (THMG).

Methods

A comprehensive literature review was carried out and *inter alia* three relevant World Health Organization Guidelines, in which the author was a member of the guideline development group and six other publications of the author, on the topic of ID and IDA in pregnancy, are included in this review. Ten studies on the detection and prevention of ID and IDA in pregnancy, carried out from 1990 to 2017 at the AOGU of the THMG are described. The objectives of these were to: describe the rates of ID and IDA in women presenting for antenatal care in 1990 and compare with the same in 2015 (24.25); describe the effectiveness of the antenatal oral iron supplementation programme in 1992 (26); measure the agreement between haematological indices obtained by different laboratories in 2000 (27) and compare with the same in 2015 (28); assess the validity of commonly used haematological indices (29); describe the effectiveness of intermittent antenatal oral iron supplementation between 1994 to 1996 (30-32) and compare it with the same in 2015/2016 (33).

Studies on the detection and prevention of iron deficiency and iron deficiency anaemia in pregnancy, carried out from 1990 to 2017 at the Academic Obstetrics and Gynaecology Unit of the Teaching Hospital Mahamodera, Galle

Prior to conducting these studies, ethical approval was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, administrative approval was obtained from the respective Directors of the THMG during the times of the studies, and the randomized control trial (RCT) carried out in 2015/2016 was registered in the Sri Lanka Clinical Trials Registry, which was established in 2006. Informed written consent was obtained from all the women who participated in these studies.

Study 1 (24)

A cross sectional analytical study was carried out in 236 consecutive pregnant women during a two months' period from January 1990, using the cyanmethhaemoglobin method, haematocrit tubes and immunoradioemetric assay technique for the measurement of their Hb, hematocrit (Hct) and serum ferritin (SF) respectively. The rates of anaemia and ID were 56% and 69% respectively, and 13% of women had ID although they were not anaemic. The Hb had a low sensitivity (63%) and a poor negative predictive value (41%) in the detection of ID.

Study 2 (25)

A cross sectional analytical study was carried out in 350 consecutive pregnant women with gestations between 12 to 20 weeks, presenting from November 2014 to January 2015. Their Hb and Hct were measured by the flow-cytometry and hydro-dynamic focusing methods and their SF was measured by the electro-chemiluminescence method. The best cut off level of SF which was associated with anaemia, obtained from a Receiver Operator Characteristics (ROC) curve, was SF < 30 µg/L (area under the ROC curve = 0.77, 95% CI 0.72 to 0.81), and it had a sensitivity of 78.3% (95% CI 65.8 - 87.9) and a specificity of 74% (95% CI 68.6 – 79.0) in detecting anaemia. Using this SF < 30 µg/L cut off, 36.9% of subjects had ID. The rate of anaemia was 16.6%.

Study 3 (26)

A prospective cohort study was carried out in 88 consecutive pregnant women with gestations between 14 to 24 weeks, during a two months' period commencing 15 May 1992. Their Hb and SF were measured, using the same methods as in study 1, before and after varying durations of antenatal, daily, oral supplements which consisted of a tablet containing ferrous sulphate 200 mg (elemental iron 60 mg) with folate 25 µg, and another tablet containing calcium lactate pentahydrate 300 mg. Women who had an initial Hb < 10 g/dl and SF < 10 ng/ml had a marked increase of Hb and SF respectively after supplementation ($p < 0.001$), and women who had supplementation for > 12 weeks had a significant increase of SF ($p < 0.05$). However, there was no significant change in the mean Hb (10.9 g/dl, sd 1.7) and mean SF (23.9 ng/ml, sd 23.5) of the 88 women. All the women had taken the iron folate supplement and the calcium supplement together, 87 had taken the supplements after a meal of unpolished rice and vegetables, and 16% with a cup of tea. Only 58% had taken the supplements correctly, as advised. Therefore, it was concluded that the compliance and method of intake of the supplements should be improved to enhance the effectiveness of the supplementation programme.

Study 4 (27)

A cross sectional analytical study was carried on two cohorts of pregnant women presenting for antenatal care in 1990; one in the second trimester - T^2 (Group A, n = 156) and the other in the third trimester - T^3 of pregnancy (Group B , n = 47). In Group A, the Hb and Hct were measured in the same blood sample at the Karapitiya Medical Faculty Laboratory (KMFL) using the cyanometh--haemoglobin method and haematocrit tubes respectively and the Kyoto Medical Laboratory Galle (KMLG) using an automated haematology analyser. In Group B, from the same blood sample, in addition to the Hb and Hct measurements carried out in KMFL and KMLG another measurement of Hb, using the cyanomethhaemoglobin method, was carried out at the Karapitiya Teaching Hospital Laboratory. In T^2, the mean Hb reported from KMFL was markedly less than the mean Hb reported from KMLG (8.3 g/dl sd 1.7 vs 11.4 g/dl sd 1.2, $p < 0.001$). There were no clinically significant differences in the mean Hct reported from the two laboratories. In T^3 there were no significant differences in the mean Hb reported from the three laboratories. Although there was slight agreement between the reports of three laboratories (Kappa 0.28, $p < 0.02$) with modest agreement between the reports from the KMFL and KMLG (Kappa 0.62, $p < 0.001$), individual differences between the reports were clearly seen when the data were plotted graphically. As the Hb measured by three different laboratories differed significantly from each other, interpreting a single Hb measurement with caution, and the use of the Hct in addition to the Hb was recommended for the diagnosis of anaemia.

Study 5 (28)

A cross sectional analytical study was carried on 350 consecutive pregnant women, with gestations between 12 to 20 weeks from November 2014 to January 2015. Their Hb and Hct were measured, in the same blood sample, by flow-cytometry and hydro-dynamic focusing methods at the Durdans Hospital Laboratory, Galle and the colorimetric method at the laboratory of the THMG. No significant differences were seen between the mean Hb values and between the mean Hct values obtained from the two laboratories. Strong, positive correlations were seen between the Hb values as well as between the haematocrit values obtained from the two laboratories ($r = 0.86$, $p < 0.001$ and $r = 0.83$, $p < 0.001$ respectively). The limits of agreement and the clinical limits of indifference between the Hb as well as between the hematocrit values obtained from the two laboratories were satisfactory but individual differences of > 10% were seen in 6.6% of results

Study 6 (29)

In 1990, another cross sectional analytical study was carried on the same two cohorts of pregnant women of Study 4. Haematological indices were measured by an automated hematology analyser and the SF was measured by immunoradiometric assay. For the diagnosis of ID, a Sf < 12 ng/L was used. Using the accepted cut off values of each haematological index for the diagnosis of anaemia, the validity of detection of ID by each haematological index was measured. The Hct, mean corpuscular volume, and the mean corpuscular haemoglobin concentration (MCHC) had a high specificity (96 %- 100%) but very low sensitivity (10%-38%). Only the mean corpuscular haemoglobin had a high sensitivity (92% in T^2 and 86% in T^3), but it had a low specificity (21% - 24%). The MCHC had the best accuracy (71% in T^3) but its accuracy in T^2 was only 64%. Only the MCHC showed a moderate agreement with the SF (Kappa 0.41, $p < 0.001$). Therefore, it was recommended that multiple haematological indices should be evaluated before diagnosing ID and deciding on subsequent supplementation or treatment of ID during pregnancy.

Study 7 (30)

In 1994, a prospective cohort study was carried on 56 consecutive pregnant women with gestations between 13 to 26 weeks. Their Hb and SF were measured, using the same methods as in studies 1 and 3, before and after the intake of an oral multi micronutrient capsule, which contained ferrous fumarate 350 mg (elemental iron 117 mg), folate 1.5 mg, cyanocobalamine 15 µg, calcium carbonate 200 mg, cholecalciferol 400 iu, and ascorbic acid 75 mg. The women were advised to take the capsule with water, once a week, at 11.00 a.m. (approximately one hour before lunch), for 12 weeks. Mebendazole 100 mg twice daily for three days was given prior to commencement of the supplementation. There was 100% compliance and no side effects were reported. There were no significant changes in the mean Hb and Hct, in the total study sample, after supplementation. However, there was a significant reduction in the mean Hb by 1.3 g/dl (sed 0.44, $p < 0.01$), in the 13 women who had Hb of ≥ 11 g/dl. Furthermore, the number of women with Hb < 11 g/dl and SF < 12 ng/ml increased from 14/56 before supplementation to 24/56 after supplementation ($p < 0.05$). Therefore, this weekly regimen was inadequate to meet the increased requirements of iron during pregnancy.

Study 8 (31)

In 1994, a prospective cohort study was carried on 77 consecutive pregnant women with gestations between 14 - 26 weeks. The method adopted was the same as in Study 7, with one important difference. The women were advised to take the supplement every Monday, Wednesday and Friday for 12 weeks. There was 100% compliance and no side effects were reported. The mean Hb increased and the number of women with Hb < 8 g/dl and SF < 12 ng/ml decreased after supplementation, especially in the 27 women who had initial Hb < 11 g/dl and SF < 12 ng/ml. ($p < 0.01$). However, the mean SF decreased by14.1 ng/ml (sed 4.9, $p < 0.05$) in the 50 women who had an initial SF > 12 ng/ml and the mean Hct decreased by 2% (sed 0.7, $p < 0.01$) in the total study sample. Therefore, although this thrice weekly regimen was beneficial in women with moderate to severe ID, it was inadequate to

meet the increased requirements of iron during pregnancy in women with borderline or mild iron deficiency.

Study 9 (32)

In 1995, a randomized controlled trial (RCT) was carried out on 92 consecutive pregnant women with gestations between 14 to 24 weeks. Their Hb, Hct and SF were measured, using the same methods as in studies 1and 3, before and after supplementation. They were treated with mebendazole 100 mg twice a day for three days, and randomly allocated to receive weekly (n = 26), three times a week (n = 35) and daily (n = 31), an oral micro nutrient capsule which was the same as in studies 7 and 8. The women were advised to take the capsule with water daily, weekly or every Monday, Wednesday and Friday, at 11.00 a.m. (approximately one hour before lunch). Compliance was checked by keeping a record of the number of capsules administered to each participant and reviewing each participant at four weekly intervals. The compliance was very good and no serious side effects were reported. There were reductions in the numbers of women with anaemia in all three groups. However, the number of women with SF < 12 ng/ml increased in the weekly supplementation group while they decreased in the daily supplementation group ($p < 0.001$). There were very high risks of developing ID in the weekly and thrice weekly supplementation groups (OR 18, 95% CI 2.8 - 115.5, $p = 0.002$, and OR 10, 95% CI 1.6 - 64.8, $p < 0.015$, respectively). There was an increased risk of developing anaemia in the weekly supplementation groups (OR 15, 95% CI 14 - 165.6, $p = 0.027$). Therefore, intermittent regimens were considered to be inadequate and continuation of the daily regimen was recommended.

Study 10 (33)

During the period December 2014 to April 2015, a RCT was carried out on 292 consecutive, non-anaemic pregnant women with gestations between 14 to 22 weeks, who presented for antenatal care, and who had been treated with mebendazole 100 mg twice a day for three days. They were randomly allocated to receive 120 mg elemental iron, 3 mg folic acid and 100 mg vitamin C weekly (n = 149) or 60 mg elemental iron, 1 mg folic acid and 100 mg vitamin C daily (n = 143). All were assessed for side effects at four weekly intervals and their Hb, Hct and SF were measured at 32 to 36 weeks gestation, with the same methods used in Study 2. Only 106 participants in each group completed the study. Between the two groups there were no significant differences in the mean duration of supplementation, the post supplementation mean Hb, Hct and SF, and the risks of developing anaemia, ID or high Hb levels, by an intension to treat analysis as well as in those who completed the trial. Side effects were significantly greater in the daily supplementation group compared to the weekly supplementation group. Therefore, in non anaemic pregnant women presenting for antenatal care, a weekly regimen was an effective alternative to a daily regimen for antenatal oral iron and folate supplementation, in preventing anaemia and ID in the third trimester.

Conclusions

Haematological indices during pregnancy need to be interpreted with caution, taking into consideration several factors. Poor compliance and unsatisfactory methods of ingestion of the daily antenatal oral iron supplement could have contributed to the high rates of anaemia and ID at term during the period 1990 to 1992. Although the agreement between haematological indices obtained from different laboratories in Galle were unsatisfactory in 2000, better agreement was observed in 2015. In pregnant women presenting for antenatal care to the AOGU of THMG, the rates of anaemia and ID have markedly decreased from approximately 56% and 69 % respectively in 1990 to approximately 17% and 37% respectively in 2015. As the rate of IDA is < 20%, weekly antenatal oral iron supplements should be adequate to improve birth outcomes in the non-anaemic women attending this clinic. Although the implementation of such a programme was not appropriate in the AOUG of THMG three decades ago, it should be considered for implementation now. A national survey should be carried out to estimate the current prevalence of anaemia among pregnant women in Sri Lanka.

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Interaction between lean, fat and bone masses among patients with chronic kidney disease; a cross-sectional comparative study

Silva EH[1], Wickramatilake CM[2], Lekamwasam S[3], Mudduwa LKB[4], Ubayasiri RA[5]

[1]*Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka.*
[2]*Department of Biochemistry,* [3]*Department of Medicine,* [4]*Department of Pathology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.*
[5]*Department of Vascular and Transplant Surgery, Teaching Hospital Karapitiya, Galle, Sri Lanka.*

Correspondence: Ms. Eranga H. Silva
e-mail: *ehsilva@ahs.ruh.ac.lk*
https://orcid.org/0000-0003-0139-8869
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ABSTRACT

Introduction: Interactions between three major body compartments; lean, fat and bone masses are essential for the maintenance of optimum bodily functions. This interaction is disturbed by factors such as advancing age, disease and drugs. The aim of the study was to understand the interaction of body compartments in chronic kidney disease (CKD) to optimise disease outcome.

Methods: Fifty patients (38 males) with CKD awaiting kidney transplant at Teaching Hospitals, Karapitiya and Kandy and 50 age and gender matched control subjects were compared. Socio-demographic data, social habits and anthropometric measurements with blood pressure was obtained. Both patients and controls underwent DEXA scan to assess body composition.

Results: Mean (SD) age of the patients was 48(9.6) years. In both groups; patients with CKD and participants in the control group, total body lean mass (TBLM) showed significant correlations with total body bone mineral density (TBBMD) (patients: r = 0.38, p = 0.008) (controls: r = 0.57, p = <0.001) and total body bone mineral content (TBBMC) (patients: r = 0.62, p = <0.001) (controls: r = 0.78, p = <0.001). No significant correlations were observed between total body fat mass (TBFM) and TBBMD or TBBMC. When regression models were fitted with TBBMD and TBBMC as dependent variables and TBLM as the independent variable, 1kg difference in TBLM was associated with 0.007 g/cm^2 (p = 0.02) and 0.010 g/cm^2 (p = 0.012) change in TBBMD in patients and controls, respectively. Furthermore, 1kg difference in TBLM was associated with 32 g (p = 0.02) and 33 g (p = 0.018) difference in TBBMC in patients with CKD and participants in the control group, respectively.

Conclusions: Similar to participants in the control group, TBLM is a better predictor of TBBMC and TBBMD compared to TBFM and TRFM in patients with end stage renal disease (ESRD) and this knowledge can be used in health promotion programs to improve bone health of these patients.

Key words: *Body composition, bone mineral content, bone mineral density, end stage renal disease, fat mass, lean mass*

Introduction

Chronic kidney disease (CKD) is a global health problem with an overall global prevalence of 13.4% (1). Derangement of bone mineral metabolism occurs in all stages of CKD, it can cause physical immobility, musculoskeletal pain and bone fractures, resulting in increased mortality, morbidity and poor quality of life (2). This group of disorders initially named as renal osteodystrophy (ROD) is now renamed as CKD – mineral and bone disorder (CKD-MBD) by considering the extra-skeletal manifestations associated with the condition (3).

