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Contents

Editorial

Troponin—Keep Up or Be Left Behind

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The dawn of the new millennium ushered in a new era for cardiac marker testing. The European Society of Cardiology (ESC) and the American College of Cardiology (ACC) redefined myocardial infarction (MI) as "myocardial necrosis caused by ischemia" and designated troponin (Tn) as the "preferred" biomarker (1). The upper reference limit (URL) of Tn was specified to be at the 99th centile of a reference population, and the assay imprecision at this level should be 10% or less. Furthermore, the National Academy of Clinical Biochemistry (NACB) and the International Federation of Clinical Chemists (IFCC) recommend that Tn results should be available within 60 minutes (2). The ESC/ACC reiterated this position in their "universal definition of myocardial infarction" (3). The American Heart Association published a monograph on "Biomarkers in Heart Disease" in 2008 (4).

A review of the historic developments in Tn testing is informative (5). Tn comprises 3 subunits: T, I, and C, each with differing action (6). TnT and TnI are cardio-specific, but TnC is also present in skeletal muscle. TnT interacts with tropomyosin, while TnI promotes the binding of myocardial actin and myosin, and TnC enhances calcium binding in the Tn complex to produce myocyte contraction. The mechanism of Tn clearance from circulation remains unclear. Renal clearance, previously implicated because elevated Tn is often found in chronic renal failure, is now deemed unlikely (7). However, the reticuloendothelial system remains a possible candidate (8).

Until recently, laboratories struggled to meet the ESC/ACC 2000 criteria for Tn assay quality, and users assumed that detectable levels of Tn indicated myocardial injury, while undetectable Tn levels implied that no MI had occurred. That paradigm has shifted (9–11). Although the TnT assay is only available from one vendor (Roche Diagnostics) and is popular in Europe, the TnI assay is available from several manufacturers and is widely used in the USA. Both are equivalent in diagnostic utility for acute MI (AMI) and risk stratification. High-sensitivity (hs) Tn assays are superior to conventional Tn assays in the early detection of AMI (12,13). In patients with definite myocardial injury but negative TnI results by conventional assay, 64% had detectable TnI values in an assay with improved detection limits (14). Moreover, hs-TnT was able to predict evolving non-ST elevation MI (NSTEMI) earlier than a standard TnT assay in patients with suspected acute coronary syndrome (ACS) and negative troponin upon admission (15). Lest doctors be lulled into treating the Tn test result rather than the patient, it is important to adhere to NACB practice guidelines (16) for using Tn assays together with the clinical symptoms, ECG changes, or imaging evidence of cardiac ischemia. It is also prudent to recall the need for a temporal rise or fall in Tn levels in MI diagnosis (2,3,5). Hs-Tn assays also detect non-ischemic causes of myocardial injury or stress, and clinicians should be mindful of this fact to institute the appropriate diagnostic and therapeutic measures (17). The issue of Tn elevations after exercise, especially in marathon runners, needs to be re-examined with hs-Tn assays, as it might indicate the need to screen such subjects. Peri-operative and post-operative care should also include hs-Tn measurements for proper interpretation and risk assessment.

In stable coronary artery disease with preserved left ventricular function (n = 3679), hs-TnT levels were correlated with poorer outcomes (cardiovascular deaths and heart failure) over 5 years of follow-up (19). In middle-aged (mean 44 years) Japanese men without overt cardiovascular risk, elevated hs-TnT levels were positively correlated with cardiovascular risk factors (20).

The current challenges in assessing troponin levels are rapid analysis and high sensitivity. Moreover, we need to meet the desired door-to-balloon time (DBT) of less than 60 minutes (2) because outcomes (30-day and 1-year) are worse in those with prolonged DBT and are particularly poor when DBT exceeds 90 minutes. This service standard requires many other coordinated steps beyond analysis. The service experience in our 800-bed acute-care general hospital is as follows. Samples drawn from the emergency room (ER) or ward are sent to the lab, along with the lab request form, via pneumatic tubes. Samples are processed immediately on receipt. After order entry into the lab information system (LIS) and a 5-minute high-speed centrifugation (more than 2500 g), samples are analysed on an automated immunoassay system (Cobas 6000, Roche Diagnostics, Mannheim, Germany). The Cobas 6000 has a new 9-minute protocol, replacing the previous 18-minute assay. With 2 identical analysers in service, we achieve work standardisation, process simplification, and 100% up-time. We found the functional sensitivity of the assay (10% inter-assay coefficient of variation) to be 11.5 pg/mL. The 99th centile cut-off in our normal ambulatory subjects (n = 380) is 15 pg/mL for hs-TnT versus 30 pg/mL for the standard TnT assay. Once available, all results are transmitted to the requesting unit via the LIS, printed locally, and also deposited into the electronic medical record (EMR). Our mean turn-around-time (TAT) for hs-TnT is now 30 minutes versus 40 minutes with the previous 18-min protocol. For Tn samples received from the emergency department, 99% of the results are available within 60 minutes.

Another issue laboratories face is whether to report hs-Tn results in ng/mL or pg/mL. With the standard TnT assay, the units used were ng/mL and the cut-points were 0.03 ng/mL, as these were the units most clinicians reading US literature were familiar with. However, hs-TnT has improved lower limits of assay detection at 0.003 ng/mL with a cut-point of 0.015 ng/mL. There is a danger of clerical or typographical error occurring with so many decimal points. We recommend the use of whole numbers to reduce the chance of error: 3 pg/mL (lower limit of detection) and 15 pg/mL (99th centile URL). In addition, the improved hs-Tn assays now require an estimate of what constitutes a real significant change in Tn values given its inherent biological and analytical variation. Clinicians need to know how their hospital Tn assays perform, and laboratories must relay this information to the clinician.

More work remains to be done. However, new information is coming out fast and furiously. We must keep apace or risk being left behind.

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Original Article

Evaluation of the Antidiabetic and Antilipaemic Activities of the Hydroalcoholic Extract of Phoenix Dactylifera Palm Leaves and Its Fractions in Alloxan-Induced Diabetic Rats

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Abstract

Background: **The antidiabetic and antilipaemic effects of Phoenix dactylifera leaf extract (PDE) and its fractions were investigated in various rat models.**

Methods: **Diabetes was induced in male Wistar rats by alloxan monohydrate. Diabetic animals were randomly divided into 8 groups (1 diabetic control and 7 treated groups). Diabetic control animals received saline (5 mL/kg) orally, whereas the treatment groups received different doses of PDE (100, 200, and 400 mg/kg), PDE fractions (50, 100, and 200 mg/kg), or glibenclamide (4 mg/kg) orally once a day for 14 days. Blood was withdrawn for glucose determination on the 1st, 6th, 10th, and 14th days. The rats were fasted overnight and then sacrificed on the 14th day; blood was collected for biochemical evaluation, including the levels of blood glucose, plasma insulin, serum triglyceride, and cholesterol.**

Results: **Subacute administration of PDE or its fractions in alloxan-induced diabetic rats significantly reduced blood glucose ($P < 0.01$). Water intake, serum triglyceride, and cholesterol also decreased in treated animals compared with the control group ($P < 0.01$). Plasma insulin level increased in the treated groups relative to the control group ($P < 0.01$).**

Conclusion: **The results suggested that PDE exhibits antidiabetic and antilipaemic effects in alloxan-induced diabetic rats.**

Keywords: *antidiabetics, antilipaemics, antioxidants, diabetes metabolism, plant extracts*

Introduction

Diabetes mellitus (DM) is a common disorder associated with markedly increased morbidity and mortality rates. DM, which affects a large number of people around the globe, can be defined as a group of metabolic diseases characterised by chronic hyperglycaemia that results from defects in insulin secretion, insulin action, or both, and causes impaired function in carbohydrate, lipid, and protein metabolism. Pharmacological treatment of DM is based on oral hypoglycaemic agents and insulin, but these clinical approaches either do not succeed in restoring normoglycaemia in most patients or fail after a variable period. Moreover, continuous use of synthetic antidiabetic drugs causes side effects and toxicity (1,2). Therefore, there is a need for natural and non-toxic antidiabetic drugs for diabetic therapy. Some studies have demonstrated the hypoglycaemic effects of different parts of various species of palm. It has been shown that an aqueous extract from the leaves of the European fan palm, *Chamaerops humilis*, decreased plasma glucose levels as well as total cholesterol and triglycerides levels (3). Additionally, hypoglycaemic compounds have been found in the roots of *Arocomia aculeata* in many studies (4–6); this coincides well with its traditional use in the treatment for diabetes among indigenous peoples in Mexico. Palm oil tocotrienol-rich fractions were found to reduce the blood glucose level and improve dyslipidaemia in streptozotocin-induced diabetic rats (7); whereas the alcoholic extract of palm seeds decreased the blood glucose and lipid concentration in male diabetic rats (8). However, a clinical trial showed that there was no significant difference in plasma glucose levels after eating

dates versus sugar cubes in patients with type 1 diabetes mellitus. Thus, eating dates in diabetic patients is not preferable to eating sugar cubes (9). A biochemical study showed that date sugar has high alpha-glucosidase, alpha-amylase, and angiotensin I-converting enzyme (ACE) inhibitory activities that correlate with its high total phenolic content and antioxidant activity (10). It has been shown that these agents have beneficial effects in the management of hypertension in type 2 diabetes mellitus (10–11).

The fruit of the plant, date, is used as a food and a beverage, as well as a remedy against various complaints in traditional medicine (8). Its leaves are also used as a folk remedy in the United States and southwest Iran to reduce blood glucose level in diabetes (8–12).

Because there have been no studies assessing the antidiabetic effect of *P. dactylifera* leaves on diabetic rats, the present study evaluates the antidiabetic and antilipaemic effects of hydroalcoholic extract from *P. dactylifera* leaves (PDE) and its fractions on normoglycaemic, glucose-induced hyperglycaemic, and alloxan-induced diabetic rats.

Materials and Methods

Animals

This study was carried out in Ahwaz Jundi Shapoor University of Medical Sciences in 2009. Male Wistar rats weighing 150–180 g were obtained from the animal house of Ahwaz Jundi Shapoor University of Medical Sciences. The rats were fed on conventional diets and water *ad libitum* and maintained under standard conditions of humidity, temperature (20–24 °C), and light–dark cycle (12-hour light:12-hour dark). The rats were randomly assigned to control and treatment groups consisting of 6 rats per group. All animal experiments were carried out in accordance with Ahwaz Jundi Shapour University of Medical Sciences Ethical Committee acts.

Preparation of PDE and its fractions

P. dactylifera leaves (barhee palm) were collected July–September 2009 from Ahwaz City (Khozestan Province, Iran); they were authenticated by Associate Professor Alemzadeh Ansari N (Department of Horticulture, Faculty of Agriculture, Shahid Chamran University, Iran) and S.S. Marashi (Institute of Date Research and Tropical Fruits, Ahvaz, Iran). The voucher specimen (No. Irandate, 10-005) is deposited in the Herbarium, Jundi Shapour University of Medical Sciences. The collected leaves were dried in shade and then powdered. The powder was macerated in 70% ethanol (1:10, w/v) for 3 days with occasional shaking. The combined ethanolic extract was filtered through cheesecloth and centrifuged at 3000 g for 15 minutes. The supernatant was concentrated and lyophilised for preservation (yield = 8.2%) and then stored at 4°C until use.

Fractionation was carried out with solvents of increasing polarity to obtain organic and aqueous fractions. Twenty grams of PDE were dissolved in about 50 mL of distilled water. The same volume of chloroform was added with vigorous shaking. The chloroform layer (lower) was collected thrice and evaporated in a rotary evaporator to give the chloroform fraction, ChCl3 Fr (yield = 16%). The other layer (upper) was again taken into a separating funnel, ethyl acetate was added, and it was separated and then evaporated in rotary evaporator to give the ethyl acetate fraction, EtOAc Fr (yield=6%). The remaining lower layer was collected and evaporated to obtain the aqueous fraction, Aq Fr (yield=11%) (13).

Acute toxicity assessment

To assess acute toxicity, the extract was orally administered in graded doses (1, 2, 4, 6, and 8 g/kg) to 5 treatment groups, while the control group received saline (5 mL/kg). All treated animals were closely observed for any abnormal or toxic manifestations and mortality up to 48 hours.

Preliminary phytochemical screening

PDE was subjected to qualitative chemical screening to identify the various major classes of active chemical constituents, namely tannins, steroid, terpenoids, saponins, flavonoids, and alkaloid.

Test For Tannins: 500 mg of extract was stirred with about 10 mL of distilled water and then filtered. Four drops (0.3 ml) of 1% ferric chloride solution were added to 2 mL of the filtrate. The occurrence of a blue–black, green, or blue–green precipitate indicated the presence of tannins (14).

Liebermann–Burchard Test For Steroids: To 0.2 g of extract, 2 mL of acetic acid was added, the solution was cooled in ice, and then concentrated H2SO4 was carefully added. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring, i.e., a glycone portion of cardiac glycoside (15).

Test For Terpenoids: 100 mg of extract was dissolved in ethanol. Then, 1 mL of acetic anhydride was added, followed by the addition

of concentrated hydrogen sulphate. A change in colour from pink to violet showed the presence of terpenoids (15).

Test For Saponins: 1 g of extract was boiled with 5 mL of distilled water and filtered. Then, 3 mL of distilled water was added to the filtrate, and the mixture was shaken vigorously for about 5 minutes. Frothing that persisted upon warming was taken as evidence of the presence of saponins (15).

Shinoda's Test For Flavonoids: 500 mg of extract was dissolved in ethanol, warmed, and then filtered. Three magnesium chips were then added to the filtrate followed by few drops of concentrated hydrochloric acid. Colour changing from pink, orange, or red to purple indicated the presence of flavonoids (14).

Ferric Chloride Test For Flavonoids: 500 mg of extract was boiled with distilled water and then filtered. Four drops (0.3 ml) of 10% ferric chloride solution were then added to 2 mL of the filtrate. A green-blue or violet colour indicated the presence of a phenolic hydroxyl group (14).

Lead Ethanoate Test For Flavonoids: 100 mg of the extract was dissolved in water and filtered. Then, 3 mL of lead ethanoate solution was then added to 5 mL of each of the filtrate. The appearance of a buff-coloured precipitate indicated the presence of flavonoids (14).

Test For Alkaloids: 100 mg of the extract was stirred with 5 mL of 1% aqueous hydrochloric acid in a waterbath and subsequently filtered. Then, 1 mL filtrate was taken individually into 2 test tubes. To the first portion, 4 drops of Dragendorff's reagent were added; the occurrence of an orange–red precipitate was taken as positive. To the second portion, Mayer's reagent was added, and the appearance of a buff-coloured precipitate indicated the presence of alkaloids (15).

Induction of Diabetes

A freshly prepared solution of alloxan monohydrate in normal saline solution was injected intra-peritoneally (150 mg/kg) to rats that had fasted overnight. After 1 hour, the animals were allowed to feed ad libitum. Their blood glucose level was checked before and 1 week after alloxan injection with a one-touch glucometer. The animals were considered diabetic when the blood glucose level reached 250 mg/dL of blood (16). Water intake was measured within 3 consecutive days (11^{th}–14^{th} day). Animals were placed in metabolic cages with food and water given ad libitum. Water intake was measured for the entire 72 hours.

Determination of blood glucose, plasma insulin, serum cholesterol, and triglyceride levels

Blood samples (20 µl) were obtained from the tail tip of fasted rats, and blood glucose levels were determined using a one-touch glucometer (Elegance, Frankenberg, Germany). Plasma insulin concentrations were measured using Insulin-EIA Test (DiaPlus Inc, USA). Serum triglyceride (TG) and cholesterol levels were analysed using commercial kits (Roche Diagnostics GmbH, Mannheim, Germany) with a Hitachi autoanalyser.

Determination of PDE hypoglycaemic activity upon acute administration

The hypoglycaemic effect of the extract after a single oral administration was tested in normoglycaemic, glucose-induced hyperglycaemic, and diabetic rats. Each group was further divided into 4 treatment groups: 1 negative control group given normal saline (0.154 mM NaCl solution), 1 positive control group given glibenclamide (4 mg/kg), and 3 test groups given PDE (100, 200, and 400 mg/kg; all suspended in the same vehicle).

Firstly, the fasting blood sugar level of each rat was determined at the beginning of the experiment after overnight fasting with free access to water. Then, the designated treatments were administered orally. For the normoglycaemic and diabetic rats, blood glucose levels were determined at 0, 30, 60, and 120 minutes after the treatments. For the glucose-induced hyperglycaemic group, animals received test samples and 30 minutes later, they were given glucose (2 g/kg b.w.) orally. Blood glucose concentrations were measured just before (0 minute) and 30, 60, and 120 minutes after the oral administration of glucose.

Determination of hypoglycaemic activity of PDE and its fractions upon subacute administration in alloxan-induced diabetic rats

The hypoglycaemic effect of the extract and its fractions ($ChCl_3$ Fr, EtOAc Fr, Aq Fr) in diabetic rats was tested. PDE (100, 200, and 400 mg/kg) and its fractions (50, 100, and 200 mg/kg each) were administered orally, once per day for 14 consecutive days. The blood glucose levels were determined on the 1^{st}, 6^{th}, 10^{th}, and 14^{th} days after the administration of the test samples, and the rats' body weights were also monitored on the same days. On the 14^{th} day, all animals were sacrificed. The blood was withdrawn for measurement of plasma insulin, serum cholesterol, and triglyceride levels.

Statistical analysis

All results were expressed as mean ± SEM for each group. Statistical analysis was performed using one-way analysis of variance (ANOVA). Tukey's test was used for multiple comparisons. The values were considered to be significantly different when $P < 0.05$.

Results

Phytochemical analysis and acute toxicity assessment

Phytochemical screening of the PDE indicated the presence of flavonoids, phenols, steroids, and saponins (Table 1). The extract was rich in flavonoids. PDE administration up to 8 g/kg did not show any toxicity to the rats.

Hypoglycaemic activity of PDE upon acute administration in normal, glucose-induced hyperglycaemic, and alloxan-induced diabetic rats

The extract at 400 mg/kg showed significant hypoglycaemic activity in glucose-induced hyperglycaemic rats ($P < 0.05$), while no significant effect was observed in normal and alloxan-induced diabetic rats (Table 2).

Hypoglycaemic activity of PDE and its fractions upon subacute administration in alloxan-induced diabetic rats

A significant antidiabetic effect of PDE at 400mg/kg was observed starting from the 6th day onwards ($P < 0.05$), and from 10th days onwards for 200 mg/kg of PDE; however, administration of extract at 100 mg/kg had not the antidiabetic effect. Dose-dependent antidiabetic activity experiments demonstrated that the extract possessed a remarkable hypoglycaemic effect at 400 mg/kg in diabetic rats ($P < 0.05$). PDE fractions at two doses, 100 and 200mg/kg, also showed a hypoglycaemic effect ($P < 0.05$), with chloroform fraction at 200 mg/kg being the most effective in reducing glucose level (Table 3).

Effects of PDE and its fractions on body weight and water intake in alloxan-induced diabetic rats

Table 4 presents the variations in body weight and water intake of the diabetic control and diabetic treatment groups after 14 days. On the 14th day, alloxan significantly reduced body weight of the diabetic control rats as compared with the 1st day ($P < 0.05$).The extract at 200 and 400 mg/kg and its fractions at 200 mg/kg attenuated this weight loss. PDE at 200 and 400 mg/kg and its fractions (aqueous, chloroform, and ethyl acetate) at 200 mg/kg also significantly decreased water intake compared with the diabetic control rats ($P < 0.05$). The extract at 400 mg/kg demonstrated a significant beneficial effect on water intake when compared with the reference drug glibenclamide.