The reduction of bone mineral density (BMD) which is a major determinant of bone strength (2) starts in the early stages of CKD and continues with the progressive decrease of renal functions and patients with end stage renal disease (ESRD) have a higher risk of fracture (2).

The associations between bone mass, lean and fat masses, are complex and vary according to age, gender, hormonal status, disease stage and drug usage. These associations are more complex in patients with CKD and there are uncertainties about the influence of obesity on bone mass among patients with CKD (4). We believe that understanding the interactions between three main body compartments; lean, fat and bone masses among patients with CKD is relevant in their long-term management. Such information would assist in designing nutritional interventions and life style modifications in patients with CKD. However, studies carried out to assess the association between bone, fat and lean masses among patients with ESRD on routine haemodialysis are sparse and we were unable to find previous studies conducted among Sri Lankans.

Methods

Patients with ESRD (stage V) awaiting renal transplant at Teaching Hospitals, Karapitiya and Kandy were included in the study. Consecutive patients were included after obtaining informed written consent. Patients who had previous fracture, metal implants or renal transplant were excluded from the study. Approval for the study protocol was obtained from the Ethics Review Committee of Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka. Fifty (38 males) age and gender matched controls were recruited for the study from the neighbourhood of the patients (neighbourhood controls) after excluding renal impairment by serum creatinine estimation. In selecting controls, chronic illnesses or following special life style modification programmes such as intense dietary interventions were excluded.

An interviewer administered questionnaire was used to collect data and additional information was gathered from personal medical records. The duration of the disease was calculated from the time of the first detection to the time of the interview. The duration of the dialysis was calculated from the first dialysis to the last dialysis session. The socio-demographic data collected included age, monthly income, education status, smoking and alcohol consumption. Patients were categorised as non-smoker (never smoked), past smoker or current smoker depending on the smoking status. Same type of categorisation was used to describe the alcohol consumption.

Standard protocols were followed when obtaining anthropometric measurements. A portable stadiometer was used to measure the height to the nearest 1 cm. Weight was measured to the nearest 0.1kg using a beam balance. A non-stretchable tape was used to measure waist and hip circumferences to the nearest 1 cm and waist to hip ratio was calculated.

Systolic and diastolic blood pressures were measured using a sphygmomanometer (*Matsuoka* Japan) after allowing the study subjects to rest for 15 minutes, an average of the two consecutive measurements was taken for analysis.

All patients underwent body composition analysis with dual energy x-ray absorptiometry (DEXA) (*Hologic Discovery QDR,* USA) scan. Bone related measurements included total body BMD (TBBMD), total body bone mineral content (TBBMC), BMDs in femoral neck, total hip and lumbar spine (L1 to L4, anterior-posterior projection). Total body lean mass (TBLM), total body fat mass (TBFM) and truncal fat mass (TRFM) were also measured using the analytical software (Version 12.4) provided by *Hologic*.

SPSS statistical software was used for the statistical analysis. Descriptive data were presented as mean (SD) while categorical data as frequencies and percentages. Non-normally distributed data were presented as median and interquartile range. Independent sample t-test was used to compare the anthropometric variables and body composition variables between the patients and controls. The association between body compartments were tested using Pearson correlation. Probability of <0.05 was considered as statistically significant.

Results

Fifty patients (38 males) with CKD and fifty age and

sex matched individuals (controls) were recruited for the study. The median (interquartile range) durations of disease and dialysis (those were on HD) were 24 (12 - 36) and 8 (4 - 12) months, respectively.

The majority of patients had completed school education up to the G.C.E. Ordinary Level and most of them had a monthly income of less than LKR 25,000. A considerable number of patients (26%) had no regular income.

There were no current smokers or current alcohol consumers among patients, however, there were 23 (46%) past smokers and 26 (52%) past consumers of alcohol (Table 1).

Collectively, a greater proportion of controls (54%) had completed G.C.E. Advanced Level or university education. The majority of controls (64%) had monthly income between LKR 25,000 to 60,000. Among controls, 26% were current smokers while 72% were current alcohol consumers (Table 1).

Table 1: Data on Socio-demographic characteristics and social habits

	Patients with CKD n = 50 (%)	**Participants in the Control group n = 50 (%)**
Education		
University or similar	4 (8%)	08 (16%)
G.C.E. Advanced Level completed	14 (28%)	19 (38%)
G.C.E. Ordinary Level completed	23 (46%)	10 (20%)
Grade > 5 – 11	7 (14%)	09 (18%)
Grade 1-5	2 (4%)	03 (6%)
Not disclosed	0 (0%)	01 (2%)
Monthly Income (LKR)		
> 25000 - < 60000	16 (32%)	32 (64%)
= 10000 - < 25000	17 (34%)	13 (26%)
= 5000 - < 10000	03 (6%)	04 (8%)
= 1000 - < 5000	12 (24%)	00 (0%)
No regular income	13 (26%)	01 (2%)
Unable to say	01 (2%)	00 (0%)
Smoking status		
Non-smoker	27 (54%)	25 (50%)
Past smoker	23 (46%)	12 (24%)
Current smoker	00 (0%)	13 (26%)
Alcohol Status		
Non-alcoholic	24 (48%)	14 (28%)
Past alcohol user	26 (52%)	00 (0%)
Current alcohol user	00 (0%)	36 (72%)

Participants in the control group and patients with CKD were similar with regards to height, weight and waist circumference. Compared to participants in the control group, patients with CKD had lower BMI and hip circumference and higher waist to hip ratio and systolic and diastolic blood pressures. Although patients with CKD had lower TBFM and TBLM and higher TRFM and body fat percentage compared to participants in the control group, only the difference in TBLM was significant. Furthermore, TBBMD, TBBMC and regional BMDs at the hip region were significantly lower among patients with CKD compared to the participants in the control group (Table 2).

In both participants in the control group and patients with CKD, TBBMD, TBBMC and regional BMDs showed significant positive correlations with TBLM. Compared to CKD patients, the correlation seen between these measurements were greater among participants in the control group (Table 3). Furthermore, inverse correlations were seen between percentage fat mass and TBBMC and TBBMD measurements. The inverse correlations seen between TBFM and TBBMC/ TBBMD values were not significant.

Table 2: Comparison of anthropometry, blood pressure and body composition between patients with CKD and participants in the control group

Measurement	**Patients with CKD (n = 50) Mean (SD)**		**Participants in the Control group (n = 50) Mean (SD)**		***p value***
Age (completed years)	45	(10)	44	(10)	0.72
Height (m)	1.62	(0.09)	1.61	(0.08)	0.54
Weight (kg)	58.4	(11.9)	62.3	(11.1)	0.09
BMI (kg/m^2)	22.1	(3.7)	23.9	(3.7)	0.01
WC (cm)	82.7	(10.5)	82.2	(9.00)	0.79
HC (cm)	89.7	(8.3)	93.8	(7.6)	0.01
WHR	0.92	(0.06)	0.87	(0.06)	<0.001
SBP (mmHg)	163.7	(27.4)	119.8	(13.9)	<0.001
DBP (mmHg)	97.4	(14.7)	79.6	(11.9)	<0.001
eGFR	9.4	(3.5)	94.7	(21.4)	<0.001
TBFM (g)	16113.1	(5229.9)	16622.8	(5242.9)	0.63
TRFM (g)	8144.3	(3162.3)	7548.8	(2617.9)	0.31
Body fat percentage (%)	27.8	(6.2)	27.0	(7.0)	0.54
TBBMC (g)	1925.8	(370.5)	2067.3	(336.5)	0.05
TBLM (g)	39011.2	(7879.4)	42444.1	(8051.7)	0.034
TBBMD (g/cm^2)	1.06	(0.11)	1.11	(0.09)	0.02
SBMD (g/cm^2)	0.93	(0.23)	0.94	(0.15)	0.88
FNBMD (g/cm^2)	0.72	(0.14)	0.85	(0.13)	0.001
THBMD (g/cm^2)	0.86	(0.13)	1.02	(0.14)	<0.001

Abbreviations: BMI - body mass index; WC - waist circumference; HC - hip circumference; WHR - waist hip ratio; SBP - systolic blood pressure; DBP - diastolic blood pressure; eGFR - estimated glomerular filtration rate; TBFM - total body fat mass; TRFM - truncal fat mass; BMC - bone mineral content; TBLM - total body lean mass; TBBMD - total body bone mineral density; SBMD - spine bone mineral density; FNBMD - femoral neck bone mineral density; THBMD - total hip bone mineral density.

Table 3: Correlations between measures of body composition among cases and controls

		TBBMC	TBBMD	SBMD	FNBMD	THBMD
TBFM	Cases	0.076	-0.093	-0.057	0.088	-0.123
	Controls	0.155	0.157	0.326*	0.157	0.275
TBLM	Cases	0.616**	0.377**	0.399	0.421*	0.217
	Controls	0.788**	0.570**	0.490**	0.547**	0.613**

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

Abbreviations: TBFM, to tal body fat mass; TBLM, total body lean mass; TBBMC, total body bone mineral content; TBBMD, total body bone mineral density; SBMD, spinal bone mineral density; FNBMD, femoral neck bone mineral density; THBMD, total hip bone mineral density.

When a regression model was fitted with TBBMD and TBBMC as dependent variables and TBLM as the independent variable, 1kg difference is TBLM was associated with 0.007 g/cm^2 (p = 0.02) and 0.010 g/cm^2 (p = 0.012) change in TBBMD in patients and controls, respectively. Furthermore, 1 kg difference in TBLM was associated with 32 g (p = 0.02) and 33 g (p = 0.018)different in TBBMC in patients and controls, respectively.

Discussion

This study showed significant positive relationships between TBLM, and total body and regional BMDs among both participants in the control group and patients with CKD. The correlations seen among participants in the control group were greater when compared with those among CKD patients. These findings indicate that muscle mass has greater influence than fat mass on total body and regional BMDs in this age group but the association is lesser among patients with CKD.

Information related to body composition analyses among patients with CKD is sparse. The observations made among CKD patients in this study are consistent with the limited literature previously published on patients with CKD on dialysis (4, 5). Previous studies have reported that lean mass is a better predictor of bone quality of patients on haemodialysis and healthy individuals (4, 6). The study by Negri, *et al*., using sixty patients on peritoneal dialysis (45 women and 20 men), found lean body mass to be the only body composition measurement to show a positive correlation with BMC among males and post-menopausal females (6).

The link between lean mass and BMD / BMC is plausible and possibly a result of dynamic load resulting from muscle contractions on bone formation (7, 8). Further, the distortions and tensions caused by muscle mediated mechanical forces on bone tissue act on osteocytes to upsurge bone strength (9). In addition, muscle mass produces stretching of collagen fibers and periosteum at the interface, resulting in the stimulation of bone growth, and higher blood flow to bone lead to an increase in bone strength, as blood flows to limbs at a level proportional to muscle mass (10).

Although it was thought that increased body fat, through peripheral synthesis of oestrogen, enhances BMD (11), recent studies have shown fat mass to have negative effects on bone mass (12). The concept of beneficial effects of mechanical loading of increased body weight over bone mass is challenged by these studies (13). In addition, it is evident that increased visceral fat is more harmful for bone tissue (12). A study by Fournie *et al,* conducted among patients on peritoneal dialysis revealed that surrogate markers of visceral fat such as waist circumference, truncal fat and leg fat were inversely related to cortical volumetric BMD (13). We found percentage fat mass to be inversely and significantly associated with BMD while inverse associations between TBFM and BMD did not reach statistical significance, probably due to sample size limitation and these findings are concordant with previous studies (13). Further,

the specific role of truncal fat over the total body fat in determining BMD cannot be ruled out.

Kirchengast and Huber, found that association between soft tissue body compartments and bone varies according to the gender (14). Their findings showed that fat mass is the best predictor of bone mass among women whereas lean mass is the best predictor among men (14). In our study, the majority of patients were men (76%) and the results obtained may have been affected by that.

One of the major strengths of our study was that we compared the associations between fat and lean masses with bone measurements in patients with CKD, age and gender matched participants in the control group. Another is the use of DEXA scan to measure the body composition parameters. It had allowed the measurement of both body composition and bone parameters non-invasively with a minimum radiation and high accuracy. Small sample size with less female representation and cross-sectional study design were the major limitations of the study.

Conclusions

Similar to participants in the control group, TBLM is a better predictor of TBBMC and TBBMD compared to TBFM and TRFM in patients with ESRD and this knowledge can be used in health promotion programs to improve bone health of these patients.

Data used for this publication will be shared upon request. Authors have no conflict of interest to declare.

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Immunohistochemical assessment of PTEN expression and its association with tamoxifen resistance in ER positive breast cancers

Wijesiri TW[1], Peiris HH[2], Mudduwa LKB[1]

[1]*Department of Pathology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.*
[2]*Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka.*

Correspondence: Dr. Thilini Wathsala Wijesiri
e-mail: *thiliniwwijesiri@gmail.com*
https://orcid.org/0000-0003-3585-3751
Submitted on 03.05.2021 and accepted for publication on 16.06.2021

ABSTRACT

Introduction: Although Estrogen Receptor (ER) positivity is a good prognostic factor in breast carcinoma (BC), a subset of patients experiences poor disease-free survival (DFS). Mutation in phosphatase and tensin homologue deleted on chromosome ten (PTEN) is identified as a poor prognostic feature in BC. This study was designed to find out the impact of lost or poor PTEN expression on ER positive BC, in terms of the recognized prognostic factors and survival outcome to find out its association with tamoxifen resistance.

Methods: This was a cross sectional study with a follow up component. BC tissue blocks submitted to our unit from 2006 to 2012 were selected. From the laboratory data, patients who had ER positive BC, undergone mastectomy, treated with tamoxifen were selected. All clinicopathological parameters, DFS and overall survival (OS) were analysed against lost or poor PTEN expression. Clinicopathological features were compared using Chi-square test. Kaplan-Meier model with log-rank test was used for the survival analysis.

Results: A total of 130 BC patients satisfied the inclusion criteria. PTEN expression was lost or poor in 82.3% (n=107) patients. PTEN expression had a positive association with the level of ER expression (p=0.011) and a negative association with Nottingham prognostic index (NPI) (p=0.045) and pathological stage (p<0.048). Only 12.1% (n=16) patients had recurrences and 7.69% (n=10) had died over a period of 51 months of mean follow up. There was no significant association between PTEN expression and survival.

Conclusions: This study showed that there is a statistically significant association between lost or poor PTEN expression and low ER expression, high NPI and stage 3 in ER positive BC. Further studies including larger study sample with a longer follow up are recommended to find out the association of PTEN with the survival in ER positive BC treated with tamoxifen.

Key words: *Breast carcinoma, ER, Immunohistochemistry, PTEN*

Introduction

Breast cancer is the second commonest cancer in the world and the most frequently occurring cancer among females (1). In the year 2012, 1.67 million new breast cancer cases were diagnosed and that is about 25% of all cancers around the globe (1). It is also the fifth leading cause of death of all cancers (1). Breast cancer is the commonest cause of cancer death in women in underdeveloped countries and the second commonest cause of cancer death in more developed regions in the world (1). In Sri Lanka, it is the leading cancer among females and accounts for 25.4% of diagnosed cancer among females (2). It also accounts for the highest cancer mortality in Sri Lankan females (2, 3).

Tamoxifen is the most commonly used selective estrogen receptor modulator (SERM) which is used for the treatment and prevention of estrogen receptor (ER) positive breast cancer and it has been the first line treatment for premenopausal patients with ER positive breast carcinoma (4). Tamoxifen acts as an anti-estrogen agent in the breast tissue. It acts by binding to ER leading to a conformational change in the receptors. This results in blockage in the expression of estrogen dependent genes. The prolonged binding of tamoxifen to the nuclear chromatin leads to decreased estrogen response by tumour cells, hence growth arrest and induction of apoptosis within the breast cancer cells takes place (5).

PTEN, also known as MMAC1 (mutated in multiple advanced cancers), is a tumour suppressor gene located at chromosome 10q23. PTEN mutation is associated with tumorigenesis, cancer progression and drug resistance and it is the second most frequently mutated gene in human cancer after p53 (6). Varieties of human tumours are known to associate with PTEN mutation, which includes glioblastoma, prostatic carcinoma, endometrial carcinoma, breast carcinoma and melanoma. Germline mutations in PTEN gene are known to cause Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (PTEN hamartoma tumour syndrome) characterised by a high risk of cancers including breast cancer. Affected female patients with CS syndrome have 25% - 50% life time risk of developing a breast carcinoma. Around 30% - 40% of sporadic breast carcinomas show PTEN loss (7).

PTEN acts as a tumour suppressor by antagonizing the phosphatidylinositol (4,5)-triphosphate kinase (PI3K)/ proteinkinase B (Akt) signaling pathway by dephosphorylating phosphorinositol 3,4,5-triphosphate (PIP3), a key signaling component of PI3K/Akt pathway and thereby modulating cell cycle progression and cell survival. The biological consequences of inhibition of PI3K/Akt pathway include stimulation of apoptosis and inhibition of cell cycle entry by halting G1 to S phase progression leading to growth inhibition (8). Therefore, mutation or reduced expression of PTEN can lead to inhibition of tamoxifen induced apoptosis leading to tamoxifen resistance in PTEN mutated breast carcinoma.

Though many studies have been done in the past to identify the role of PTEN gene mutation in various cancers, its prognostic significance in breast cancer is not sufficiently investigated. A few studies have found out that reduced PTEN expression in breast cancer have a significant relationship with tumour size, pathological stage, lymph node metastases and ER and Progesterone Receptor (PR) status (9, 10).

The aim of the study was to investigate the role of PTEN gene as a prognostic marker in ER positive breast cancer patients by analysing immunohistochemical expression of PTEN and its association with recurrence of disease, survival, stage, grade, tumour size and hormonal receptor status and to find out its association with tamoxifen resistance.

Methods

This was a cross sectional study with a follow up component which included 130 breast cancer patients. Breast cancer tissue blocks submitted to our unit from 2006 to 2012 were selected. Using data from the laboratory database and co-investigator's database, patients who had ER positive BC, undergone mastectomy, treated with neo-adjuvant tamoxifen therapy were selected. Wax blocks with perished tissue, haematoxylin and eosin (H&E) slides showing autolytic changes and patients who were stage IV at presentation were excluded from the study. All relevant clinical parameters were retrieved from the histopathology reports at the Department of Pathology, Faculty of Medicine, University of Ruhuna and the survival data were retrieved from the co-investigator's data base.

Following definitions were used to define Recurrence Free Survival (RFS) and Overall Survival (OS) which are the same definitions that were used to define the above endpoints in the said data base that contains patients follow up details.

Recurrence free survival (RFS) - Time from the date of diagnosis to the date of confirmation of development of local, regional and/ or distant recurrences (11).

Overall survival (OS) - Time from the date of diagnosis to the date of death due to any reason (11).

Date of diagnosis of the disease - Date of diagnosis or confirmation of breast carcinoma by Fine Needle Aspiration Cytology (FNAC), tru cut, and incision or excision biopsy; whichever was done first.

Date of recurrence - Date of diagnosis of recurrence by histology, cytology or radiology; whichever was done first.

Tissue microarray (TMA) blocks were prepared from the wax blocks with breast cancer tissue for the PTEN assessment. Normal breast tissue was taken as the control.

Immunohistochemistry

PTEN immunohistochemistry was done manually with anti-PTEN antibody (monoclonal, mouse anti human, clone 6H2.1, dilution 1:100, Dako) with EnVision system (HRP labeled Polymer, Dako) and chromogen Dako Dab liquid. Immuno-histochemistry staining was performed according to the protocol which was optimised and validated for PTEN, in our laboratory.

The sections were taken on to poly-L-Lysine coated slides and were incubated overnight at a temperature of 60ºC. Then the slides were deparaffinized and hydrated by passing through Xylene and graded series of alcohol. Antigen retrieval was performed by pressure cooking in pH 9 buffer. Then the sections were treated with endogenous peroxidase blocking buffer for 15 minutes to block the endogenous peroxidase activity. As the next step, sections were incubated overnight in the humidified chamber with 6H 2.1 PTEN primary antibody (dilution 1 : 100). Afterwards, they were washed in PBS buffer twice and treated with the secondary antibody (EnVision system). After washing, Dab substrate buffer solution (freshly made) was added to the sections to reveal the PTEN antibody. Following this step, the sections were again washed with PBS buffer and counterstained with Harris Haematoxyline and differentiated with acid alcohol and mounted with DPX.

Interpretation of Staining for PTEN

PTEN immunohistochemical expression can show cytoplasmic and/or nuclear localization (12). In our study it was predominantly cytoplasmic and normal glandular epithelium was taken as the control as it shows immunoreactivity for PTEN. Duct epithelial cells and myoepithelial cells showed strong cytoplasmic staining for PTEN. Stromal cells and inflammatory cells also showed strong cytoplasmic staining for PTEN which were useful as internal controls.

Scoring for PTEN immune-expression was done according to a semi-quantitative scale, introduced by Andrade *et al.,* which is based on intensity of immunohistochemical staining (12). According to the intensity of staining, the tumours were divided in to three groups. Staining intensity of normal duct epithelial cells was taken as the control (Figure 1). The group assigned as "0" had no staining (Figure 2a), group assigned as +1 had reduced staining (Figure 2b) and group assigned as +2 had equal staining intensity (Figure 2c), compared to normal duct epithelial cells (Table 1).

Table 1: Scoring of PTEN immune expression by tumour cells

PTEN expression (compared to normal duct epithelial cells) by tumour cells	Score
No staining	0
Reduced staining intensity	+1
Equal staining intensity	+2

Statistical analysis

Statistical analysis was done by using *SPSS20* software. Chi-square test was used to determine the associations between different variables. Recurrence free survival and Overall survival were calculated by the Kaplan-Meier survival estimates and log rank test. The level of significance was set at 0.05.

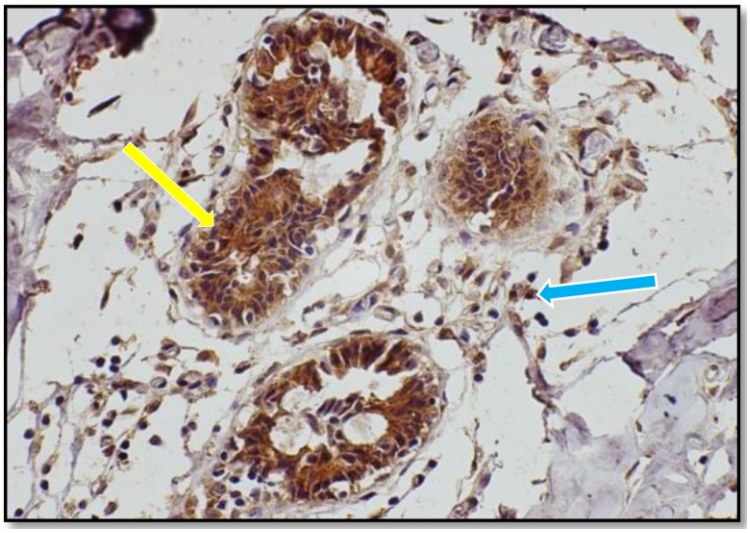

Figure 1: Normal breast tissue showing strong cytoplasmic positivity for PTEN (yellow arrow). Background inflammatory cells also show cytoplasmic positivity for PTEN (blue arrow), which was useful as an internal control. (H&E x 40)

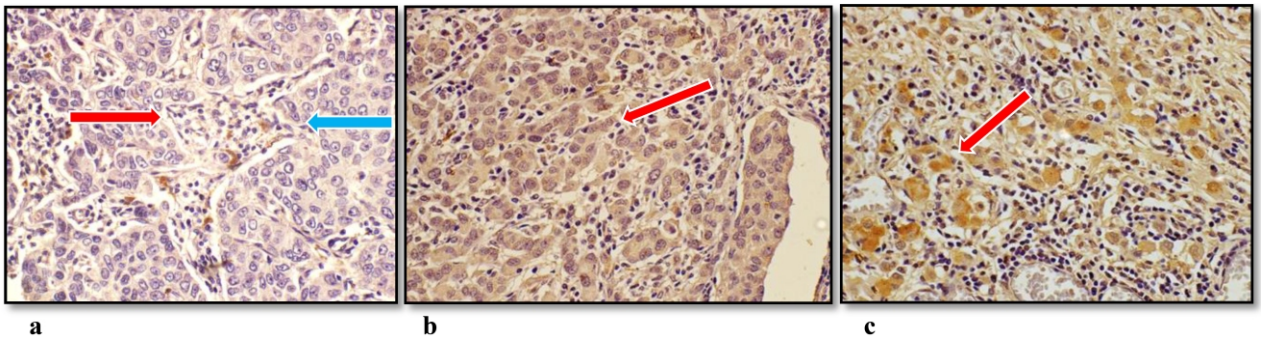

Figure 2: a. Score 0 = staining undetectable in tumour cells (red arrow). Background inflammatory cells show cytoplasmic positivity (blue arrow),
b. Score 1 = staining weaker than normal duct epithelial cells (red arrow),
c. Score 2 = staining equal to that of normal duct epithelial cells (red arrow) (H&E x 40)

Results

Clinicopathological findings

The study sample consisted of 130 patients with ER positive breast cancer who had underwent modified radical mastectomy. Majority of the patients were between ages 36 and 60 years (71%) and the mean age at diagnosis was 53 years. All patients have been treated with standard adjuvant tamoxifen therapy. Mean patient follow-up period was 51 months (6-93 months). At the completion of the study, 10 patients had died and 120 were alive. In the study group, 117 (90%) patients had duct carcinomas while 10 (7.69%) patients had lobular carcinomas and only 3 (2.3%) patients had mucinous carcinomas. Out of the total number of 130 patients, lymph node (LN) metastasis was present in 69 (54.3%), whereas only 58 (45.7%) were nodes negative. Disease recurrence occurred in 16 (12.1%) of patients while 113 (86.9%) had no recurrence at the end of the follow up. Out of those who had recurrences, 14 (10.8%) had metastasis and two had local recurrence (1.5%). Patients' characteristics and tumour characteristics are shown in Table 2.

Table 2: Patients' characteristics

Characteristics	Number	Percentage
Tumour size		
< 2 mm	55	44%
2 - 5 mm	64	51%
> 5mm	7	5%
Histological type		
Duct	113	90%
Lobular	10	8%
Other	7	2%
Nottingham Grade (NG)		
1	27	21%
2	75	58%
3	26	20%
Poor fixation	2	1%
Nottingham prognostic index (NPI)		
< 3.4	30	25%
3.4 - 5.4	72	59%
> 5.4	20	16%
Lymph node stage		
1	111	78%
2	16	12%
3	10	8%
Pathological stage		
I	28	22%
II	64	52%
III	32	26%

ER, PR and Her2 phenotype

All patients were ER positive (100%) and Her 2 negative (100%) while 113 (87.6%) patients were PR positive.

PTEN immunophenotype

PTEN expression was positive (Score 2+) in 23 (17.7%) patients while it was negative (Score +1 or Score 0) in 107 (82.3%) patients. PTEN immuno-expression was analysed in relation to clinicopathological parameters. No correlation was found between PTEN expression with patients age category (p=0.301), tumour size (p=0.178), histological type, lympho-vascular invasion (p=0.232), Nottingham grade (p=0.46), LN metastasis (p=0.106), PR expression (PR=0.127), recurrence of the disease (p=0.304). However, 26/27 (95.7%) in the low ER expression category (Allred score 3 and 4) was PTEN negative, while PTEN negativity was observed in 81/103 (78.6%) patients in ER high expression category (Allred score 5 to 8). Therefore, PTEN negativity was more frequent in tumours with low ER expression (p=0.023) demonstrating a strong positive association of PTEN expression with low and high ER expression. In contrast, PTEN negativity was frequent among tumours with high NPI score than low NPI score; 22/23 (95.7%) tumours with a high NPI score (>5.4) were PTEN negative, whereas only 78/99 (78.8%) tumours in the low NPI score category had PTEN negativity. These results exhibit a negative association of PTEN expression with NPI score (p=0.045). Similarly, PTEN negativity was significantly more frequent in stage 3 tumours than stage 1 and 2 (30/32vs72/92) p = 0.0480. This also highlights the negative association of PTEN expression with pathological stage of the tumour.

Survival analysis

PTEN expression was analysed against overall survival (p=0.713) (Figure 3a) and recurrence free survival (p=0.452) (Figure 3b) and failed to demonstrate a significant association. Survival analysis in relation to PTEN expression was also done in separate groups of patients according to LN stage, pathological stage, ER expression and NPI value, but failed to demonstrate a significant relationship.

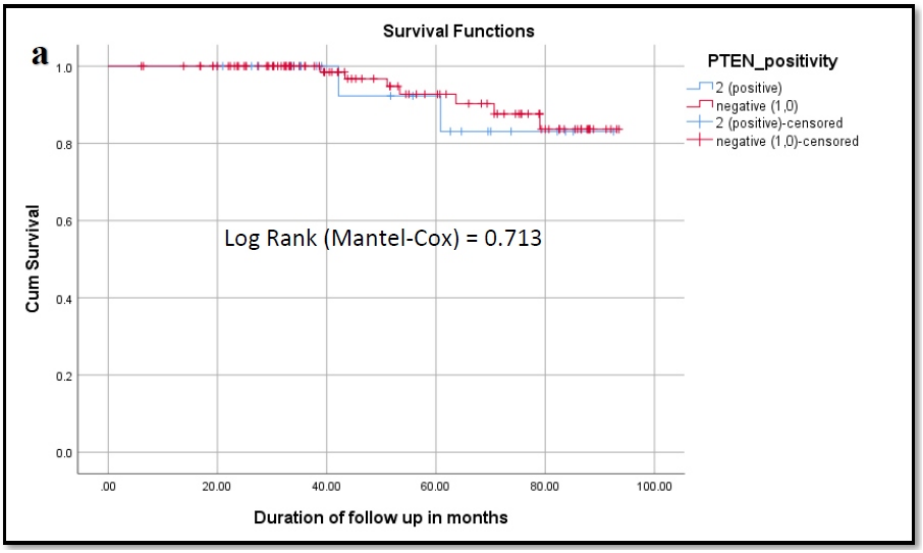

Figure 3: **a.** Comparison of overall survival in PTEN negative and positive groups

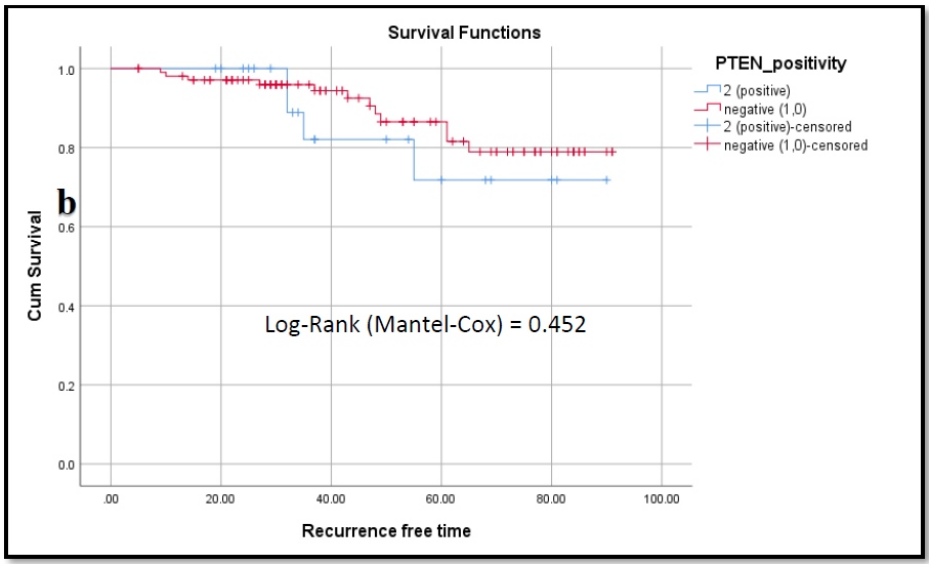

Figure 3: b. Comparison of Recurrence free survival in PTEN negative and positive groups.