Effects of PDE and its fractions on plasma insulin and serum lipid levels in alloxan-induced diabetic rats

As shown in Table 5, subacute administration of PDE at 200 and 400 mg/kg and its fractions (aqueous, chloroform, and ethyl acetate) at 200 mg/kg increased plasma insulin levels in the treated groups compared with the diabetic control group ($P < 0.01$). Administration of PDE and its fractions for 14 days also decreased the

Table 1: Phytochemical analysis of the hydroalcoholic extract of *P. dactylifera* leaves

Chemical group	Results
Tannins	-
Flavonoids	+
Saponins	+
Alkaloids	-
Steroids	+
Phenols	+
Terpenoids	-

Positive sign (+) indicates presence, negative sign (-) indicates absence.

Table 2: Acute hypoglycaemic effect of the hydroalcoholic extract of *P. dactylifera* on normoglycaemic, glucose-induced hyperglycaemic, and alloxan-induced diabetic rats

Group	Dose (mg/kg)	Mean blood glucose concentration ± SEM, mg/dL (% inhibition)			
		0 min	30 min	60 min	
Normoglycaemic					
Control	-	86.7 ± 5.7	97.3 ± 7.1 (-12.0)	97.3 ± 6.6 (-12.0)	75.0 ± 6.1 (12.8)
Glyben	4	80.0 ± 1.8	78.6 ± 1.3 (2.5)	75.4 ± 0.8 (6.3)	71.2 ± 0.3[α] (11.2)
PDE	100	88.2 ± 1.8	92.3 ± 2.1 (-4.0)	86.7 ± 1.5 (2.2)	85.8 ± 1.3 (3.4)
	200	87.3 ± 6.2	93.3 ± 7.9 (-6.0)	88.7 ± 6.4 (-1.0)	89.0 ± 6.1 (-2.2)
	400	85.3 ± 3.2	91.2 ± 4.1 (-7.0)	90.0 ± 2.6 (-5.0)	87.2 ± 2.5 (-2.3)
Glucose-induced hyperglycaemic					
Control	-	84.6 ± 4.2	131.1 ± 7.9 (-44.0)	118.3 ± 9.4 (-59.0)	113.0 ± 7.2 (-65.5)
PDE	100	91.5 ± 2.3	136.0 ± 3.1 (-43.0)	125.5 ± 1.8 (-37.0)	115.5 ± 1.2 (-26.0)
	200	88.2 ± 4.42	134.2 ± 6.1 (-52.0)	120.0 ± 7.4 (-36.0)	116.5 ± 7.0 (-31.0)
	400	83.6 ± 3.5	130.8 ± 5.7 (-56.0)	107.3 ± 6.6 (-29.0)	87.1 ± 8.2[α] (-4.8)
Diabetic					
Control	-	384.5 ± 14.9	406.8 ± 13.3 (-5.5)	398.3 ± 7.4 (-3.6)	287.0 ± 7.2 (24.5)
Glyben	4	394.5 ± 32.2	347.0 ± 9.9 (11.9)	347.0 ± 8.9 (11.5)	126.0 ± 5.2 (69.1)
PDE	100	360.0 ± 16.5	338.2 ± 11.0 (6.2)	332.0 ± 11.0 (7.8)	310.5 ± 9.5 (13.9)
	200	389.8 ± 27.9	389.3 ± 26.7 (1.0)	402.5 ± 23.6 (-3.0)	335.3 ± 3.3 (25.5)
	400	381.8 ± 21.8	397.8 ± 20.7 (-4.0)	412.8 ± 21.4 (-8.0)	209.3 ± 9.5 (46.0)

[α] $P < 0.05$ indicates significant difference in comparison with control group using one-way ANOVA followed by Tukey's test.
Abbreviation: Glyben = glibenclamide, PDE = hydroalcoholic extract of *P. dactylifera*

Table 3: Subacute hypoglycaemic effect of the hydroalcoholic extract of *P. dactylifera* and its fractions (aqueous, chloroform, and ethyl acetate) on alloxan-induced diabetic rats

Group	Dose (mg/kg)	Mean blood glucose concentration ± SEM, mg/dL (% inhibition)			
		1st day	6th day	10th day	14th day
Control	-	378.8 ± 22.4	368.2 ± 16.4	371.6 ± 12.3	360.2 ± 14.2
Glyben	4	342.4 ± 14.2	203.6 ± 11.4$^{\alpha}$ (40.3)	195.0 ± 5.8$^{\alpha}$ (43.0)	155.6 ± 4.2$^{\alpha,\beta}$ (55.0)
PDE	100	400.8 ± 20.9	350.0 ± 20.5 (12.5)	321.0 ± 18.0 (20.0)	275.4 ± 15.4 (31.7)
	200	374.4 ± 21.0	267.0 ± 16.5 (28.7)	180.3 ± 16.3$^{\alpha}$ (51.9)	158.0 ± 13.7$^{\alpha,\beta}$ (57.7)
	400	422.0 ± 23.9	180.2 ± 13.4$^{\alpha}$ (57.3)	131.2 ± 6.4$^{\alpha}$ (68.8)	108.2 ± 5.7$^{\alpha,\beta}$ (74.4)
Aq Fr	50	328.0 ± 19.0	296.0 ± 11.2 (9.8)	276.8 ± 8.2 (15.9)	272.6 ± 3.6 (17.1)
	100	352.7 ± 14.4	301.0 ± 17.2 (14.4)	267.5 ± 14.3 (24.2)	213.3 ± 12.0$^{\alpha}$ (39.5)
	200	455.8 ± 23.9	245.4 ± 14.2$^{\alpha}$ (46.2)	153.6 ± 11.5$^{\alpha}$ (66.4)	104.0 ± 5.2$^{\alpha,\beta}$ (77.2)
ChCl3 Fr	50	395.3 ± 15.5	370.0 ± 16.1 (7.4)	333.3 ± 24.4 (15.7)	292.7 ± 22.6 (26.1)
	100	380.7 ± 15.1	286.7 ± 10.3 (24.8)	183.8 ± 7.7$^{\alpha}$ (27.5)	145.2 ± 6.9$^{\alpha,\beta}$ (48.3)
	200	471.8 ± 28.3	222.0 ± 11.2$^{\alpha}$ (52.8)	126.4 ± 11.9$^{\alpha}$ (70.0)	93.0± 4.6$^{\alpha,\beta}$ (80.2)
EtOAc Fr	50	318.3 ± 11.4	296.5 ± 13.1 (6.9)	280.8 ± 9.2 (12.0)	251.3 ± 14.1 (21.1)
	100	357.9 ± 13.4	297.5 ± 11.5 (16.7)	214.2 ± 6.9 (41.1)	185.2 ± 4.6$^{\alpha}$ (48.8)
	200	421.6 ± 19.4	274.3 ± 16.5$^{\alpha}$ (35.0)	214.2 ± 17.1$^{\alpha}$ (49.2)	142.4 ± 6.2$^{\alpha,\beta}$ (66.3)

$^{\alpha}$ $P < 0.05$ indicates significant difference in comparison with 1st day while $^{\beta}$ $P < 0.05$ indicates significant difference in comparison with 6th and 14th day using one-way ANOVA followed by Tukey's test. Abbreviation: Glyben = glibenclamide, PDE = hydroalcoholic extract of *P. dactylifera*, Aq Fr = aqueous fraction, ChCl3 Fr = chloroform fraction, EtOAc Fr = ethyl acetate fraction

serum levels of cholesterol and triglyceride in the treated groups compared with the diabetic control animals ($P < 0.01$).

Discussion

In the present study, treatment with PDE and its fractions had significant antihyperglycaemic and antilipaemic effects. Considering the increase in plasma insulin concentration that was detected, the antihyperglycaemic activity of this extract may (at least in part) occur via the release of insulin from the pancreas. In our study, we observed that PDE and its fractions decreased blood glucose in alloxan-induced diabetic rats. The mechanism of action of the extract and its fractions could be similar to that of hypoglycaemic sulphonylureas, which promote insulin secretion by closure of K^+–ATP (adenosine 5-triphosphate) channels. This results in membrane depolarisation and increased Ca^{2+} influx, and it is a key initial step in insulin secretion. In this context, a number of other plants have also been reported to have antihyperglycaemic and insulin-stimulatory effects (18,19). Because alloxan is known to destroy

Table 4: Effect of the hydroalcoholic extract of *P. dactylifera* and its fractions (aqueous, chloroform, and ethyl acetate) on body weight and water intake in alloxan-induced diabetic rats

Group	Dose (mg/kg)	Body weight (g)		Water intake (mL/day)
		Initial 1st day	Final 14th day	
Control	-	208.0 ± 6.8	183.8 ± 4.2[α]	223.5 ± 15.3
Glyben	4	193.0 ± 3.4	198.0 ± 2.2	80.3 ± 3.2[α]
PDE	100	186.8 ± 6.6	161.5 ± 7.7[α]	210.0 ± 11.8
	200	201.4 ± 9.9	204.4 ± 13.2	71.2 ± 4.2[α]
	400	213.8 ± 7.1	209.5 ± 8.3	52.2 ± 3.2[α]
Aq Fr	200	180.2 ± 8.5	185.3 ± 6.5	48.6 ± 2.8[α]
ChCl3 Fr	200	172.8 ± 4.3	175.4 ± 6.1	50.0 ± 3.3[α]
EtOAc Fr	200	204.9 ± 11.2	210.0 ± 12.3	63.7 ± 5.1[α]

[α] $P < 0.05$ indicates significant difference in comparison with initial body weight using one-way ANOVA followed by Tukey's test. Abbreviation: Glyben = glibenclamide, PDE = hydroalcoholic extract of *P. dactylifera*, Aq Fr = aqueous fraction, ChCl3 Fr = chloroform fraction, EtOAc Fr = ethyl acetate fraction

Table 5: Effect of the hydroalcoholic extract of *P. dactylifera* and its fractions (aqueous, chloroform, and ethyl acetate) on plasma insulin level, serum cholesterol and triglyceride levels in alloxan-induced diabetic ratss