Discussion

The aim of the study was to investigate the role of PTEN gene as a prognostic marker in breast cancer patients by analysing immunohistochemical expression of PTEN and analysing its association with recurrence of disease, stage, grade, tumour size and hormonal receptor status and to find out the association of PTEN with the survival in ER positive breast carcinomas treated with tamoxifen.

Only two studies, in the past have evaluated the PTEN expression in breast carcinoma with the tamoxifen resistance and they have demonstrated a poor survival in breast cancer patients with PTEN mutation compared to non-mutated ones (13, 14). Both the studies included small numbers of patients, 49 and 100. Both used immuno-histochemistry to evaluate the PTEN status, out of

those, one study had used genomic studies (fragment analysis) to evaluate the PTEN gene (14). Recurrence rate for breast cancer in both the studies were significantly high (57% and 47.9%). Survival studies must have a sufficient follow up to capture enough events and thereby ensure there is sufficient power to perform statistical tests. Although the number of participants is small in these two studies, because of longer follow up periods the comparatively higher number of events may have given a sufficient statistical power to the study.

Our study, which included 130 patients, did not demonstrate a relationship with PTEN expression and overall survival or recurrence free survival. However, the study showed that PTEN expression positively correlates with the level of ER expression (high and low). This means that PTEN negative patients are most likely to have low ER expression. Studies have proven that patients with high ER expression respond well to endocrine therapy compared to low expressers (15). In addition, our study demonstrated that PTEN expression negatively correlates with NPI value (patients with >5.4 and $\leq$5.4) and pathological stage of the tumour (patients in stage 1,2 and 3). It is well known that breast cancer prognosis is poor with tumours having NPI scores >5.4 as well as stage 3 tumours, compared to tumours in stage 1 and 2. Therefore, this study gives evidence favouring that loss of PTEN is a prognostic feature which signifies poor prognosis among breast cancer patients. Moreover, our study further gives evidence to support that PTEN can be lost even in patients with well-known good prognostic feature; ER positivity.

One of the above studies (13) also demonstrated a positive association of PTEN with ER expression and a negative association with LN metastasis and tumour recurrence but none of those studies showed a correlation of NPI and pathological stage with the PTEN expression.

Recurrence rate in our study group was very low (12.1%) compared to the above studies (57% and 47.9%) which may be the main reason why our study does not demonstrate a relationship with PTEN expression and survival. The number of events in the current study cohort appears not sufficient to substantiate an existing relationship. It can also be related to the mean follow-up period, which was nearly 4 years (51 months). In the other two studies the mean follow-up period was 72 (6 years) and 114 months (10 years) respectively (13, 14). The follow up period in our study is unlikely to be influencing the relationship between PTEN expression and survival because the study group which followed up to 6 years also demonstrated a significant association with loss of PTEN expression and survival, which was only around 2 years longer than our study. The discrepancy in the recurrence rate could be due to the fact that breast cancer patients are being managed well in the Sri Lankan health care system compared to the other two countries, Canada and Serbia, in the above study groups. This is further explained by the fact that in Sri Lanka, five year breast cancer survival is around 78.8%, which is not low compared to the USA figures (90%).

In this study the percentage of PTEN mutation (Low or absent expression) was 82.3%, which was higher than previously reported values (57% and 44.9%). The observed discrepancy can be related to the study population, which was a different population in a different part of the world as previous two studies were done in European countries, which has a different genetic composition. Other important reason for this discrepancy can be due to the sensitivity of the immunohistochemical analysis. In our thorough literature review, standardized reliable and reproducible methods for measuring PTEN expression on formalin-fixed tissue was lacking. The only study that was found was by Andrade *et al.,* who had developed a protocol for assessing PTEN status in formalin fixed breast cancer sample immunohistochemically. Our study was the first time that immunohistochemistry was used to evaluate PTEN status in tissues in Sri Lanka. Before commencing the study we have optimised and validated the immunohistochemistry method for PTEN.

In conclusion, though this study did not demonstrate a relationship between reduced PTEN protein expression with recurrence free survival and overall survival in tamoxifen treated patients, it was able to give further evidence that PTEN expression can be used as a prognostic marker in ER positive breast cancer patients as it showed a positive association with the level of ER expression

and negative association with NPI score and the pathological stage of the tumour. Further, this study also confirmed that a fraction (four fifths) of breast cancer patients that are categorized to have a better prognosis (ER Positivity) can have a poor prognostic feature; loss or poor PTEN expression. We would recommend further studies recruiting a larger study sample with a longer follow up to find out the association of PTEN with the survival in ER positive breast carcinomas in order to find out any relationship between loss of PTEN and tamoxifen resistance.

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The relationship between platelet count and the onset of leaking phase in dengue patients

Nishshanka P[1], Kodikararachchi RW[2], Karunanayake A[3]

[1]*Postgraduate Institute of Medicine, Colombo, Sri Lanka.*
[2]*Department of Clinical Medicine, Teaching Hospital Karapitiya, Galle, Sri Lanka.*
[3]*Department of Physiology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.*

Correspondence: Dr. Amaranath Karunanayake
e-mail: *a2222nath@gmail.com*
https://orcid.org/0000-0001-5836-6329
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ABSTRACT

Introduction: The complications of dengue are related to reduced intravascular volume following plasma leakage. Thrombocytopenia or platelet dysfunctions are detected in all stages of the illness and are related to the clinical outcome. The main objective of the study was to correlate absolute platelet count to the onset of leaking phase in dengue patients who had entered into leaking phase after the hospital admission.

Methods: One hundred and sixteen patients (age range 14-74 years, median age 33 years) who entered the leaking phase after admission were recruited to the study. Dengue infection in patients was confirmed with either the presence of positive NS1 or positive IgM antibodies. Serial ultrasonography was done from admission until the leaking phase is detected. Platelet counts were assessed twice per day just prior to the ultrasonography.

Results: There were 47 (40.5%) females and 69 (59.5%) males among the recruited patients. The mean number of days at which leaking was detected following onset of fever was 4.9 ± 0.9 days. The mean platelet count at the onset of leaking phase was 30,051 ± 17,023 /µL. Age and the platelet count at the time of onset of leaking phase was significantly negatively correlated (whole group r = -0.323 $p < 0.001$; in females r = -0.369, $p = 0.011$; males r = -0.280 $p = 0.020$). Platelet count at the time of onset of leaking phase was significantly lower in the patients older than 30 years of age (27,577.5 ± 17,361.4) compared to those of the patients younger than 30 years (33,955.6 ± 15,888.8) (p=0.032). Upper limit of the range of platelet count at the onset of leaking phase was 77, 000/ µL which was slightly lower in females.

Conclusions: According to this study, the value of platelet count as a predictor of the progression of dengue infection depends on the age of the patients. Young patients are more prone to develop leaking phase with higher platelet counts than the older patients.

Key words: *Thrombocytopaenia, dengue, leaking phase*

Introduction

Dengue viral fever is the most prevalent mosquito-borne disease in the world.

The first case of dengue in Sri Lanka was reported in 1960 (1). During the past few decades, the Sri Lankan population has gone through several epidemics and is currently experiencing annual dengue epidemics (2). The incidence and the severity of the disease are significantly increased during recent years resulting in high mortality.

Many dengue patients are asymptomatic and may not progress into the critical phase. The critical phase

occurs around the time of defervescence associated with an increased tendency for capillary leakage. The leaking phase manifests as a rise in haematocrit, pleural effusions, ascites or haemorrhage (3).

Therefore, dengue patients should be closely monitored for the development of plasma leakage. As a prognostic indicator, ultra sound scans (USS) could be used to assess patients at risk for entering the critical phase. Gallbladder wall thickening due to oedema which can be detected in USS is considered the earliest sign of plasma leaking in dengue haemorrhagic fever (4). The haematological parameters are useful in predicting the development of critical phase within the next 24 to 48 hours (5).

The platelet count has value as potential indicator of clinical severity in dengue (6, 7). A rapid decline in platelet count or a platelet count less than 150,000/µL blood is an alarming sign of dengue (6). The mechanisms involved in thrombocytopenia are not fully understood (8). Bone marrow suppression (9, 10) and increased peripheral destruction (11, 12) are the proposed mechanisms for thrombocytopenia. Furthermore, number of studies have documented platelet dysfunction in dengue (13, 14).

The value of platelet count as a predictor of critical phase has been evaluated in several studies. The rapid decline of platelet counts before the critical phase has been reported in both adult and paediatric studies (15-17). Several studies showed gallbladder wall thickening which was significantly associated with lower platelet counts (17-20). Santhosh *et al.* have reported gallbladder wall oedema in 97.8% of patients whose platelet count was less than 40,000/µL (19).

There have been no studies done in Sri Lanka on the value of platelet count as a predictor of the critical phase. The main objective of this study was to assess the relationship between ultrasonographic evidence of earliest leakage and corresponding platelet count in dengue patients who had entered into leaking phase after the hospital admission.

Methods

This descriptive cross-sectional study was conducted at Teaching Hospital, Karapitiya, over a period of six months. All patients with confirmed dengue fever with positive NS1 antigen or IgM antibodies and developed leaking phase after the admission into two medical wards were included in the study. Patients with dengue leaking phase on admission, patients with other aetiologies for thrombocytopenia such as cirrhosis, splenomegaly, autoimmune disorders, myelodysplastic syndromes or malignancies and patients who were on thrombocytopenic drugs were excluded from the study.

After obtaining informed written consent, the data were collected by the principal investigator using a pre-tested questionnaire.

All dengue patients underwent serial ultrasonography on admission and then twice a day until leaking phase was over. Ultrasonography was performed by a medical registrar experienced in detecting dengue leaking phase under the supervision of a consultant radiologist. The consultant radiologist repeated all doubtful scans. USS studies were performed with 6 hours of fasting to optimize gallbladder visualization. Gallbladder wall thickening was used as a radiological sign to detect the leaking phase. Wall thickness of more than 3 mm was considered as gallbladder wall thickening (18). All ultrasound examinations were performed with a portable machine (*L&T symphony model*) using 3.5 MHz and 5 MHz probes. Gallbladder wall thickness was measured by using a previously reported and validated ultrasound method (21). Blood samples were collected 30 minutes before each scan using standard procedures in to EDTA tubes and immediately analysed using Mindry 6068 and *Sysmex* XN 1000 automated full blood count analysers. Once the leaking was detected latest platelet count was taken for the analysis.

The data were analysed with *PSPP* version 0.83-g5f9212 statistical software (Free Software Foundation, Inc. htttp//fsf.org/). Mann Whitney U test was used to assess the significance between variables. $P < 0.05$ was considered statistically significant.

The ethical approval for the study was obtained from the Ethics Review Committee of Faculty of Medicine, University of Ruhuna. Approval was taken from the Director, Teaching Hospital, Karapitiya for data collection and written, informed consent was obtained from the patients or the guardian in case of patients less than 18 years of age.

Results

Of one hundred sixteen patients who had entered into leaking phase after the hospital admission, 47 (40.5%) were females and 69 (59.5%) were males. The median age of females was 36 years and males were 32 years. Majority (55%) of the patients were from Galle Municipality area. Demographic characteristics and variables related to the disease course are given in Table 1.

The duration of hospital stay was significantly higher in female patients than in male patients (p = 0.043). However, gender and the duration of the fever on admission was negatively correlated with the duration of the hospital stay (r = -0.344; p = 0.018). Furthermore, there was no gender-related difference in the duration of fever on admission.

The duration of fever on admission was negatively correlated with the duration of hospital stay (r = -0.410; p < 0.001) and platelet count at the onset of leaking phases (r=-0.200; p=0.031). Furthermore, the duration of fever on admission was positively correlated with the duration of fever at the onset of leaking phase (r = 0.201; p = 0.031).

However, among the male patients, the duration of fever on admission was negatively correlated with the mean duration of hospital stay (r = -0.490; p < 0.001) and mean platelet count at the onset of leaking phases (r = -.331; p = 0.005). The platelet count at the onset of leaking phases was positively correlated with the duration of the hospital stay (r = -0.252; p = 0.037).

Correlation of the mean duration of fever on admission, during the hospital stay and at the onset of leaking phase according to the age group is given in Figure 1.

The mean number of days the leaking phase was detected after the onset of fever was 4.9 ± 0.9 days. There was no statistically significant association between the duration of fever at the onset of leaking phase and gender (p = 0.247) (Figure 2). Mean platelet count at the onset of leaking phase was 30,051 ± 17,023. There was no significant association of platelet count at the onset of leaking phase and the gender (p = 0.478).

There was a significant negative correlation between the age and the platelet count at the time of onset of leaking phase in the sample (r = -0.323 p = <0.001; females r = -0.369; p = 0.011 and males r = -0.280; p = 0.020) (Figure 3). Upper limit of the range of platelet count at the onset of leaking phase was slightly lower in females.

Table 1: Demographic characteristics and variables related to the disease course

Demographic characteristics	Male (n=69)	Female (n=47)	Total (n=116)
Age (median)in years	32	36	33
Age range (years)	14 - 69	15 - 74	14 - 74
Mean duration (SD) of the hospital stay (days)	5.1 (1.4)	5.6 (1.5)	5.3 (1.5)
Range of duration of hospital stay (days)	3 - 10	4 - 10	3 - 10
Mean duration (SD) of fever in days on admission	4.0 (1.5)	4.2 (1.6)	4.1 (1.5)
Mean duration (SD) of fever in days at the onset of leaking phase	4.8 (0.9)	5.0 (0.9)	4.9 (0.9)
Mean platelet count (cells/ μL) (SD) at the onset of leaking phase	30449 (16554)	29468 (17855)	30051 (17023)
Range of platelet count at the onset of leaking phase (cells/ μL)	3,000 - 77,000	7,000 - 74,000	3,000 - 77,000

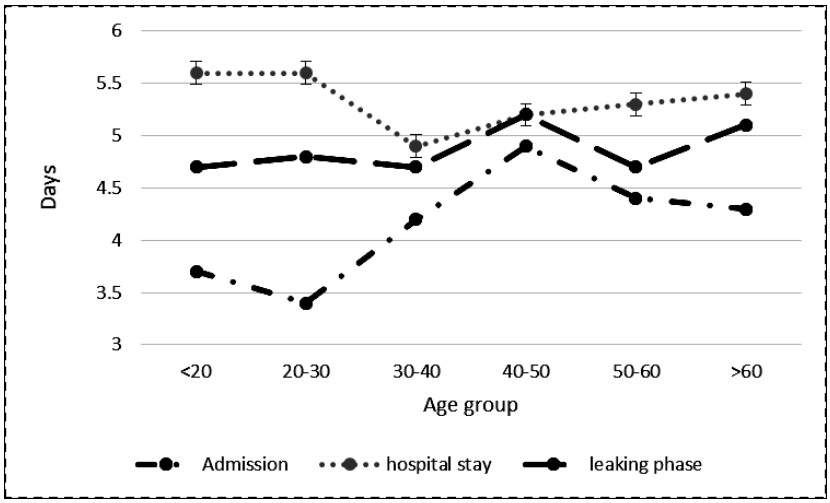

Figure 1: Mean durations of fever; on admission, during hospital stay and at the onset of leaking phase according to the age group

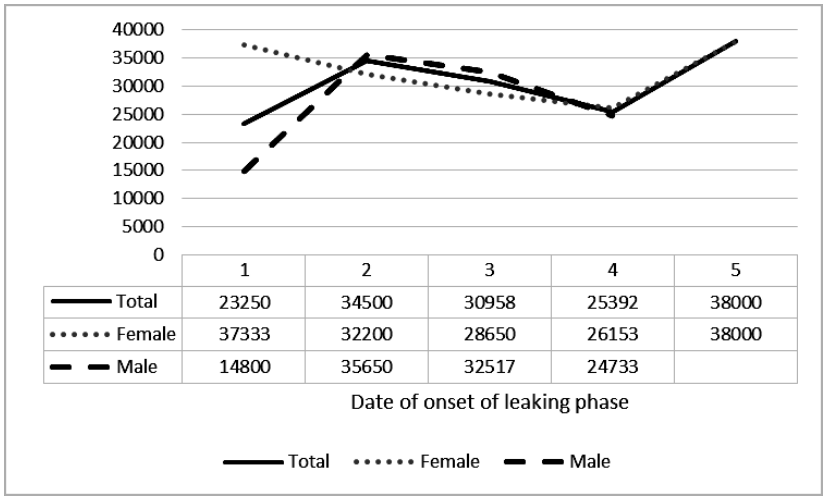

Figure 2: Correlation between mean platelet count at the onset of leaking phase and mean duration of the fever at the onset of leaking phase

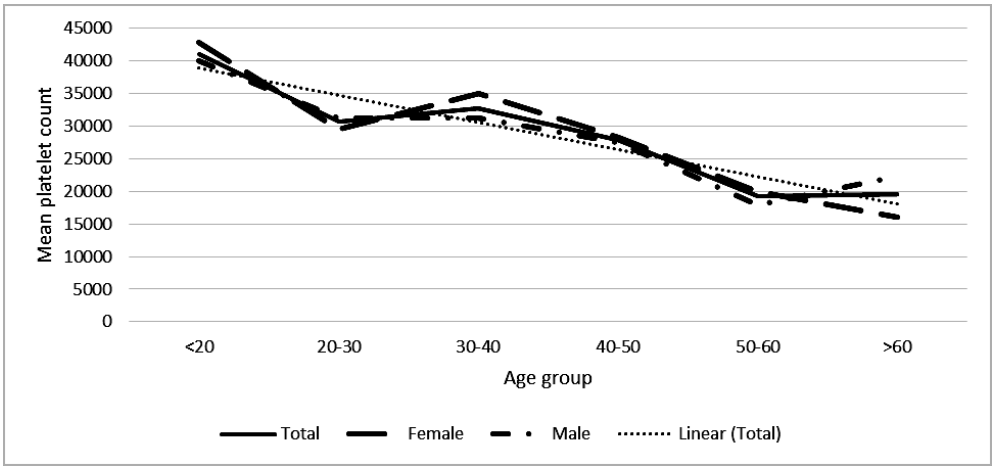

Figure 3: Correlation between age and the platelet count at the time of onset of leaking phase

The platelet count at the time of onset of leaking phase was significantly lower in patient older than 30 years of age (27,577.5, SD 17,361.4) than patients younger than 30 years of age (33,955.6, SD 15, 888.8) ($p = 0.032$).

Discussion

This study shows that the value of platelet count as a predictor of the progression of dengue fever depends on the age of the patients.

Age and gender have been studied as factors forecasting the severity of dengue. Female gender and young age have been found as risk factors for mortality (22). The duration of hospital stay was significantly longer in female patients than male patients in the current study which would support the finding of preponderance of females among severe dengue cases but not among mild cases (23). It has been suggested that risk of female gender reflects either a more robust cellular immune response or a higher intrinsic susceptibility to capillary permeability in females than males (24).

The age-stratified analyses in previous studies have demonstrated the difference in clinical and laboratory manifestations of dengue between elderly and younger adults. It was observed that children less than 10 years of age are more prone to increased vascular permeability, leading to shock (24). In our cohort, the age and the platelet count at the time of onset of leaking phase was significantly negatively correlated. This shows that the young patients are more prone to develop leaking phase with higher platelet counts than the older patients.

Similar to our findings, several studies have reported that platelet count ≤ 100,000/μL increased the risk of leaking (25, 26). Furthermore, platelet count decreases rapidly before patients enter the state of shock. This emphasizes that in young patients leaking phase may develop at higher platelet counts than in elderly patients.

Upper limit of the range of platelet count at the onset of leaking phase was in this study was 77,000/μL, which was slightly lower in females. Considering the higher limit of the mean platelet count at the onset of leaking phase as observed in the current study, we propose that the leaking phase must be anticipated when the platelet count reaches 80,000/μL. National Guidelines recommend that platelet counts less than 100,000/μL indicate that the patient might enter the critical phase (onset of plasma leakage) and this is supported by many other studies (27, 29). This study further supports the above recommendation.

The mean duration of fever at the onset of the leaking phase was 4.9 ± 0.9 days with a wide range in platelet count. These findings emphasize the importance of early detection and continuous monitoring of dengue patients. Early identification of the patients with risk improves the outcome. Furthermore, it may enhance the efficient allocation of hospital resources by diverting resources for patient with impending threat for severe disease.

Conclusions

The value of platelet count as a predictor of the progression of dengue fever depends on the age of the patients. Younger patients are more prone to develop leaking phase with higher platelet counts than the older patients.

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Characteristics of cases of fatal drowning at a tertiary care hospital in Sri Lanka

Ariyarathna HTDW, Jayathilaka ELPA

Department of Forensic Medicine, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.

Correspondence: Dr. H. T. D. W. Ariyarathna
e-mail: *ariyaratna@sjp.ac.lk*
https://orcid.org/0000-0002-4266-5508
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ABSTRACT

Introduction: Deaths due to drowning largely manifest as preventable accidents or suicides. This study deals with characteristics of cases of fatal drowning presented to a tertiary care hospital in Sri Lanka.

Methods: A descriptive study was conducted retrospectively based on postmortem reports of deaths due to drowning available at the Colombo South Teaching Hospital from September 2015 to September 2020. The study reviews characteristics of fatal drowning cases reported to the institution including their epidemiology, features, injury patterns and associated factors.

Results: Drowning accounted for 1.35% (n = 65) of total postmortems performed. The male to female ratio was 8:1. Children below 15 years of age contributed to 7.7% of the deaths. Accidental deaths were the commonest followed by suicide and homicide respectively. Evidence of alcohol consumption was noted in 30.8% (n = 20) and the majority of the drownings occurred in the sea. No putrefaction was seen in 58.5% (n = 38) bodies. The characteristic froth was noted in 43.1% (n = 28) of the study sample and there had not been a significant association between the presence of froth with the medium of water. Facial and chest injuries were the commonest associated injuries. Four cases of late death (near drowning) were reported. Twenty-six (40%) deaths could have been prevented.

Conclusions: This study helps to emphasize an existing problem in Sri Lanka. The prevention of drowning is mostly an achievable goal in Sri Lanka as long as the risk factors and epidemiology are known along with an adequate emphasis.

Key words: *Drowning, epidemiology, prevention*

Introduction

Deaths due to drowning is not unusual to a forensic pathologist. Deaths that ensue submergence of a person in water is considered as drowning. When the manner or circumstance of death is considered, drowning could be accidental, suicidal, homicidal, or unascertained. Drowning leads to different outcomes. Some victims who drown mange to survive without any ill effects. The events of drowning may cause immediate death, delayed death, morbidity or even brain death resulting in a vegetative status. The latest Sri Lankan Annual Health Bulletin (2019) does not specifically mention the mortality and morbidity data of immersion incidents. (It mentions only overall 'injury' data that include poisoning and other consequences of external causes (S00-T98) (1).

The Indoor Morbidity and Mortality Record (IMMR) is one of the most important records available in the country for the evaluation of the health status of Sri Lanka. Indoor Morbidity and Mortality Reporting of Sri Lanka should be commended at this point, as it attempts to classify injuries and diseases according to the International Statistical Classification of Diseases and Related Health Problems (ICD), but with certain deficiencies. Drowning incidents are just grouped under injuries in the IMMR, but no real picture or the ICD are depicted therein (2). Moreover, an agreed terminology in relation to drowning is essential for an effective comparison to be done (3). According to the World Health Organization (WHO), though drowning is easily preventable, an adequate study and consideration have not been given to this subject. Even global estimations are considered to be significantly lower than the real incidence of drowning (4).

The author is of the view that there should be a readily available data set for fatal drowning in a country, as some of the cases are easily preventable. Furthermore, the existing patterns should be available before implementing any of the preventive measures. Following postmortem examinations, ways of preventability from drowning were considered. This exercise showed that most of the deaths were unwarranted. Although the underlying causes for drowning are multifactorial, it is always beneficial to have a detailed national database, as many strategies can be implemented to reduce the incidence of such tragedies. However, there is no national database on deaths due to drowning in Sri Lanka. Furthermore, there is no proper emphasis on preventability and no detailed reports or published studies are available highlighting the aspect of preventability.

This study was primarily designed to study characteristics of cases of fatal drowning reported to the institution, encompassing their epidemiology, characteristic features, injury patterns and associated factors.

Methods

A descriptive study was carried out retrospectively based on the reports of postmortem examinations performed at the Colombo South Teaching Hospital from September 2015 to September 2020. Post-mortem records of all confirmed cases of deaths due to drowning during the study period were selected for the study. The study reviewed characteristics of fatal drowning including its epidemiology, features, injury patterns and associated factors. Data was analysed with *SPSS*™ version 21 and $p < 0.05$ was considered as statistically significant. Ethical approval for the study was obtained from the Ethics Review Committee of Colombo South Teaching Hospital.

Results

A total number of 4804 postmortem examinations were performed during a period of 5 years. Out of them, 65 deaths were due to drowning and they accounted for a 1.35% of all the postmortem examinations performed. There were 58 males and 7 females within the study sample (male : female ratio 8 : 1). There were only four incidents, where the victims died only following admission (near drowning). All the other victims were dead on admission (n = 61, 93.8%). The mean age was 42.7 (SD ± 23.53) years. The highest number of deaths were reported in the age category of above 70 years followed by the age category of 16 - 20 years showing a biphasic distribution. For the ease of comparison with other studies done locally and internationally, the age groups were divided into three classes; less than 15 years, 16 - 30 years and more than 30 years. The highest number of deaths (n = 38, 58.5%) were reported in the above 30-year age group (Table 1).

Table 1: Age distribution of the study sample

Age group	Frequency (n)	Percentage (%)
Below 15 years	5	7.7
16 years - 30 years	22	33.8
Above 30 years	38	58.5
Total	65	100

Table 2: The circumstances/manner of death in the study sample

Circumstances of death	Frequency (n)	Percentage (%)
Accidental	41	63.1
Suicidal	14	21.5
Homicidal	1	1.5
Unascertained	9	13.8
Total	65	100.0

Most deaths were accidental (n = 41, 63.1%), followed by suicides (n = 14, 21.5%). One death was a homicide (1.5%), involving a 43-year-old male. Nine deaths (13.8%) could not be definitively categorized and they were categorized as "unascertained" (Table 2).

There was a considerable gap between males and females in accidental deaths (male to female ratio 7.3:1) and suicidal deaths (male to female ratio 6.1: (1). No females were found in the categories of homicide and unascertained drowning deaths (Figure 1).

State of Alcohol consumption before the drowning

It was ascertained at the postmortem examination by the time of death that 20 (30.8%) victims had consumed alcohol whereas 30 (46.2%) had not. In 14 cases (21.5%) alcohol consumption status before drowning could not be ascertained.

Table 3 outlines the site of drowning and the presence of typical froth. Sea was the commonest location for drowning (n= 30, 46.2%) followed by wells (n=16, 24.6%). Other locations included swimming pools, lakes, ponds, and water ditches (n=19, 29.2%). Twenty-eight bodies (43.1%) showed the presence of typical froth while thirty-five of them (53.8%) did not show such froth. There was no significant association between the medium of drowning and the presence of froth around the nose and mouth.

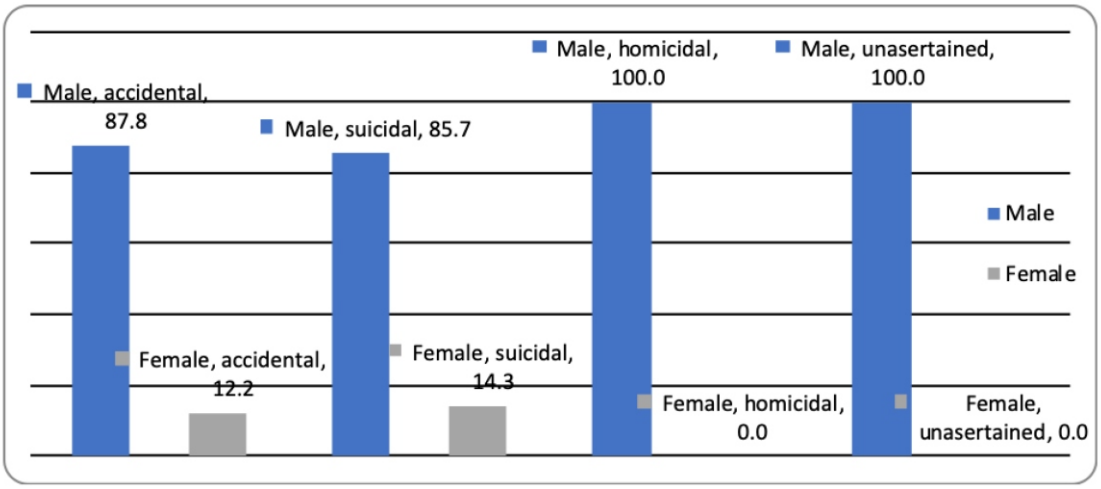

Figure 1: Gender distribution of circumstances/manner of death

Table 3: The association of the site of drowning and the presence of typical froth

Factor	Typical froth				Significance
	Yes		No		
Location of drowning					
Sea	14	(50.0%)	15	(42.9%)	$p = 0.785$
Well	6	(21.4%)	10	(28.6%)	
Other	8	(28.6%)	10	(28.6%)	

Table 4: Distribution of antemortem and postmortem injuries on the body

Places	Ante-mortem injuries		Postmortem injuries	
	n	%	n	%
Face	9	56.25	5	27.77
Limbs	5	31.25	8	44.44
Abdomen	-	-	2	11.11
Chest	2	12.50	3	16.66
Total	16	100	18	100

* Some bodies had both antemortem and postmortem injuries, where as 41 bodies did not have any injury

Antemortem and/ or postmortem injuries were noted in 24 (36.9%) individuals at sites such as the face, chest, abdomen, and limbs. No injuries were noticed in 41 (63.1%) bodies. When considering antemortem injuries alone, face was the commonest region affected (n = 9, 56.25%) followed by the limbs (n = 5, 31.25%), whereas in postmortem injuries the limbs (n = 8, 44.44%) were the commonest body region affected followed by the face (n = 5, 27.77%). Injuries seen over the abdomen were solely of postmortem in origin and this was the body region that had minimally been affected when considering all injuries. When considering each body region and comparison between antemortem and postmortem injuries, the face received more antemortem injuries compared to postmortem injuries (9 vs, 5), and the limbs received more postmortem injuries compared to antemortem injuries (8 vs. 5).

There had been four live admissions (6.2%) following the incident of immersion (near drowning) and later they had succumbed to injuries.

At the time of the postmortem examination, mild/moderate putrefaction and severe putrefaction were noticed in 29.2% (n = 19) and 12.3% (n = 8) of the cases respectively.

Of the 65 cases studied 40% (n = 26) were identified by the respective forensic pathologists as preventable deaths while 12.3% deaths were identified as unpreventable.