Chemical group	Dose (mg/kg)	Insulin (µIU/mL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Control	-	3.5 ± 0.2	121.2 ± 3.1	131.0 ± 6.3
Glyben	4	6.1 ± 0.4[α]	90.2 ± 2.9[α]	96.0 ± 2.4[α]
PDE	100	3.8 ± 0.3	116.5 ± 3.2	135.2 ± 5.5
	200	5.1 ± 0.4[α]	98.0 ± 1.5[α]	105.0 ± 3.4[α]
	400	7.2 ± 0.4[α]	88.2 ± 1.2[α]	72.6 ± 2.2[α]
Aq Fr	200	6.5 ± 0.4[α]	93.8 ± 2.5[α]	90.6 ± 1.8[α]
ChCl3 Fr	200	5.4 ± 0.2[α]	90.2 ± 2.3[α]	83.6 ± 1.8[α]
EtOAc Fr	200	7.2 ± 0.4[α]	89.7 ± 3.1[α]	75.3 ± 2.4[α]

[α] $P < 0.01$ indicates significant difference in comparison with control group using one-way ANOVA followed by Tukey's test. Abbreviation: Glyben = glibenclamide, PDE = hydroalcoholic extract of *P. dactylifera*, Aq Fr = aqueous fraction, ChCl3 Fr = chloroform fraction, EtOAc Fr = ethyl acetate fraction

pancreatic β-cells, the present findings appear to be in consonance with an earlier suggestion by Jackson and Bressler (20) that sulphonylureas have an extra-pancreatic, antihyperglycaemic mechanism of action secondary to their insulin-secreting effect and the attendant glucose uptake into (and utilisation by) tissues.

The antidiabetic effect of PDE and its fractions also may be due to the effect of active flavonoids, phenols, steroids, and saponins; these compounds may scavenge free radicals liberated by alloxan in diabetic rats (21,22). Hypoglycaemic effects have been reported for some plants that contain flavonoids (22,23).

Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Insulin is a potent inhibitor of lipolysis because it inhibits the activity of hormone-sensitive lipases in adipose tissue and suppresses the release of free fatty acids. In diabetes, enhanced activity of this enzyme increases lipolysis and releases more free fatty acids into circulation. Increased fatty acid concentrations also increased the

β-oxidation of fatty acids, producing more acetyl-CoA and cholesterol in diabetics. The hypocholesterolaemic activity of PDE and its fractions after subchronic administration may be due to a number of mechanisms, including a) stimulation of cholesterol-7-alpha-hydroxylase (CYP7A1), which converts cholesterol into bile acids; b) inhibition of HMG-CoA reductase; and/or c) inhibition of cholesterol absorption from the intestines due to the formation of complexes with compounds such as glycosides and saponins (24–26). A reduction in triglyceride levels may be due to decreased lipogenesis and increased lipolytic activity by activation of the hormone-sensitive lipase (27) or lipogenic enzymes (28), and/or activation of lipoprotein lipase (29), as is observed in antidiabetic plants such as Ormodica charantia (29), Artemisia herba alba (27), and *Ceasalpinea bondecella* (30), which exhibit hypolipidaemic activity. Furthermore, several plant constituents, including flavonoids, are known to decrease triglyceride level (31).

Our results suggest that the extract and its fractions have insulin-like activity. It is possible that the hypolipidaemic effects of the extract may be related to this effect. In the present study, acute toxicity was tested up to the high concentration of 8 g/kg (20 times more than the therapeutic dose). Even at this dose, the extract did not exhibit any sign of toxicity. The main purpose of a preliminary acute toxicity study is to provide some idea of conspicuous behavioural changes and/or death, and PDE did not exhibit any toxic symptoms in this limited toxicity evaluation in male rats. A review of the literature shows that studies have not been carried out on this plant with regard to other pharmacological properties or phytochemistry.

Dehydration and loss of body weight have been associated with diabetes mellitus (32). In diabetic rats, increased water intake and decreased body weight were observed. This indicates a polydipsic condition and loss of weight due to excessive breakdown of tissue proteins (33). The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats (34) as well as proteins, which might lead to muscle wasting (35). Oral administration of PDE and its fractions for 14 consecutive days to diabetic rats decreased their water intake and improved body weight. These effects could be due to better control of the hyperglycaemic state in diabetic rats. Decreased fasting blood sugar improves body weight in alloxan-induced diabetic rats (36,37). PDE caused hypoglycaemia in alloxan-induced diabetic rats, thus validating the traditional use of *P. dactylifera* leaves in southwest Iran for the treatment of diabetes. As many antidiabetic drugs do not correct dyslipidaemia, the observed hypocholesterolaemic and hypotriglyceridaemic effects of the extract in alloxan-induced diabetic rats unveils PDE potential in the management of diabetes because the extract may also reverse dyslipidaemia associated with diabetes and prevent cardiovascular disease complications (38).

Conclusion

The findings of the current study showed that PDE and its fractions have a hypoglycaemic effect in alloxan-induced diabetic rats. In addition, they were highly effective in managing the complications of diabetes mellitus such as hyperlipidemia and weight loss. The antidiabetic effects of PDE and its fractions may be mediated through an increase in insulin secretion, the stimulation of glucose uptake and glycogen synthesis by cells, and/or protection of pancreatic β-cells from alloxan- and glucose-induced oxidative stress.

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Authors' Contributions

Conception and design, drafting of the article, final approval of the article: SAM, MKGN
Obtaining of funding, analysis and interpretation of the data, critical revision of the article: SAM
Provision of study materials, collection and assembly of data, administrative, technical, or logistic support: KJ, MJ, HB

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Original Article

Abnormal Microvascular Reactivity with Hypercholesterolaemia in Pregnancy

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Abstract

Background: **Post-occlusive skin reactive hyperaemia (PORH) is a model used to assess microvascular reactivity. This study aims to compare PORH response among pregnant hypercholesterolaemic patients with age and gestational age-matched controls.**

Methods: **This cross sectional study involved 17 hypercholesterolaemic, pregnant women and 20 pregnant controls entering their early third trimester. Laser Doppler fluximetry (LDF) was used to measure skin perfusion. The process of PORH was performed by occluding the upper arm with an occlusion cuff at 200 mmHg for 3 minutes. Skin perfusion was recorded before, during, and after occlusion release. Baseline perfusion, time to achieve peak perfusion (Tp), peak perfusion after occlusion release ($PORH_{peak}$), and maximum change in perfusion due to occlusion ($PORH_{max}$) were recorded.**

Results: **Serum total cholesterol (TC) was significantly different ($P < 0.001$) between the 2 groups: 7.25 (SEM 0.18) mmol/L for hypercholesterolaemic women and 5.54 (SEM 0.15) mmol/L for the control group. There were no significant differences in their baseline, $PORH_{peak}$, and $PORH_{max}$. However, Tp in the hypercholesterolaemic group was significantly increased ($P = 0.024$) compared with the controls at 14.9 (SEM 0.6) seconds and 13.1 (SEM 0.5) seconds, respectively.**

Conclusion: **Pregnant hypercholesterolaemic patients showed an abnormal microvascular reactivity response. Tp with ischemia was significantly increased compared with normocholesterolaemic controls.**

Keywords: *hypercholesterolaemia, laser Doppler flowmetry, microcirculation, pregnancy, reactive hyperaemia*

Introduction

Hypercholesterolaemia is an important cardiovascular risk factor and a potent factor contributing to the progression of atherosclerosis (1). It is associated with endothelial dysfunction and reduced endothelium-dependent responses in the forearm and coronary circulation (2,3). Maternal hypercholesterolaemia during pregnancy has been reported to be associated with enhanced fatty streak formation in human foetuses (4). The hypercholesterolaemic condition during pregnancy may also modify vascular and placental functions, contributing to complications during pregnancy or to the offspring. It has been shown that pregnant rats fed a diet enriched in cholesterol showed 4-fold increase in abortions, 2-fold increase in neonatal mortality, smaller litter size, and a lower birth weight of pups (5).

Post-occlusive skin reactive hyperaemia (PORH) is the increased of blood flow after temporary occlusion of the arterial blood flow. The hyperaemic response to an ischaemic block is thought to be endothelium-dependent, involving myogenic response and release of metabolic factors such as nitric oxide (NO) and prostaglandins. Assessment of PORH by laser Doppler fluximetry (LDF) is a simple, non-invasive and reproducible method to assess microvascular health (6,7). Besides providing information on baseline blood flow measurements, the time-course of the cutaneous response to post-ischaemic

reactive hyperaemia can give information on changes occurring in the microcirculation (8). Cutaneous post-ischaemic reactive hyperaemia has been used to detect microvascular reactivity differences between smokers and non-smokers (9), diabetic patients and controls, as well as between subgroups of diabetic patients (10). More recently, post-hyperaemia flux was reported to be significantly lower, and time to achieve peak post-ischaemic response was slower in diabetics compared with controls (11). Farkas et al. have demonstrated that hypertensive patients have a lower maximal change in skin blood flow with reactive hyperaemia compared with controls (12).

Normal pregnancy is characterized by reduced peripheral vascular resistance and blood pressure, increased cardiac output, and subsequent increase of blood flow in the systemic and pulmonary circulations. Brachial artery flow-mediated dilatation was found to be increased from the first trimester, reaching the highest value in the last trimester compared with non-pregnant values. However, no studies have directly compared microvascular or skin vascular reactivity using reactive hyperaemia method between healthy non-pregnant and pregnant women.

In pregnant women, vascular reactivity has been shown to be altered in skin vessels of pre-eclamptic patients (13). In normotensive pregnant subjects, the reactive hyperaemia response was nearly as pronounced as the maximal vasodilatation of the vessels induced by local heating. However, in patients with pre-eclampsia, the reactive hyperaemia response reached was only half of the maximal vasodilatory capacity. Pre-eclamptic women were also shown to have lower post-occlusive reperfusion compared with controls, besides having lower basal blood flow (14).