Discussion

According to the WHO, drowning is considered as the third leading cause of death due to unintentional injury worldwide and it is responsible for 7% of all injury-related deaths (5). Drowning in general is an area to which a lesser degree of emphasis has been paid to compile accurate data (4,5). Some key characteristics of fatal drowning were identified in this study. Drowning-related incidents are handled under the category of preventable injuries in Sri Lanka, and it is also included as a type of unintentional injury along with road traffic accidents, poisoning, falls, and burns (6).

Drowning is a major public health problem and thus an effective preventive mechanism is mandatory (7). To achieve this, it should include the study all cases of drowning in a given country detailing underlying causes and/or risk factors. That task is impossible when there are no accepted definitions with consistent coding that can be compared locally and internationally. Therefore, a proper data analysis system is needed to be implemented in our country. From a definitional perspective, the WHO define drowning as “death by asphyxia due to submersion in a liquid medium.” Near-drowning is defined as “immediate survival after asphyxia due to submersion.” (8) It may end up with either survival or death (9).

Since there are no accurate statistics in Sri Lanka on drowning-related deaths, it is worthwhile discussing about the victims of drowning as a whole. Some victims do not warrant any hospital admission though they actually experienced the eminent threat.

The rest of the patients may need hospital admissions. Out of those, a proportion of patients may not experience any ill effect and they recover without any residual damage ("live discharges"). Another proportion of patients may experience permanent brain damage resulting in delayed death. In a real sense, those findings are not counted as cases of drowning. This partially explains why the real number of victims who experienced drowning may be far more than the figures reported in hospital statistics. The author is of the view that not only the fatal cases but also these types of cases too need to be analysed to study its epidemiology and the underlying factors for drowning. It is the responsibility of the forensic pathologists to highlight such important aspects to the stakeholders. The IMMR is prepared by using statistics provided by government hospitals. But according to the Annual Health Statistics of Sri Lanka, around 60% of deaths are encountered in non-hospital settings. Such facts should also be kept in mind while considering the national statistics (1).

The present study revealed that fatal drowning constitutes a little more than one percent of the total postmortem examinations conducted at the institution during the given period. There were two peaks observed in the age distribution. According to the author's experience, the first peak in the 16-20 years age group could have been due to the reason that young males tend to consume alcohol and swim during their leisure time causing drowning. The second peak may be due to suicides and accidental causes with advancing age.

The WHO state that nearly 60% of all drowning deaths were "below the age of 30 years" (4). In our sample, only a 41.54% (n = 27) were below 30 years. The number of children below 15 years was 5 (7.7%)**.** A study done in Australia has revealed that the maximum morbidity of children of 0-4 years is 2.63 per 100,000 peoplen (10). The findings of our study is also compatible with a previous study done in Sri Lanka and indicated the lowest drowning rate in the 5-14 year age group (11).

A male preponderance was observed in this study under discussion and the same is confirmed with both local and international literature. In a cross-country analysis performed in Sri Lanka by Mathews *et al*, it was revealed as 4:1 (12). According to a systematic review, males are at greater risk, and it accounted for 45,240 events (75%) compared to 15,295 female events (25%) from 50 out of 62 studies (13).

The manner/circumstances of death play a significant role in deaths due to drowning. All manners of death presented here are commoner in males with a considerable gap between females. A systematic review by Tyler *et al.,* who studied the epidemiology of drowning in low- and middle-income countries also showed that accidental death is the most common category in all instances studied (14). A study done in in Sri Lanka has revealed that the commonest circumstance was accidental as expected and it amounted to 57.6%, while suicide 22%. In the same study, circumstances were unascertained in 20.3% and no homicides were reported (15).

Colombo is a city where the sea is closer to any given point and it could well be the most common site for drowning. Out of the accidental deaths, two deaths were due to drowning in swimming pools; a five-year-old boy while participating in a wedding ceremony with his parents, had accidentally drowned. The second swimming pool death was an accidental drowning after alcohol consumption and the victim was also a male of thirty years. The author wishes to highlight the preventability of these two deaths. With a four-sided fence and parental supervision, the death of this 5-year-old child could have been prevented. Related laws of swimming pool safety have not been implemented in Sri Lanka yet. The United States Centers for Disease Control and Prevention (CDC) has identified various predisposing factors related to swimming pool deaths and injuries. Subpar swimming skills, absence of four-sided fencing around the pool, lack of supervision, alcohol use and seizure disorders are some of the risk factors (16). Stakeholders should implement strict laws at swimming pool facilities in Sri Lanka along with four-sided fencing. It is recommended to employ a lifeguard/ pool attendant as well.

The study identified four cases with some survival time following the initial incident of drowning. Such cases are termed as cases of "near drowning". The majority had reportedly died during the incident. One case had been treated for a day in a ward and

the other three had only been treated for a few hours. The management of cases of near drowning may need ventilation and it is still useful to have statistics in those victims as well (17).

The cause of death of drowning is ascertained by excluding all other possible causes. The presence of typical froth is considered more or less a specific feature in drowning. The present study revealed the typical froth in a little less than half of the deaths. Fine, white, coarse and copious froth is considered as a significant finding in relation to deaths due to drowning. It was attempted to find whether there is a significant difference in presence of froth depending on the medium of drowning. No significant difference was identified**.** In a study done in the Netherlands, only 16.1% of cases had shown the characteristic froth and it was only in freshwater drowning (18).

Alcohol consumption before death was identified in approximately one third of individuals and thirty individuals had not consumed any alcohol in our study. Since there was no reliable evidence, it was not possible to comment on the remainder of the study sample. According to the Ministry of Health Sri Lanka, human behaviour is identified as a key risk factor for the occurrence of drowning (6). In another Sri Lankan study, 21% of individuals had consumed alcohol before accidental drowning (15).

Decomposition is a frequent occurrence observed in dead bodies that are recovered from water. Only a 41.5% of dead bodies showed putrefactive changes in our study. Decomposition changes were noticed in 52% of cases in a 10-year retrospective study done in India (19).

A dead body recovered from water may show injuries either in the forms of antemortem or as postmortem injuries. According to Farrugia *et al.*, traumatic injuries are not common in subjects with a history of drowning. Only seven of them had sustained traumatic soft tissue injures (20). Fornes *et al.,* have emphasized that differentiation between antemortem and postmortem injuries is difficult at times due to multitude of reasons. The absence of well-defined criteria is one such reason (21). The analysis of postmortem injures sustained with immersion is also helpful for forensic pathologists during the exercise of injury interpretation. Apart from macroscopy, histology also plays an important role in differentiation of antemortem and postmortem injuries. In non-putrefied dead bodies microscopic examination of body parts may contribute to differentiate antemortem drowning from postmortem drowning. Histological examination of lung tissues may provide clues. In addition to trauma, state of the dress, effort made to rescue the body, adiposity, postmortem contact of the body against surfaces, animal predation and the amount of air in the lungs, and bowels before drowning are a few factors that determine the nature of postmortem injury patterns (22).

In Australia, with more or less the same population as in Sri Lanka, numbers of drowning deaths were very low, despite the population engaging more in water sports and water-related activities (23). The Sri Lanka Life Saving, Life Saving Victoria in Australia and the Australian High Commission in Sri Lanka have declared 3.5 deaths per 100,000 people in Sri Lanka from 2016-2018 (24). However, the present study deals with small numbers and therefore comparison is not feasible.

One of the key concerns of this study is to study the preventability of drowning in the cases studied. The respective forensic pathologists have mentioned that 40% of the cases could have been prevented and 12.3% deaths have been identified as unpreventable. The death of young individuals who can contribute to the economy of the country negatively affects the work force. According to the latest reports available in Sri Lanka, about 800 Sri Lankans annually die due to drowning and it is the second leading cause for deaths due to unintentional injury (6). Therefore, forensic pathologists must perform evidence-based studies to convey the important aspects of drowning to prevent such unwarranted deaths.

Recommendations

- The relevant age groups are to be addressed to prevent premature/ unnecessary deaths.
- The vulnerable areas of the sea should be secured with much wider publicity.
- Strict rules and regulations should be imposed to prevent swimming pool-related deaths.

- The underlying causes/ risk factors contributing to the death of children by drowning should be studied.
- The contribution of alcohol consumption to drowning needs to be studied quantitatively or by performing blood tests in every possible case.
- The exact burden of deaths due to drowning is to be identified with more emphasis to develop accurate databases representing the whole country.
- A compulsory and detailed reporting system of all the deaths due to drowning with possible risk factors should be implemented and a national database needs to be maintained.
- Cases of near-drowning and live discharges following immersion incidents too need to be included for the drowning statistics of the country with uniform definitions, to be on par with the international nomenclature.

Conclusions

One of the most important facts that were highlighted in this study is that a little less than the half of deaths studied could have been prevented. The data collection and analysis related to drowning is not yet carried out, up to a desired standard in order to arrest and prevent the existing threats effectively in Sri Lanka. Under injury prevention strategies, drowning is a preventable type of death. Therefore, a proper emphasis must be paid for taking preventive measures through a careful analysis of details surrounding the case. A Judicial Medical Officer/ Forensic pathologist certainly can contribute to achieving this target as long as a proper emphasis is given.

Limitations

The sample size of the study is a major limitation. The study reflects only one tertiary care hospital in the Western province of the country making it impossible to generalize the study findings.

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A case of iodide mumps following contrast CT imaging; an exploration of its pathogenesis

Aravinthan M[1], Jayasinghe S[1,2]

[1]*Professorial Medical Unit, National Hospital of Sri Lanka, Colombo, Sri Lanka.*
[2]*Department of Clinical Medicine, Faculty of Medicine, University of Colombo, Sri Lanka.*

Correspondence: Dr. Mythily Aravinthan
e-mail: *mailmythi@gmail.com*
https://orcid.org/0000-0002-2335-8553
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Introduction

Iodine containing contrast media are widely used in diagnostic and therapeutic procedures like angiography, contrast-enhanced computed tomography (CT) and angioplasties. The adverse events to contrast media range from allergic reaction to anaphylaxis and contrast nephropathy. 'Iodide Mumps' refers to benign swelling of the salivary glands following the use of iodide contrast which was first described in 1956 by Sussman and others from Israel (1). Since then, over seventy-five cases of the same have been reported (2). But its incidence is postulated at 1 - 2% (3). Awareness of this rare event and its benign course helps avoid unnecessary anxiety for the patient and the attending medical team.

Case Presentation

A 64-year-old male with type 2 diabetes mellitus, hypertension, chronic kidney disease (CKD) stage III (eGFR - 34 ml/min/1.73 m^2) and Child-Pugh B alcoholic chronic liver cell disease underwent a contrast enhanced Computed Tomography (CT) scan to evaluate a suspicious lesion of the liver. The procedure involved the use of 100 ml of low osmolar non-ionic iodide contrast ioxehol (*Omnipaque* 300; 300 mg/ml iodide). Around 8 hours after the procedure he noted a rapid painless swelling of his neck. The patient was highly anxious but denied any difficulty in swallowing or breathing. Clinical evaluation revealed an afebrile patient with bilateral symmetrical non tender enlargement of the submandibular salivary glands without erythema or warmth (Figure 1). His vital signs were stable and there were no signs of angioedema or anaphylaxis. Basic laboratory investigations were within the normal range (WBC - 7000 /µl; Hb - 11 g/dl; platelets - 170 x 10^3/ µl; CRP < 6 mg/dl; ESR - 18 mm/hr; serum potassium - 4.8 mmol/l; serum sodium - 139 mmol/l; serum creatinine - 1.8 mg/dl). An ultrasound scan of the neck confirmed diffusely enlarged submandibular glands with milder enlargement of bilateral parotids without any calculi or other abnormalities involving the thyroid or lymph nodes. There was no evidence of facial nerve palsy. Trauma and other viral or bacterial infectious causes were excluded by history, normal inflammatory markers and infection screen (mumps/ EBV and HIV serology). With the available findings a clinical-radiological diagnosis of 'iodide mumps' was made. Oral paracetamol 1 g as and when needed was given for the mild discomfort which he complained subsequently. He was kept under observation for 24 hours for delayed reactions and was discharged with a follow up at two weeks. The swelling had started to reduce by two days and was completely resolved at two weeks.

Figure 1: Bilateral submandibular swelling

Discussion

Circulating Iodide is concentrated in the thyroid gland and all the exocrine secretions containing chloride ions including saliva, sweat, tears, gastric and pancreatic juices. Iodide enters these sites via the sodium-iodine symporter; an intrinsic membrane protein and is thought to have important biological roles in the form of an anti-oxidant and local mucosal protective agent (4). Under normal circumstances, most iodide is excreted unchanged by the kidneys and the remainder via the above secretions. Although the exact pathogenesis is unclear, it is postulated that high concentrations of iodide can cause local mucosal oedema and ductal obstruction leading to a non-inflammatory swelling of the salivary glands (3, 5). Submandibular and Parotid are the most affected but thyroiditis and pancreatitis can also occur (2, 6, 7). Iodide mumps is different from 'iodism' which refers to chronic dose dependent systemic iodine toxicity (1). Iodism is more severe and involves systemic features like fever, anorexia, cachexia, nausea, vomiting, skin lesions known as iododerma and inflammation of respiratory and gastrointestinal mucosae including salivary glands. Iodism can be fatal whereas iodide mumps refers to the acute idiosyncratic manifestation which is benign (1).

The contrast in this case, iohexol, is a non-ionic water-soluble iodine compound that is excreted unaltered, predominantly through the kidneys. Following rapid intravenous injection, peak blood levels occur immediately followed by a rapid fall within five to ten minutes and the vascular compartment half-life is around twenty minutes. Approximately 90% of injected iodide is excreted in urine within the first 24 hours (8). The urinary and biliary excretion possibly affected by coexistent CKD and cirrhosis could have led to the delay of higher peak levels of the drug occurred accompanied by prolonged half-life. There is little data on the concentration of iohexol in the salivary gland. However, it is reported that concentration of iodine in salivary glands is 30 times of that of plasma (8).

According to a meta-analysis, age, osmolarity of contrast, volume of contrast or the renal function do not influence the occurrence of this reaction and it is idiosyncratic in nature. The onset of symptoms can be immediate or delayed maybe up to 5 days. Often it is a painless swelling resolving without sequelae as in our case. Tenderness is seen in up to 49% with few experiencing sense of choking and swallowing difficulty (2). Facial nerve palsy secondary to parotid swelling is by far the most sinister sequelae reported (2).

Symptoms completely resolve over a median duration of 3 days with a delay of up to 72 days in few cases (2). Prophylactic and therapeutic interventions using steroids, non-steroidal anti-inflammatory agents, antihistamines, saline gargle, warm soaks and dialysis have no influence on the occurrence or the time to recovery (10). Older age and longer time to onset of symptoms haven been associated with delayed resolution of swelling (2).

The stereotypic reaction is known to recur with repeated exposure to iodide contrast sometimes with increasing severity but has been well tolerated without any sequelae (3). It can occur in those without any known allergy and can occur de-novo in those who have previously tolerated iodide contrast uneventfully (3).

Conclusions

'Iodide mumps' is a benign, obvious, under--diagnosed complication of post-iodide contrast media exposure. This can be diagnosed clinical-radiologically. Despite causing discomfort and intense anxiety, reassurance alone suffices avoiding un-necessary interventions.

Authors disclose no conflicts of interest. The written informed consent has been obtained from the patient to publish this case report with photographs.

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A case of Poncet's disease; a mimicker of rheumatoid arthritis

Dharmaratne CJ[1], Jayasena DP[1], Sobana VM[1], De Silva UD[1], Muhunthan S[2]

Department of Clinical Medicine, Teaching Hospital Karapitiya,Galle, Sri Lanka.
Department of Respiratory Medicine, Teaching Hospital Karapitiya, Galle, Sri Lanka.

Co-Correspondence: Dr. C. J. Dharmaratne; e-mail: *charukad@gmail.com*
https://orcid.org/0000-0002-2370-1606
Dr. D. P. Jayasena; e-mail: *pravja1988@gmail.com*
https://orcid.org/0000-0002-9966-3773
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Introduction

Tuberculosis (TB) has a variable clinical presentation affecting the lungs, lymph nodes, pleura, gastrointestinal tract, meninges, joints, and bones. Musculoskeletal involvement is the most common manifestation of extrapulmonary TB, seen in 10-19% of cases (1). Although septic TB arthritis is a well-recognized manifestation of extrapulmonary TB; there is another rare form of reactive arthritis known as Poncet's disease (PD). PD is an aseptic non-erosive reactive arthritis that occurs with active extra-articular TB without any long term complications (2). It was first described by Antonin Poncet in 1897 as a polyarthritis secondary to visceral TB (3). The pathogenesis of PD remains unknown, however, it is strongly suspected to be due to M*ycobacterium tuberculosis* inducing a T helper 1 cell-mediated cross-reaction between tuberculoprotein and cartilage proteoglycans which trigger inflammatory arthritis (3). Therefore, antigen molecular mimicry and cross-reactivity have been implicated in the pathogenesis of PD in genetically predisposed individuals with HLA DR 3 and HLA DR 4 haplotypes (3-5). PD typically presents as a symmetrical large and small joint polyarthritis during an active TB infection with aseptic joints mimicking inflammatory arthritis (3). The focus of TB in majority of cases was TB lymphadenitis (3, 4).

There are no specific investigations to diagnose PD, hence it is diagnosed by clinical assessment and exclusion of other probable causes of inflammatory arthritis (3, 4). PD is a frequently misdiagnosed entity even in high TB prevalent countries due its unusual and uncommon presentation (6). Misdiagnosis and treatment as inflammatory arthritis with immunosuppressive medication will lead to dissemination of TB (7). Commencement of anti-TB treatment completely resolves symptoms within weeks to months and furthermore, confirms the diagnosis of PD (1, 3, 4). To the authors' knowledge, this is the first reported case of PD in Sri Lanka.

Case presentation

A 66-year-old male street vendor presented with low-grade evening pyrexia, fatigue, night sweats, loss of appetite and weight for 4 months. He had active arthritis effecting bilateral proximal interphalangeal joints (PIPs), metacarpophalangeal joints (MCPs), wrist joints (WJs), elbow joints (EJs), knee joints (KJs), ankle joints (AJs) and metatarsophalangeal (MTPs) joints for 3 months despite treatment with analgesics and had affected most of his activities of daily living. The pain worsened with activity but no associated morning stiffness. There was no history of diarrhoea, eye symptoms, skin rashes and genitourinary symptoms or personal/ family history of tuberculosis or any other comorbidities. He had a history of smoking, approximately 5 packets of cigarettes per year and did not consume alcohol.

His body mass index (BMI) was 16 kg/m^2. He was pale, afebrile, and anicteric. There was no palpable

lymphadenopathy. His cardiovascular, respiratory, abdominal, and nervous system examinations were normal. Musculoskeletal system examination revealed active synovitis and limited range of movements in the above-mentioned joints. Full blood count (FBC) showed anaemia of chronic disease (Hb - 9 g/dl) with normal WBC (7.3 x 10^9/L) and neutrophil count (4.2 x 10^9/L) with thrombocytosis (platelet - 572 x 10^9/L). The ESR was 130 mm/hr and CRP was 20 mg/dl.

He had markedly elevated rheumatoid factor (RF) of 380 IU/ml and anti-cyclic citrullinated peptide (anti-CCP) titre was 110 IU/ml. Moreover, antinuclear antibodies (ANA), antineutrophil cytoplasmic antibodies (ANCA), anti-streptolysin O titre (ASOT), serum calcium, bone profile markers and HIV antibodies were negative. Right KJ synovial fluid analysis showed leucocytes (8 x 10^9/L) with polymorph predominance and no crystals. The synovial fluid gram stain, bacterial and TB cultures were negative. Synovial biopsy revealed mild chronic inflammatory cell infiltrate with no histological evidence of TB.

Radiographs of hands, wrists, knees, and ankle joints did not reveal any erosive changes and chest X-ray revealed left upper lobe cavitory lesions with mild volume loss (Figure 1). Three sputum samples were negative for acid-fast bacilli. However, he had a strongly positive Mantoux test of 16 mm induration. His liver function, renal function and serum electrolytes were normal.

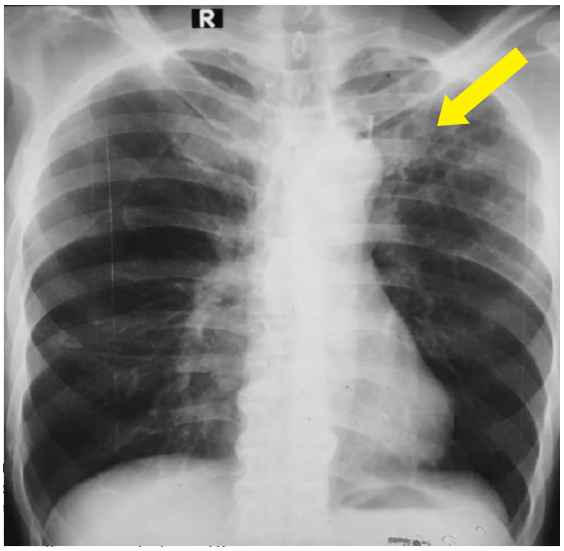

Figure 1: Left apical cavitation demonstrated on Chest X-ray (arrow)

The high-resolution computed tomography chest showed thin-walled cavities with fibrosis and volume loss in the left apico-posterior segment suggestive of post-pulmonary tuberculosis. Following case discussion with the pulmonologist, we proceeded with bronchoscopy where the bronchoalveolar lavage (BAL) for TB polymerase chain reaction (PCR) revealed a moderate yield of TB DNA and BAL culture subsequently grew *Mycobacterium tuberculosis.*

After multidisciplinary team discussion including general physician, respiratory physician and Rheumatologist, patient was commenced on the category 1 anti-TB treatment (isoniazid 5 mg/kg, rifampicin 10 mg/kg, pyrazinamide 25 mg/kg and ethambutol 15 mg/kg) according to the World Health Organisation (WHO) tuberculosis guidelines (8). At three months his joint pains have completely resolved. He was continued on anti-TB treatment for 6 months in total and fully recovered.

Discussion

PD is a very rare entity, where no more than 200 cases published in the literature (7). So far there have been no cases of PD reported in Sri Lanka, to the authors' knowledge. Whilst symmetrical aseptic polyarthritis involving large joints remain the classic presentation of PD, the majority of cases were reported to be asymmetrical non-destructive oligoarthritis with predominant ankle involvement (3). Joint symptoms could last from three days to six years (3). Associated constitutional symptoms were fever, malaise and weight loss (1, 3). Moreover, cervical and axillary lymphadenopathy along with erythema nodosum were observed in up to 6% of patients with PD.

Despite RF and anti-CCP antibodies being useful markers in the diagnosis of rheumatoid arthritis, they were found to be positive in patients with mixed cryoglobulinaemia, TB, infective endocarditis, primary biliary cirrhosis and malignancy (1, 9). Tuberculosis infections in particular were associated with seropositive RF (8-60%) and anti-CCP (7-39%) antibodies (1, 9). Furthermore, seropositivity of RF and anti-CCP antibodies have been reported in PD similar to our patient fulfilling American College of Rheumatology and European League Against

Rheumatism criteria for Rheumatoid arthritis (1, 8, 9). Therefore, RF and anti-CCP seropositive arthritis should not automate to the diagnosis of rheumatoid arthritis (1, 2, 8, 9). There are no standard definitions and diagnostic criteria widely used in clinical practice to identify PD (3, 4). Thus diagnosing PD is made by exclusion of other causes of inflammatory polyarthritis (3, 4, 9). However there is a proposed diagnostic criterion by Sharma and Pinto (Table 1) who applied it in their case series, yet it needs to be validated in prospective cohort studies and different population groups (3).

Despite the elevated inflammatory markers, the aseptic nature of his involved joints was confirmed by the sterile synovial fluid cultures and absent caseating granulomatous necrosis in the synovial biopsy, thus making TB arthritis an unlikely diagnosis. PD arthritis occurs concomitantly with the onset of infection accompanied by extra-articular manifestations whereas reactive arthritis occurs 1 - 4 weeks after the infection (1-3, 9). Unlike reactive arthritis, PD is a non-erosive arthritis sparring axial and sacroiliac joints (3). PD significantly improved with anti-TB treatments over several weeks in contrast to reactive arthritis which requires non-steroidal anti-inflammatory drugs, glucocorticoids or DMARDs (full term) with a slower recovery over 6 months (1, 2, 9).

Although it is possible to consider the co-existence of pulmonary TB and rheumatoid arthritis there was clear evidence of rapid remission of polyarthritis and dramatic decline of inflammatory markers following anti-TB treatment. Therefore this outcome favoured PD, unlike rheumatoid arthritis where his polyarthritis would have persisted despite anti-TB therapy (1, 9). Further, according to Sharma and Pinto's diagnostic criteria, our patient is a definite case of PD (3).

Conclusions

PD is a rare form of aseptic arthritis in the presence of pulmonary or extrapulmonary TB which mimics classic inflammatory arthritides such as rheumatoid arthritis and there are no validated diagnostic criteria that make it challenging to diagnose (1, 3, 9). This case study emphasizes the importance of including PD as a differential diagnosis in patients presenting with fever and polyarthritis or oligoarthritis especially in countries with high TB prevalence (4, 9).

Table 1: Sharma and Pinto's diagnostic criteria for Poncet's disease

Essential criteria	Inflammatory, non-erosive, non-deforming arthritis Exclusion of other causes of inflammatory arthritis
Major criteria	Concurrent diagnosis of extra-articular tuberculosis Complete response to antitubercular therapy
Minor criteria	1. Mantoux positivity 2. Associated hypersensitivity phenomenon, such as erythema nodosum, tuberculids or phlyctenular keratoconjunctivitis Absence of sacroiliac and axial involvement
	For diagnosis: **Definite** – Essential + two major **Probable** – Essential + one major + three minor **Possible** – Essential + one major + two minor, or – Essential + three minor

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A case of Proteus syndrome with a large atrial septal defect

Kariyawasam JP[1], Kankananarachchi I[1], Naotunna C[1], Wakwella HC[1], Gunawardane TPJ[1], Gunasekara C[2]

[1]University Paediatric Unit, [2]Paediatric Cardiology Unit, Teaching Hospital Karapitiya, Galle, Sri Lanka.

Correspondence: Dr. J. Prathibha Kariyawasam
e-mail: *prathibhaka85@gmail.com*
https://orcid.org/0000-0002-7404-8264
Submitted on 31.05.2021 and accepted for publication on 10.08.2021

Introduction

Proteus syndrome is named after the Greek god 'Proteus - meaning 'polymorphous' to characterise the variable and changing phenotype of this condition. It was first described by Cohen and Hayden in 1979. Only limited literature is available globally (1). The estimated prevalence is <1 / 1,000,000 live births (2).

It is inherited as a somatic mutation in the oncogene AKT1 encoding an enzyme (AKT 1 kinase) mediating cell proliferation and apoptosis. AKT1 kinase regulates cell growth proliferation, differentiation and cell survival (1,3). As a result of the mutation, there is an alteration of local production or regulation of tissue growth factor receptors causing abnormal growth in a patchy or segmental distribution. The disease affects bone, connective tissue, fatty tissue, skin, central nervous system and internal viscera.

Most of the time, these patients are born normal at birth and show progressive changes of growth postnatally within 6 to 18 months coming to a plateau in the adolescence (4).

Herein, we report a child whose abnormal physical features were not detected until 12 years of age. She has features suggestive of Proteus syndrome with another rare association of this syndrome, a cardiac defect.

Case presentation

A 12-year-old apparently healthy girl from a rural low-income family was referred to the paediatric cardiology clinic for further evaluation of a systolic murmur detected at the school medical inspection. She is the third born to healthy non-consanguineous parents of a low-income family. She was born at term via normal vaginal delivery with a birth weight of 3000 g. Her antenatal period had been uneventful. Her neonatal period and throughout, up to the presentation has been free of medical or surgical problems except for one episode of lower respiratory tract infection and wheezing at three years of age. She attends the local government school and has average school performance. Her development history and immunization coverage are age appropriate and the family history does not give any significant detail in relation to her condition.

Examination revealed a girl with no facial dysmorphism, weight at 10th to 25th centile, height at 3rd to 10th centile (within mid parental height), body mass index of 17 kg/m^2 (25th to 50th centile). The most striking features were webbed neck (Figure 1), scoliosis, and two large cystic lumps over the back of the chest (Figure 2) which were said to be congenital and non-progressive, a sacral pit and a small epidermal naevus just lateral to the sacral pit. Cardiovascular system showed normal blood pressure for her age with a fixed split in 2nd heart sound. There was a grade three ejection systolic murmur at the left upper sternal edge.

Her respiratory and abdominal examination revealed normal findings. She was in Tanner stage 3 in her pubertal development. Her body habitus was unusual with wasting of the left side of the body and hemihypertrophy of the right side (Figures 1 - 4). She had normal intellectual performance and satisfactory social skills.

With regards to investigations, 2D echocardiogram revealed a large ostium secundum atrial septal defect (ASD) shunting left to right, dilated right atrium and right ventricle without significant pulmonary hypertension (Figure 5). Skeletal survey revealed bilateral cervical ribs and scoliosis.

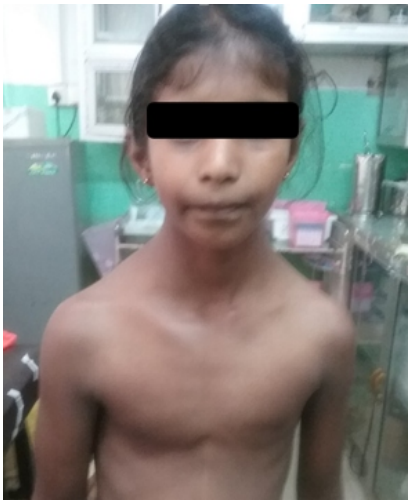

Figure 1: Webbed neck and proteus syndrome facial features

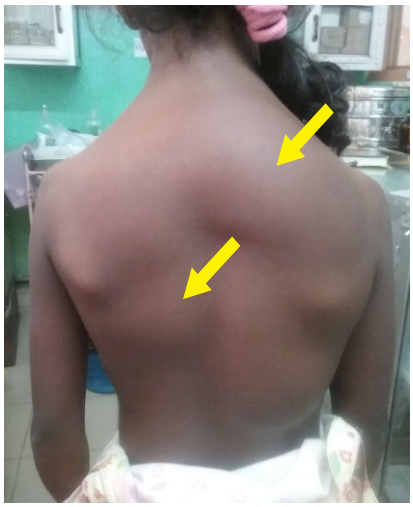

Figure 2: Two large cystic lumps (yellow arrows) over the back of the chest

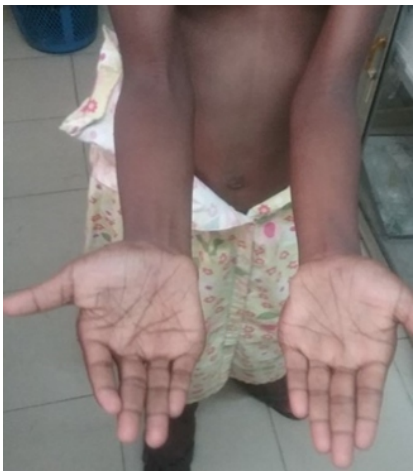

Figure 3: Upper limb wasting of the left side with hemihypertrophy on the right side