Dyslipidemia has been shown to impair microvascular reactivity in non-pregnant subjects (7,8,15,16). The effect of hypercholesterolaemia on the microvascular bed has not been studied in pregnant women. It is not known whether the difference in reactivity between normocholesterolaemic and hypercholesterolaemic patients persists in the presence of altered cardiovascular, hormonal, and metabolic adaptations occurring during pregnancy. Thus, this study aims to compare microvascular reactivity between pregnant women with hypercholesterolaemia and age-matched as well as gestational age-matched controls. LDF and the PORH response were used to assess microvascular reactivity in these women.

Materials and Methods

Subjects

This prospective, cross sectional study involved 17 pregnant hypercholesterolaemic patients and 20 age and gestational-aged matched women. Ethical approval for the conduct of this study was obtained from the Ethical Committee of Universiti Sains Malaysia. This study was conducted following the principles stated in the Declaration of Helsinki; all subjects voluntarily signed an informed consent form.

Subjects were recruited from the Obstetrics and Gynaecological Clinic of Hospital Universiti Sains Malaysia when they presented between 25–33 weeks gestation. Pregnant women with significant cardiovascular diseases (such as hypertension, arrhythmia, and heart failure), diabetes mellitus, history of previous and current gestational diabetes, multiple pregnancies, foetal anomalies, and serious maternal illnesses (such as liver and renal diseases) were excluded. All subjects underwent a modified oral glucose tolerance test (MOGTT) to exclude gestational diabetes. None of the subjects were taking any vasoactive medication such as non-steroidal anti-inflammatory drugs, corticosteroids, or lipid-lowering drugs. Women were categorized into the hypercholesterolaemic group if their serum total cholesterol (TC) was equal to or more than 6.5 mmol/L. At this cholesterol level, the risk of developing coronary artery disease is substantially increased (17).

LDF and PORH response

The LDF DRT4 system (Moor Instruments, Axminster, UK) was used in this study to non-invasively measure the forearm skin blood perfusion. This equipment was used together with the DP1T-V2 skin laser probe (Moor Instruments, Axminster, UK). LDF of skin blood flow is based on measurement of the Doppler shift principle wherein photons of laser light are scattered by moving blood cells to produce a Doppler shift on the reflected light. The outcome is generally termed as 'flux' and expressed in arbitrary perfusion units (AU).

Laser Doppler measurements on the pregnant women were carried out in the morning in a quite room with room temperature maintained at 23 (SD 1) °C. Upon arriving, subjects lay down for at least 15 minutes for acclimatization before their blood pressure was taken using an automated

blood pressure sphygmomanometer (Omron, Japan). The right arm of the subject was placed on a cushion and supported by a hand supporter to reduce involuntary hand movements. A sphygmomanometer cuff (Accoson, UK) was placed around the right upper arm 1–2 cm above the ante-cubital crease. Laser Doppler probes were then fixed on the volar surface of the right forearm distal to the sphygmomanometer occlusion cuff. After a stable skin perfusion was obtained, baseline skin perfusion flux was recorded for 1 minute. Forearm blood flow was then performed by inflating the pneumatic pressure cuff placed on the right upper arm to a supra-systolic pressure of 200 mmHg for 3 minutes. After 3 minutes, the occlusion cuff was rapidly deflated and perfusion flux was recorded for at least 2 minutes. Both the skin perfusion and temperature were measured continuously before, during and after occlusion by the laser Doppler probes. Skin perfusion was allowed to return to baseline; an interval of at least 15 minutes (or until baseline flux was achieved, whichever was longer) was allowed before a repeat of the procedure. The following parameters were measured: minimum baseline perfusion, the time required to reach peak flow after occlusion release (Tp), peak perfusion reached after occlusion release ($PORH_{peak}$), and maximum change in perfusion after occlusion compared with baseline ($PORH_{max}$) which was calculated as $PORH_{peak}$ minus minimum baseline perfusion.

The average of 2 readings for each parameter was recorded. A single operator was responsible for performing the reactive hyperaemia procedure throughout this study to reduce inter-operator variability. The intraday and interday coefficient of variations for $PORH_{max}$ and Tp at our laboratory was 4.77% and 6.5%, and 8.89% and 6.87%, respectively (6).

Blood sample for the measurement of serum TC was taken after an overnight fast. Serum TC was measured at the Chemical Pathology Laboratory, Hospital Universiti Sains Malaysia, using commercially prepared reagents (Enzymatique Endpoint, Randox) on the Hitachi Model 902 auto-analyser (Japan). The within and between run coefficient of variation for determining serum TC at the laboratory was less than 2%.

Statistical analysis was performed using the SPSS version 12 (SPSS Inc., Chicago, IL). Results are presented as mean and SEM; $P < 0.05$ defined statistical significance. Differences between groups were evaluated using the independent *t* test or Mann–Whitney U test, where applicable.

Results

Mean age and gestational age for the subjects were 32.8 (SEM 1.1) years (ranged 21–45 years) and 28.4 (SEM 0.3) weeks (ranged 25–33 weeks), respectively. Baseline characteristics for the controls and hypercholesterolaemic patients are shown in Table 1. Apart from the serum TC values, there were no significant differences between the controls and hypercholesterolaemic subjects in their age, gestational age, blood pressure, blood count values, body mass index, and fasting plasma glucose values. There was also no significant difference in their skin temperature during the PORH procedure.

Arterial occlusion induced by the occlusion cuff around the upper arm and inflated to 200 mmHg for 3 minutes resulted in a significantly lower perfusion recording compared with baseline. After occlusion release, the blood flow increased rapidly to a peak value approximately 5-fold compared with baseline; but the perfusion decreased again towards baseline soon after.

Both groups showed significant increases in $PORH_{peak}$ compared with baseline ($P < 0.001$ in both groups). Baseline perfusion, Tp, $PORH_{peak}$, and $PORH_{max}$ for the normocholesterolaemic and hypercholesterolaemic groups are shown in Table 2. There were no significant differences between the 2 groups in baseline perfusion, $PORH_{peak}$, and $PORH_{max}$. There was however, significant difference between the 2 groups for Tp; the hypercholesterolaemic group showed significantly longer Tp compared with the controls.

Linear regression analysis was performed to assess the relationship between serum TC and Tp. There was a positive significant correlation ($P = 0.015$, $r = 0.4$) between the 2 parameters; higher serum TC values were associated with longer Tp.

Discussion

We found that the presence of hypercholesterolaemia in pregnancy prolonged the Tp with the process of PORH compared with the age and gestational age-matched controls. Microvascular reactivity among non-pregnant, hypercholesterolaemic subjects has been reported in the literature (7,8,16,18). Binggeli et al. reported that post-ischaemic skin blood flow measured using the LDF was markedly reduced in hypercholesterolaemic patients compared with healthy controls and statin improved the post-ischaemic hyperaemia in the subjects after an average of 6 months of treatment (7).

Table 1: Demographic and laboratory characteristics of controls and hypercholesterolaemic patients

	Controls n = 20	Hypercholesterolaemic patients n = 17
Age (years)	32.60 (1.29)	33.00 (1.91)
Body mass index (kg/m^2)	27.13 (0.92)	26.75 (1.38)
Gestational age (weeks)	28.15 (0.49)	28.71 (0.49)
Fasting plasma glucose (mmol/L)	4.03 (0.14)	4.23 (0.14)
Serum total cholesterol (mmol/L)	5.54 (0.15)	7.25 (0.18)[a]
Systolic blood pressure (mmHg)	103.6 (2.1)	108.2 (2.9)
Diastolic blood pressure (mmHg)	63.8 (1.9)	66.9 (2.3)
Blood hematocrit (10^9/L)	33.98 (0.56)	34.7 (0.49)
Glycosylated hemoglobin (%)	5.10 (0.15)	5.01 (0.18)
Skin temperature during reactive hyperaemia (°C)	28.6 (0.23)	28.6 (0.21)

Data are expressed in mean (SEM). [a]$P < 0.001$ indicates highly significant difference in serum total cholesterol for hypercholesterolaemic patients in comparison with controls (independent t test).

Table 2: Microvascular perfusion values in normocholesterolaemic and hypercholesterolaemic pregnant patients during post-occlusive reactive hyperaemia

	Controls n = 20	Hypercholesterolaemic patients n = 17
Tp (seconds)	13.1 (0.5)	14.9 (0.6)[a]
Baseline perfusion (AU)	10.3 (1.3)[b]	11.0 (1.9)[b]
$PORH_{peak}$ (AU)	49.1 (3.7)	51.1 (4.7)
$PORH_{max}$ (AU)	38.8 (3.3)	40.1 (4.2)

Data are expressed in mean (SEM). [a]$P = 0.024$ indicates significant difference in Tp for hypercholesterolaemic patients in comparison with controls (independent t test), whereas [b]$P < 0.001$ indicates highly significant difference in $PORH_{peak}$ in comparison with baseline for both groups (paired t test).
Abbreviation: AU = arbitrary perfusion units, $PORH_{max}$ = maximum change in perfusion after occlusion compared with baseline, $PORH_{peak}$ = peak perfusion reached after occlusion release, Tp = time required to reach peak flow after occlusion release.

Unlike the study by Binggeli et al., no difference in peak hyperaemic response was seen in our study. Two possible reasons may explain this difference; firstly, the mean age of our subjects was 33 years compared with theirs, which was 42 years. It is possible that older subjects experience more vascular function abnormality with hypercholesterolaemia compared with the younger age group. The older age group may also have been exposed to a longer duration of high lipid levels. Secondly, our subjects were all female and pregnant, whereas in their study, 17 out of 19 subjects were males and there were no pregnant women. Most of their subjects were also on other drugs, mostly cardiovascular-related, such as acetyl salicylic acid, antihypertensives, and diuretics, indicating that some of these patients may already had some vascular/endothelial dysfunction present. In the study by Binggeli et al., Tp achieved by each group was not reported. Stulc et al. have observed that microvascular reactivity in hypercholesterolaemic patients without coronary artery disease was not different from controls. Microvascular reactivity was, however, reduced in hypercholesterolaemic patients with coronary artery disease (16). Patients with hypertriglyceridemia were reported by Tur et al. to show cutaneous microcirculatory changes in the forearm. Untreated hypertriglyceridemic patients have significantly lower peak flow compared

with controls and patients treated with the lipid-lowering drug, bezafibrate (8). The hyperaemic reaction was also faster in the bezafibrate-treated group compared with the other 2 groups. Apart from our current study, there are no studies assessing the effect of hypercholesterolaemia on microvascular reactivity in pregnancy.