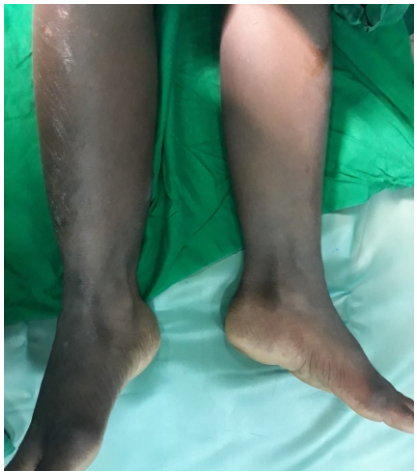

Figure 4: Lower limb wasting of the left side with hemihypertrophy on the right side

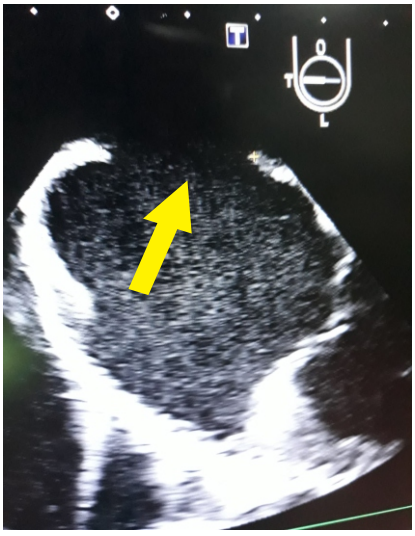

Figure 5: Transesophageal echocardiogram (TOE) showing large OS ASD (20 mm) (yellow arrow)

Table 1: Revised diagnostic criteria for Proteus syndrome

To make a diagnosis of PS, one must have all the general criteria, and various specific criteria	
General criteria	***Specific criteria***
All the following: ▪ Mosaic distribution of lesions ▪ Sporadic occurrence ▪ Progressive course	Either: ▪ Category A or ▪ Two from category B or ▪ Three from category C
Specific criteria categories	
A. 1. Cerebriform connective tissue naevus	**C.** 1. Dysregulated adipose tissue Either one: (a) Lipomas (b) Regional lipohypoplasia
B. 1. Linear epidermal naevus	2. Vascular malformations One or more: (a) Papillary malformation (b) Venous malformation (c) Lymphatic malformation
2. Asymmetric, disproportionate overgrowth One or more: (a) Limbs (b) Hyperostosis of the skull (c) Hyperostosis of the external auditory canal (d) Maga spondylo dysplasia (e) Viscera: spleen / thymus	3. Lung cyst 4. Facial phenotype All: (a) Dolichocephaly (b) Long face (c) Down slanting palpebral fissures and / or ptosis (d) Low nasal bridge (e) Wide or anteverted nares (f) Open mouth at rest

Ultrasound scan over the lumps were suggestive of cystic hygroma extending from the back of the neck. Pelvic ultrasound showed small uterus and ovaries.

Her karyotype was 46, XX. Nerve conduction studies were normal and her MRI brain and spine excluded any malformations. Considering the presence of hemihypertrophy, skeletal abnormalities, lymphatic malformations, skin manifestations and hypoplastic uterus, the clinical diagnosis was made as Proteus Syndrome. The mutation analysis was not done due to financial constraints. The child underwent ASD device closure without any complications.

Discussion

According to the Biesecker and colleague's consensus criteria set forth in 1999 and revised in 2006, when establishing the diagnosis of Proteus syndrome clinically, general and specific criteria should be fulfilled (3, 5) (Table 1).

To diagnose Proteus syndrome clinically, all the general criteria and one specific criterion from category A; or two specific criteria from category B; or three specific criteria from category C is required. Apart from general criteria, our patient satisfied category B with the presence of epidermal naevus and asymmetric, disproportionate overgrowth of ≥ 1 of limbs.

When clinical criteria do not fulfill the diagnosis, identification of pathogenic variant in AKT1 by molecular genetic testing in affected tissue parts is recommended.

Proteus syndrome, due to it's complexity and variability in presentation can be easily missed as already happened in this case. Even though there had been progressive hemi hypertrophy, enlargement of cystic lesions on the back, no attempt was made to diagnose the condition in previous health care encounters until she presented with a large ASD. Cardiac abnormalities are rare in this syndrome unlike in Sotos syndrome which is also a paediatric overgrowth syndrome with congenital heart defects (e.g. ASD, VSD). Some studies on echocardiography / cardiac MRI findings in proteus syndrome have shown that fatty infiltration of intraventricular septum (6, 7), but index case did not reveal any abnormal cardiac finding in myocardium on her echocardiogram. She did not reveal any cardiac arrhythmias clinically or on her 12 lead ECG. She had a large OS ASD with evidence of right sided volume overload. She underwent an ASD device closure successfully without any complications.

Management of skeletal overgrowth and connective tissue naevi which are disfiguring and affecting mobility and functioning needs orthopaedic, dermatological and plastic surgical assistance.

As in our child, more than two thirds of patients with Proteus syndrome have normal intellectual functions. But there are reported cases with hydrocephalus, hemimegaloencephaly, absent corpus callosum and craniosynostosis (4). One case reported previously in Sri Lanka some years back had hemimegalencephaly (8). Being a sporadic mutation and low fertility rate in patients warrant no inheritance of the syndrome in families. Proteus syndrome is one condition with several other overlapping syndromes that carry the risk of having loss of function gene mutations in a tumour suppressor gene (PTEN). These similar syndromes are named PTEN Hamartoma Tumour Syndromes. Therefore, patients diagnosed to have Proteus syndrome should be followed up for malignancy surveillance (9). Most specifically associated tumours are monomorphic adenomas of the parotid glands and bilateral ovarian cystadenomas (2). Not only tumours but there are other complications; progressive skeletal deformities, invasive lipomas, deep vein thrombosis and pulmonary embolism (10).

With developments in genetic studies, we now have facilities to detect AKT1 pathogenic variant in cultured amniocytes in suspected fetuses with segmental overgrowth, skeletal and CNS abnormalities (11).

Conclusions

Presence of unusual physical features of a child warrants proper clinical evaluation and investigations which will aid the diagnosis. Conditions like, the Proteus syndrome if diagnosed early can be managed with multidisciplinary involvement and timely interventions. As such syndromes are not usually detected antenatally or at birth progressive changes can be only picked up if proper examination is done during medical encounters. Disfiguring conditions with associated complications including malignancy in a child needs appropriate counseling and proper long term follow up.

The informed written consent of the parents has been obtained to publish this case report with photographs of the child.

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Common iliac artery mycotic aneurysm due to melioidosis

Wedagedara VM, Nuwan C, Ubayasiri RA

Department of Vascular and Transplant Surgery, Teaching Hospital Karapitiya, Galle, Sri Lanka.

Correspondence: Dr. V. M. Wedagedara
e-mail: *venurawedagedara@gmail.com*
https://orcid.org/0000-0001-6883-5061
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Introduction

Mycotic aneurysms are rare but they have a high morbidity and mortality (1). They are due to infection of the vascular wall by bacteria, not by fungi as the term implies (2). Hence the term "mycotic" is misnomer. Commonly non-hemolytic type streptococci, staphylococci, pneumococci and *Salmonella* spp. cause this infection (3). Melioidosis is very rare to cause mycotic aneurysms (4). The formation of a mycotic aneurysm is initiated by lodging of bacteria on the inner surface of the vessels or the vasa vasorum (5). Thereafter, local inflammation progresses and make the vessels fragile, leading to rupture.

Mycotic aneurysms occur most frequently in the aorta followed by cerebral, superior mesenteric, femoral, splenic, hepatic and brachial arteries (6). Risk factors include hypertension, emotional stress, aging, changed environment, allergy and smoking and diseases such as rheumatic fever (3).

Melioidosis is considered as a very rare disease in most parts of the world. But it is endemic in Southeast Asia and Northern Australia (4). *Burkholderia pseudomallei*, the organism that melioidosis was reported as the most common causative agent of mycotic aneurysm in North-Eastern Thailand (4). It is a granulomatous infectious disease associated with high mortality and morbidity despite prompt diagnosis and treatment (5). This bacterium is commonly found in soil and water. Infection is primarily via direct contact and inoculation of contaminated water or soil classically in paddy fields (4). The inoculation period of this organism ranged from 2 days to 26 years (4). Apart from a mycotic aneurysm in superficial femoral artery due to mixed bacterial species, there are no reported cases on mycotic aneurysms caused by melioidosis in Sri Lanka (7). We report a case of 66-year-old patient who presented with a ruptured common iliac artery mycotic aneurysm.

Case presentation

A 66-year-old retired police officer presented with acute onset of dull pain in the right iliac fossa radiating to the upper thigh. Pain was persistent and did not resolve with simple analgesics. He was afebrile and there was no vomiting, anorexia or loss of appetite. His bowel habits were normal. He had no family history of malignancy. There was mild pallor in him and he had a pulse rate of 110 bpm. His blood pressure was 100/70 mmHg. There was a pulsatile tender mass over the right iliac fossa. The dorsalis pedis and posterior tibial pulses on the right side were not felt. An urgent contrast CT angiogram of both lower limbs was done (Figure 1).

CT showed leaking common iliac artery saccular aneurysm on the right side. The patient was prepared for urgent exploratory laparotomy. Meanwhile, subcutaneous morphine 5 mg was given as an initial dose. He was kept fasting and given intravenous normal saline 1 litre over 30 minutes as the blood pressure dropped to 70/50 mmHg. Furthermore, 2 units of crossed match blood were given perioperatively. The broad spectrum antibiotic, meropenem 1g IV three times per day was started after obtaining blood for culture.

Subsequently damage control surgery was done. There was a leaking right common iliac artery aneurysm and a large hematoma over the right iliac fossa. Proximal aortic control was taken. Aneurysmal sac was opened and debrided. Proximal common iliac artery was ligated. Femoral arteries were explored bilaterally. Femoral to femoral cross over bypass grafting were done with 8mm polytetrafluroethylene (PTFE). The distal pulses were felt and confirmed by triphasic Doppler wave study after surgery.

Patient was in intensive care for 13 days postoperatively and had hospital-acquired pneumonia which was successfully treated. No other complications developed. The aneurysmal tissue sample was positive for melioidosis. Blood cultures were negative. 2D Echocardiogram did not show features of bacterial endocarditis. The patient was discharged with oral co-trimoxazole 960 mg and oral doxycycline 100 mg twice daily for three months after continuing ceftazidime 1g intravenously three times a day for 3 weeks.

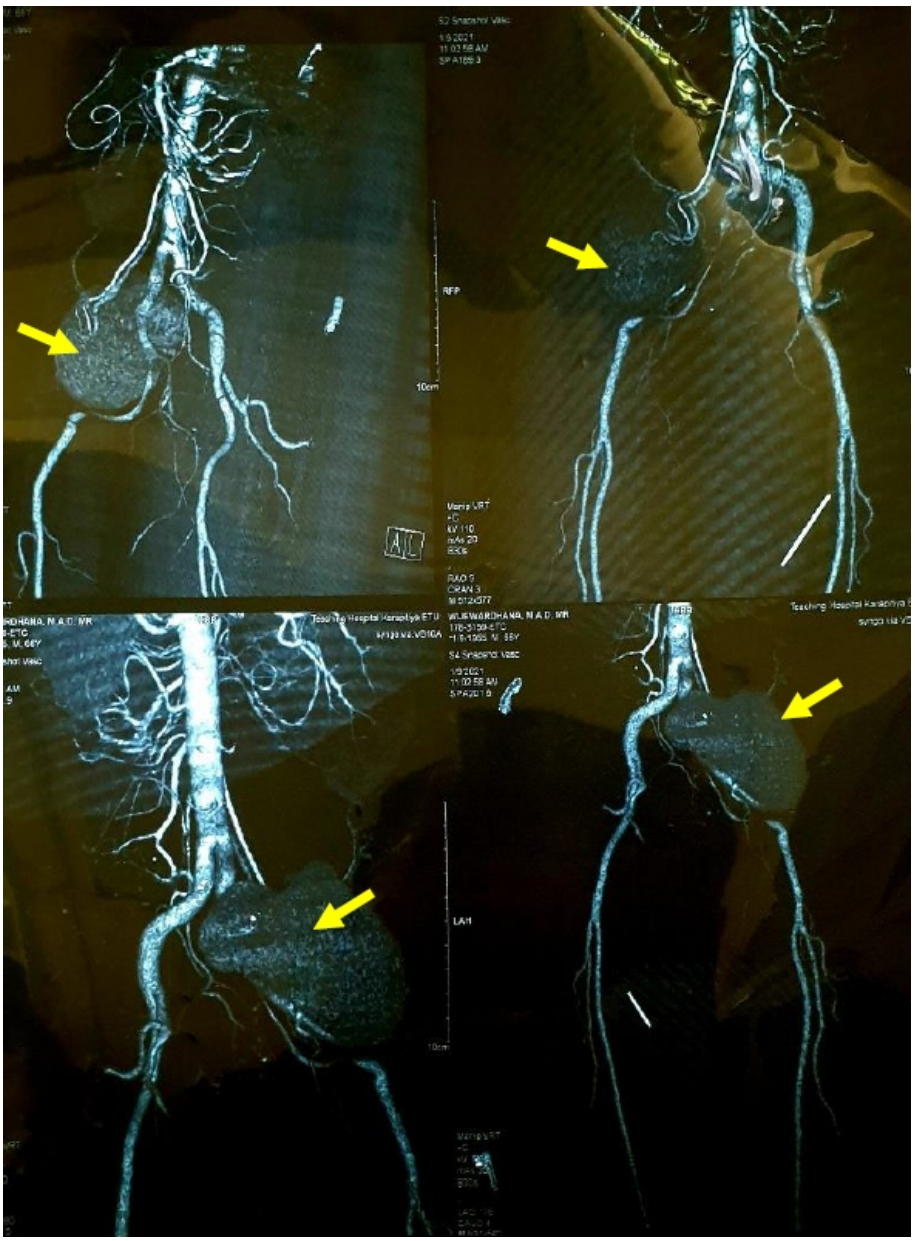

Figure 1: CT angiogram of both lower limbs

Discussion

Although mycotic aneurysms are uncommon, they should be considered as important since they tend to grow rapidly and rupture (3). The subsequent surgical procedures are difficult due to inflamed vessels and the prognosis is likely to be poor at the end. Most mycotic aneurysms occur in patients less than 40 years of age in contract to arteriosclerotic aneurysms (3). The bulk of the mycotic aneurysms involved the peripheral arteries and are seven times frequent than aortic mycotic aneurysms (2). Among them, femoral artery aneurysms were the commonest while there were only a few reported cases of common iliac artery aneurysms (2).

In the literature, there are several theories that describe the pathogenesis of mycotic aneurysms. Currently accepted theory is the lodging of bacteria over the intima of vasa vasorum which supply the major vessels. Local ischemia will sets in and it initiates local inflammation and subsequent degeneration of the vessel wall (3, 8). Ultimately the vessel wall will be fragile enough to rupture. There are two types of mycotic aneurysms according to the pathogenesis. They are primary and secondary types. Primary mycotic aneurysms are due to direct extension of an adjacent suppurative focus while secondary mycotic aneurysms are due to septic emboli due to endocarditis (9).

There are cases of mycotic aneurysms of aortic arch, subclavian artery and femoral arteries by mixed bacteria. But common iliac mycotic aneurysms have not been documented. Melioidosis is a rare but well documented cause of mycotic aneurysms and is described to occur in around 1% of affected patients (10). Therefore, mycotic aneurysms are not a very common complication of melioidosis. All the previous cases in the literature were treated by early surgical interventions which was similar to our case. This case illustrates a very rare disease which carries high morbidity and mortality rates. Thus early diagnosis is imperative despite of the rarity.

Conclusions

Melioidosis mycotic aneurysms are exceedingly rare. But due to the disastrous nature of the infection, early diagnosis and prompt treatment with prolonged antibiotics must be implemented. It is imperative to treat these aneurysms with early surgical intervention. A clinical suspicion of melioidosis mycotic aneurysm is pivotal in Sri Lanka where paddy fields are abundant.

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