Haak et al. reported that Tp during PORH was shorter in hyperlipidaemic patients after treatment with the statin fluvastatin; this was associated with a 22.8% reduction in serum TC level (18). Gomes et al. reported that Type 1 diabetic patients took longer to reach peak flux during hyperaemia response compared with age-matched controls (19). The mean age of their subjects was 33 years. Similar to our study, no difference in $PORH_{max}$ was observed between the 2 groups (19). Similarly, Tur et al. had demonstrated that in diabetic patients, $PORH_{peak}$ was lower than non-diabetic controls; diabetics also had longer Tp compared with controls (10).

There are a few possible mechanisms whereby hypercholesterolaemia contributes to prolonged Tp. Functional or a combination of functional with structural vascular changes might produce these microvascular abnormalities. Firstly, elevated serum TC may increase blood viscosity and increase the Tp. Secondly, there may be failure of prompt vasodilatation by smooth muscle cells in response to ischemia in hypercholesterolaemic subjects. During reactive hyperaemia, ischaemic stimulation forces the endothelium to release vasodilating substances such as NO and prostaglandins. In normal subjects, the response to occlusion and release would be fast and efficient, but in hypercholesterolaemia, the endothelium may take a longer time to respond, causing a longer time for reperfusion. This may be due to impairment in the diffusion of NO into the smooth muscle of the vessel wall. Hypercholesterolaemia has been reported to impair endothelium-dependent relaxation in the microcirculation and macrocirculation in experimental animals and humans (20,21). This may be due to reduced synthesis of endothelium-derived relaxing factors, or altered membrane receptor coupling mechanisms that affected the release of these factors. Since NO normally interferes with the action and synthesis of endothelin (an endogenous vasoconstrictor), the lack of NO activity may favour the vascular expression of endothelin. Thirdly, hypercholesterolaemia may cause a reduction in vasodilating substances produced by the endothelium by creating a condition of oxidative stress. Free radicals, such as superoxide anion, have been known to scavenge endothelium-derived relaxing factor (22). Lastly, structural changes in the blood vessel, such as wall thickening, may also reduce the speed of response to ischaemia.

The primary limitation of this study in that only the serum TC value was available, while the detailed lipoprotein results were not. However, the results from this study will hopefully form the basis for more detailed study in this area. Another limitation of this study is that the duration of hypercholesterolaemia in these patients is unknown; some women may be more relaxed in their dietary habits during pregnancy. However, the strength of this study is that, apart from the serum TC values, there was little difference in characteristics of the controls and the hypercholesterolaemic subjects, wherein both groups were screened for impaired glucose tolerance, they were not on any vasoactive drugs, none of the hypercholesterolaemic subjects had ever been treated with lipid lowering agents, and neither group had any cardiovascular diseases.

Conclusion

We conclude that hypercholesterolaemia in pregnancy affects microvascular reactivity by increasing the time needed to achieve peak perfusion after temporary arterial occlusion. More detailed study in this population correlating detailed lipid profile, microvascular reactivity, placenta blood flow, and pregnancy outcome is suggested.

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Authors' Contributions

Conception and design, obtaining of funding, critical revision and final approval of the article: AHGR, NAAG, NMZNM, ARW
Provision of patients: AHGR, NAAG, NMZNM
Collection and assembly of data, analysis and interpretation of the data, administrative, technical, or logistic support: AHGR, ASAR
Drafting of the article: AHGR, ASAR, ARW

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Original Article

Hyperendemicity of Onchocerciasis in Ovia Northeast Local Government Area, Edo State, Nigeria

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Abstract

Background: **Onchocerciasis is a chronic parasitic infection caused by the filarial nematode, *Onchocerca volvulus*. The objective of this study was to determine the prevalence, endemicity, and symptomatic effects of the disease in Ovia Northeast Local Government Area.**

Methods: **The prevalence of onchocerciasis was investigated in Ovia Northeast Local Government Area of Edo State, Nigeria, between March 2008 and June 2009 using the standard skin-snip method. A total of 2020 subjects, who had visited various primary health centres located in each community, were enlisted using randomised sampling, and the data were analysed using the Chi-squared (χ^2) test and logistic regression.**

Results: **A Of the 2020 individuals examined, 1674 (83%) harboured microfilaria in their skin tissues. On the basis of the standardised scale for microfilaria prevalence—less than 10% is considered sporadic, 10%–29% is considered hypoendemic, 30%–59% is considered mesoendemic, and 60% and above is considered hyperendemic—the prevalence (83%) reported in this study led to the disease being classified as hyperendemic. Females were more frequently infected than were males, and this was statistically significant ($P < 0.001$). Prevalence was also found to increase with age, and this correlation was significant ($P < 0.001$). The prevalence of the clinical features of the disease in the local government area was 87.5% for leopard skin, 84.16% for itching, and 75.42% for nodules.**

Conclusion: **A prevalence of 83% was observed and considered hyperendemic. Female gender and age (50 years or more) were significant risk factors that affected the prevalence of onchocerciasis. The findings demonstrated the hyperendemicity of infection and the need for urgent attention with ivermectin treatment and other control measures.**

Keywords: *endemics, Nigeria, Onchocerca volvulus, onchocerciasis, parasitology, prevalence, risk factors*

Introduction

Onchocerciasis is a recognised public health threat caused by the filarial nematode *Onchocerca vovulus*. In 2001, about 18 million people are infected with the disease, of which 99% live in Africa (1). However, an estimated 37 million cases were reported in 2006, with 90 million people at risk in Africa (2). Nigeria is currently the most endemic country in the world (3). The disease has a wider distribution in Nigeria than previously believed; about 10 million cases were reported (4). Onchocerciasis in Nigeria is transmitted solely by members of the *Simulium damnosum* complex. They are widespread in the savannah, forest-savannah, mosaic, and forest areas of Nigeria (5). The disease is responsible for immense suffering, as it may cause severe and worrisome onchocercal skin disease, as well as disfiguring and embarrassing conditions, such as hanging groin and genital elephantiasis. The disease may also manifest with blindness in most endemic areas in Africa, especially in the rainforest belt (6). The Ovia Northeast Local Government Area (LGA) is one of the 18 LGAs in Edo State, Nigeria, that were identified through a pilot survey as having the disease, and ivermectin drug distribution to the communities by the Community-based Distribution of Ivermectin (CBDI) commenced around 1998 to reduce the pathological burden of the disease, particularly blindness. However, the true prevalence and symptomatic effects of infection have not been studied in detail among the communities in the LGA, a fact that necessitated the present study. This study investigated the prevalence, endemicity, and symptomatic effects of the infection in 40 communities of the LGA and provided data that could contribute to a programme for controlling the disease.

Materials and Methods

Study Area

The study was carried out in Ovia Northeast LGA of Edo State, Nigeria. The LGA lies between latitudes 5°40' and 7°40' North and longitudes 5°00' and 6°30' East. The main river, the Ovia River, flows through all of the communities in the LGA. A total of 40 communities were involved in this study: Abumwenre, Agbenikaka, Agheanokpe, Aihuobabekun, Egbatan, Egbetta, Ewudu, Igbekhue, Iguadolor, Iguelegbon, Iguobo, Iguomo, Iguoshodinbudin, Isioho, Isiuwa, Iyekeze, Iyowa, Obazuwa/Odighi, Odiguetue, Ogbesse, Oghobahon, Okada, Okhunmwun, Okokhuo, Oluku, Ora, Orogo, Osasinwionba, Owan, Igbe, Ugbineh, Ugbogiobo, Ugbokun I, Ugbokun II, Ugbuwe, Uhen, Uhiere, Uhogua, Utekon, and Utese.

Sampling technique

The prevalence of onchocerciasis was investigated in the 40 communities located in Ovia Northeast LGA of Edo State, Nigeria, between March 2008 and June 2009 using the standard skin-snip methods. A total of 2020 subjects above the age of 5 years, who had visited various primary health centres in each community, were recruited using simple random sampling. Informed consent was obtained from all study subjects. A blood-free skin snip was taken from individuals using a 2 mm bite corneo-sclera punch (E1802, Holt Storz, Germany) (6) and processed according to a previously described technique (7). Briefly, the collected snip was placed in saline solution and incubated at room temperature for 4 hours. The saline solution was then placed on a clean slide with a coverslip and viewed under a microscope. The presence of the microfilariae was taken as a positive result, while its absence was taken as a negative result. Thorough physical examinations of individual volunteers were performed by a physician. The clinical assessment of the sample population was conducted behind translucent curtains. The assessment of itching was confirmed from subjects' responses and reported as present or absent, while nodule assessment was done by inspection and palpation using hands from head to toe, with special attention to the ribcage, iliac crest, greater trochanters, knees, and scapula. In palpation, mobility and attachment to the overlying and underlining skin structures were carefully inspected to eliminate neoplastic lesions such as nodules resulting from onchocerciasis, which are usually inflammatory. The leopard skin assessment was reported as present or absent upon examination of the lower limbs for the presence of depigmentation of the skin.

Statistical analysis

Data were analysed using the Chi-square (χ^2) test and logistic regression with the GraphPad InStat (GraphPad Software, San Diego, CA).

Results

A total of 1674 subjects tested positive for microfilaria out of a population of 2020 subjects from whom skin snips were obtained. Table 1 shows the prevalence of clinical manifestations of onchocerciasis, with leopard skin having the highest prevalence (87.5%), followed by itching (84.16%), and nodules (75.42%). Nodules had a significantly lower prevalence ($P < 0.001$) compared with other clinical manifestations. Females had a significantly higher prevalence of infection ($P < 0.001$) than did their male counterparts (93.1% vs. 74.5%, respectively). Generally, age affected the prevalence of onchocerciasis with individuals in the 10–19 years age group being the least affected, while individuals of more than 50 years old experienced the highest prevalence (Table 2). Communities in Ugbogiobo and Utese were heavily infected, while surrounding communities were moderately infected.

Discussion

This study recorded an overall prevalence of 83% in the Ovia Northeast LGA. This prevalence is inconsistent with previous studies (8–11). According to McMahon et al. (12), the onchocerciasis programme classifies outbreaks as sporadic, hypoendemic, mesoendemic, or hyperendemic based on the standardised microfilaria prevalence being less than 10%, 10%–29%, 30%–59%, and 60% and above, respectively. Therefore, Ovia Northeast LGA is classified as hyperendemic in view of the prevalence rate of 83% (12). The high prevalence rate recorded in Ovia Northeast LGA may be due to long-term infections. The majority of the subjects examined had lived in the community for more than 10 years, and they may have been continuously bitten by *Simulium damnosum* (vector) throughout the years. Most of the subjects were also involved in farming and hunting, which exposes them to bites from the vector. The presence of leopard skin and palpable nodules were indications of a

Table 1: Prevalence of clinical manifestations of onchocerciasis in Ovia Northeast Local Government Area

Clinical manifestation	Patients evaluated, *n*	Patients affected, *n* (%)	95% CI
Itching	669	563 (84.16)	0.814, 0.870
Nodular	590	445 (75.16)	0.719, 0.789 [a]
Leopard skin	761	666 (87.52)	0.852, 0.898

[a] Statistical analyses were done using Chi-square (X^2) test with $P < 0.001$ indicating significance in comparison with itching and leopard skin.

Table 2: The association between prevalence of onchocerciasis and various risk factors

Characteristics	Patients evaluated, *n*	Patients affected, *n* (%)	OR	95% CI	*P* value
Gender					
Male	1109	826 (74.50)	0.217	0.162, 0.290 [a]	< 0.001
Female	911	848 (93.10)	4.612	3.453, 6.160 [b]	< 0.001
Age (years)					
≤ 9	146	105 (71.92)	0.044	0.015, 0.127 [c]	< 0.001
10–19	332	214 (64.46)	0.031	0.011, 0.086 [c]	< 0.001
20–29	424	356 (83.96)	0.090	0.033, 0.251 [c]	< 0.001
30–39	459	400 (87.15)	0.012	0.042, 0.326 [c]	< 0.001
40–49	423	367 (86.76)	0.113	0.040, 0.316 [c]	< 0.001
≥ 50	236	232 (98.31)	-	-	-

Statistical analyses were done using Chi-square (X^2) test with $P < 0.001$ indicating significance.
[a] Comparison with female patients as reference.
[b] Comparison with male patients as reference.
[c] Comparison with age group of 50 years and above as reference.

long-standing infection. These conditions were determined by frequent bites of the Simulium damnosum.

More females (30.4%) than males (25.8%) were affected by itching. The prevalence of itching increased with age and was the greatest in patients who were 20–29 years. Thereafter, there was a slight decrease in itching as the age of patients increased. It is important to note that there was a deviation in the pattern of progression of itching, in which the prevalence increased with age and then flattened out at 30 years and above (10,13,14). This may be due to migration of residents in and out of the LGA in search of jobs in the urban centres or the movement of subjects from less endemic communities into the LGA for farming. Additionally, according to the CBDI, the subjects who were 30 years and older had higher ivermectin-compliance than other age groups.

Generally, the nodular prevalence recorded was significantly lower than that observed with other clinical manifestations. The skin snip (microfilaria) yields higher frequency of positive results for infection than does the observation of nodular manifestation (16). This was also observed in this study. The nodular prevalence recorded in females was higher (24.9%) than that of males (19.7%). This is however, different from an earlier report (17). The prevalence of nodules also increased with age, reached its peak in the 20–29 year age group, and then decreased slightly as age increased. This is different from other previous reports (10,13,14,17), in which the nodular rate increased continuously with age and then remained constant for patients aged 30 years and higher. Leopard skin is a chronic onchocercal dermatitis that results from the accumulation of microfilariae that degenerate in the upper dermis and consequently lead to acute–chronic inflammation due to long-standing infection. In this study, leopard skin had the highest occurrence compared with itching and nodules. A similar observation was made by other researchers in Okpuje, Edo State, Nigeria (11). This

may be attributed to the fact that most subjects with leopard skin had spent a considerable time (about 20 years and more) in these communities such that they were exposed continuously to this parasitic infection.

More females than males were infected by the disease, and this was statistically significant ($P < 0.001$). However, this observation differed from the findings of earlier reports (11,18). The difference in infection rate could be explained by the domestic exposure and behavioural patterns of the inhabitants. The males are more involved in farming, and the farmlands are far away from streams that serve as *Simulium* breeding sites. Furthermore, the females have more contact with the stream as they fetch water for domestic use and cassava fermentation (10).

In this survey, age significantly affected the prevalence of onchocerciasis ($P < 0.001$). This finding is consistent with those of earlier reports (8,11,17–19). The age-related prevalence recorded in this survey probably arose because onchocerciasis takes time to manifest.

The high prevalence of onchocerciasis, despite the administration of yearly ivermectin therapy in the LGA, may indicate resistance to ivermectin. However, this conjecture may require molecular resistance studies to confirm, especially in a country plagued by counterfeit drugs.

Conclusion

This study revealed a high prevalence of onchocerciasis (83%); this prevalence increased with age and was higher in females. Leopard skin and itching were the most reliable clinical manifestations.

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Authors' contributions

Conception and design, obtaining of funding, provision of study patients, collection and assembly of the data, analysis and interpretation of the data, drafting of the article: AFO
Statistical expertise, critical revision of the article, final approval of the article: OCE

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Original Article

A Three-Dimensional Computed Tomography Analysis of Craniofacial Asymmetry in Malaysian Infants with Cleft Lip and Palate

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Abstract

Background: **The application of three-dimensional computed tomography (3D CT) to analyse craniofacial morphology in individuals with cleft lip and palate (CLP) enables detailed assessments to be made of asymmetry in the region of the cleft and in regions distant from the cleft. The aim of this study was to compare craniofacial morphology in a sample of Malaysian infants with unoperated CLP with a control sample of unaffected Malaysian infants.**

Methods: **The study sample comprised 29 individuals: 10 with unilateral CLP (UCLP), 5 with bilateral CLP (BCLP), 7 with cleft lip and primary palate (CLPP), and 7 with isolated cleft palate (ICP). The control sample consisted of 12 non-cleft (NC) infants. All subjects were between 0.4 and 12.2 months of age. Nine mid-facial and 4 nasal bone landmarks were located on 3D CT scans and compared to a midline reference plane, which was created using the landmarks basion, sella, and nasion. Unpaired *t* tests and *F* tests were used to compare means and variances between sample groups, whereas paired *t* tests were used for comparisons within the UCLP and NC groups.**

Results: **Differences in variances of some mid-facial breadths and nasal bone dimensions were found in both male and female cleft groups when compared to the NC sample. In the UCLP group, some nasal bone and facial breadth dimensions were larger than in the NC sample and the nasal bone tended to deviate to the contralateral side of the cleft.**

Conclusion: **: CLP affects the size and orientation of the nasal bones and is associated with an altered morphology of some facial bones at positions distant from the region of the cleft.**

Keywords: *cleft lip, cleft palate, facial asymmetry, infant, radiology, three-dimensional imaging, tomography*

Introduction

Patients with orofacial clefts present with a variety of problems including dental anomalies, malocclusions, disorders of speech and hearing, and secondary facial deformities (1,2). Non-syndromic cleft lip, with or without cleft palate, is relatively common. It demonstrates a prevalence that ranges from 0.04 to 0.79 per 1000 live births (3) and 1 in every 500 to 550 live births, with the highest rates observed among the Asians (4). Although functional or iatrogenic factors are generally thought to affect normal facial morphology and growth potential (5,6), it is understood that there is an underlying genetic basis for the formation of clefts (7). Specifically, the *MSX1* gene has been associated with cleft palate, and the *MSX1* and *TGFβ3* genes have been associated with cleft lip, with or without cleft palate (7,8). Conversely, other researchers have found little evidence supporting these findings (9). Changes in facial growth and development in cleft children likely reflect the combined effect of genes and the environment; that is, clefts result from multifactorial influences that affect the growth potential of the face and the overall symmetry of the soft tissues and facial bones (5). Regardless of the pathogenesis or genetics, anomalous developmental conditions, such as cleft lip and palate (CLP), are often associated with increased levels of asymmetry, which have been described as fluctuating or directional asymmetry (10). Fluctuating asymmetry refers to small, random differences in size between sides

of the body, for example the face, and is thought to reflect developmental instability, whereas directional asymmetry involves a consistent trend in which one side is larger or smaller than the other and may be influenced by homeobox genes (10–12). The assessment of facial asymmetry is an important component of evaluating the success of surgical repair in CLP and is linked to psychological issues such as perceptions of attractiveness and intelligence (13). Therefore, the present study included an assessment of asymmetry by comparing landmark measurements from the left and right sides of the face.

Methodologically, cephalometric and panoramic radiographs have traditionally served as the primary option for the radiographic analysis of craniofacial morphology. However, there are limitations in the measurement of asymmetry using two-dimensional (2D) radiographs, such as the super-imposition of structures and the reliance on machine positioning relative to the external auditory meati, which can be asymmetric within individuals (14). Hence, three dimensional (3D) imaging techniques have been developed to overcome the shortcomings of conventional 2D methods and were applied in the present study; specifically, 3D computed tomography (CT) was used. Other available 3D imaging techniques include morphoanalysis, laser scanning, stereolithography, 3D ultrasonography, 3D facial morphometry, digigraph imaging, Moiré topography, and contour photography (1). Data obtained with 3D CT scanning can be used for soft and hard tissues analysis, whereas methods based on laser techniques are used mainly for the analysis of soft tissue surfaces. Consequently, 3D CT scanning was deemed most suitable for data collection in our study.

The overall aim of this study was to compare the craniofacial morphologies in a sample of unoperated Malaysian infants with CLP with those in a sample of age-matched, unaffected, non-cleft (NC) Malaysian infants. Differences in morphologies of the nasal bones were emphasised. A midline plane constructed from the landmarks basion (ba), sella (s), and nasion (n) was used to assess asymmetry in the selected craniofacial variables in both the CLP and the NC groups.

Materials and Methods

The Malaysian patient database established at the Australian Craniofacial Unit (ACFU), Adelaide Women's and Children's Hospital, provided the 3D CT scans of the subjects. The Malaysian cleft sample comprised 29 randomly selected individuals (12 females, 17 males): 10 with unilateral CLP (UCLP), 5 with bilateral CLP (BCLP), 7 with cleft lip and primary palate (CLPP), and 7 with isolated cleft palate (ICP). The control (NC) sample consisted of 12 Malaysian infants (4 females, 8 males) with no craniofacial abnormalities. Ideally, CT scans obtained from normal individuals would provide the ideal control group; however, the radiation dose involved in acquiring CT scans of healthy individuals cannot be justified. There should be sufficient medical and diagnostic reasons for performing a CT investigation. Hence, the NC subjects in the present study were patients with medical conditions that were sufficiently significant to justify the performance of CT scans (for example, meningitis and mild hydrocephalus). However, these conditions did not cause abnormalities in craniofacial growth and morphology (15), as confirmed by preliminary comparisons of the cranial base and facial dimensions of individuals with mild hydrocephalus and of other controls, which revealed estimates within the normal measurement range. All individuals included in the study were of Malay ethnicity. The age of the cleft patients ranged 1.1–12.2 months with a mean of 3.8 (SD 2.5) months, whereas the age for the NC group ranged 0.4–11.9 months with a mean of 4.8 (SD 2.8) months. Ethical approval was obtained from the Adelaide Women's and Children's Hospital Research Ethics Committee.

The Persona software package developed at the ACFU was utilised for 3D reconstruction of the craniofacial images and determination of the 3D coordinates of osseous landmarks on a silicon graphics computer workstation. This package enables the display of the CT scan data simultaneously around a 3D marker in windows showing axial, sagittal, and coronal sections, and it provides an accurate 3D reconstruction of the external craniofacial bones and the cranial base. The Persona software package enables the 3D positions of landmarks to be located with high precision, which allows the automatic generation of slices through selected points. The thickness of the scan data slices can vary 1.25–2.00 mm. Preliminary analyses using 68 landmark comparisons (61 distances, 7 angles) indicated the presence of random measurement errors ranging 0.2–1.1 mm for distances between landmarks, whereas the random errors for angular variables ranged 1.0°–2.7° (15). In general, the measurement errors were considered relatively small and unlikely to bias the results.

In the present study, 13 osseous landmarks

were located on the mid-facial region of subjects due to their close proximity to the clefts (Table 1, Figure 1) (16,17). A midline reference plane was created using the following landmarks: ba, s, and n (Figure 2). Breadth variables were then estimated by measuring the distances and angles between nasal osseous landmarks (Figure 3).

The influence of gender was investigated by comparing variables between male and female subjects in both cleft and NC samples. To explore the presence of any association between the side of the cleft and the direction of nasal bone deviations, the UCLP and NC samples were compared as follows: bilateral variables that coincided with the location of the cleft were measured, and asymmetry was assessed by subtracting the ipsilateral from the contralateral measurements.

The data were screened and subsequently corrected for outliers when necessary. Double determinations were performed to assess the magnitude of any systematic or random errors, and Dahlberg statistics were calculated for each variable (18).

Although the 2 groups were as closely matched for age as possible, the age range in the cleft group was slightly greater than that in the NC group. Additional age adjustments were applied to the data following the regression analyses of each variable against age in both the cleft and NC samples. Hence, all of the presented data are age-adjusted.

Comparison of the mean values and variances between male and female cleft and NC groups were performed using unpaired t tests and F tests with a significance level of $P < 0.05$. Comparisons between measurements on right and left sides of the face within the UCLP group and within the NC sample were conducted using paired t tests. The R (R Foundation for Statistical Computing, Vienna, AT) and Excel (Microsoft Corporation, Redmond, WA, US) statistical programmes were used to analyse the collected data.

Results

Male cleft and NC samples

Table 2 shows selected landmark distances which revealed the greatest differences in mean values between male cleft and NC samples. Cleft males exhibited greater distances from mid-face landmarks (snml, orl, gol, ztl, ztr, and ofl) to the midline reference plane (na–s–ba) and greater breadth distances (ofl–ofr, gol–gor, and ztl–ztr) than did NC males but none of these differences in mean values was significant statistically. However, 5 of the variables (gol, ztl, ztr, gol–gor, and ztl–ztr) displayed significantly unequal variances ($P < 0.05$), with variances in NC males exceeding those in cleft males.

Female cleft and NC samples

Table 3 presents selected landmark distances and angles which showed the greatest differences in mean values between female cleft and NC samples. All variables were larger in the female cleft group than the NC group, including the distances from mid-face landmarks to the midline plane (inmr, eul, gor, mal, pol, and ztr) and the mid-facial breadths (mal–mar). Dimensions of the nasal bone that showed the largest differences between the female cleft group compared with the NC group were na–n and inmr. Angulations depicted by the variables snml–n–snmr and inml–na–inmr were also larger in the female cleft group compared with the NC group. None of these differences in mean values was significant statistically, although the difference in mean values for inmr to the midline plane and snml-n-snmr both reached borderline significance ($P = 0.05$). Five of the variables (eul, mal, ztr, mal–mar, and n–na plane) showed significant heterogeneity in their variances ($P < 0.05$ each), with variances in NC females exceeding those in cleft females for all variables except n-na plane. . The nasal bone in the female cleft group showed a significant deviation to the right compared with the NC sample ($P < 0.05$) and this angle also showed significantly greater variability in the cleft group compared with the NC group ($P < 0.05$).

UCLP and NC samples

Comparison of the UCLP and NC samples (prior to consideration of the cleft location) revealed several statistically significant differences in mean landmark distances, as reported in Table 4. All nasal bone dimensions were larger in the UCLP group than the NC group, with significant differences in dimensions na–n, snmr–n, and snml–snmr ($P < 0.05$ each). Facial breadth distances were also larger in the UCLP group than the NC group, with significant differences in dimensions orl–orr and ztl–ztr ($P < 0.05$ each).

Table 5 presents the bilateral variables that were associated with significant differences between right and left sides of the face when the location of the cleft was considered. To determine cleft-side (ipsilateral) to non-cleft-side (contralateral) dimensional differences,

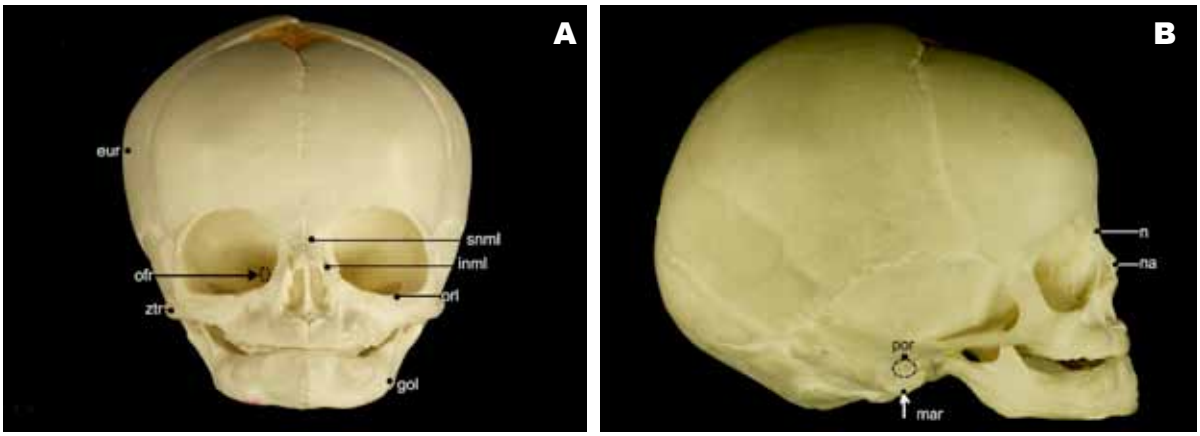

Figure 1: (A) Frontal and (B) right lateral views of a skull depicting osseous landmarks described in Table 1 (excluding sella and basion)

Table 1: Description of the 13 osseous landmarks identified on three-dimensional computed tomography scans (16,17)

Landmark	Abbreviation	Definition
Nasale	na	Tip of the nasal bone
Superior naso-maxillare l/r	snml/snmr	Most superior point on the naso-maxillary suture
Inferior naso-maxillare l/r	inml/inmr	Most inferior point on the naso-maxillary suture
Nasion	n	Most anterior point on the fronto-nasal suture (when the suture was not clearly identifiable, the deepest point on the nasal notch was substituted)
Sella	s	Centre of the sella turcica
Basion	ba	Mid-sagittal point on the anterior margin of the foramen magnum (at the saddle point)
Gonion l/r	gol/gor	Point on the angle of the mandible located by bisection of the angle formed by the mandibular line and the ramus line
Orbitale l/r	orl/orr	Most inferior point on the infraorbital margin
Zygo-temporale l/r	ztl/ztr	Mid-point of the bony concavity formed between the frontal and temporal processes of the zygomatic bone
Optic foramen l/r	ofl/ofr	Centre of the anterior opening of the optic canal
Porion l/r	pol/por	Most superior point on the margin of the external auditory meatus
Mastoidale l/r	mal/mar	Most inferior point on the mastoid process
Euryon l/r	eul/eur	Most lateral point on the skull

Letters l and r denote left and right, respectively.

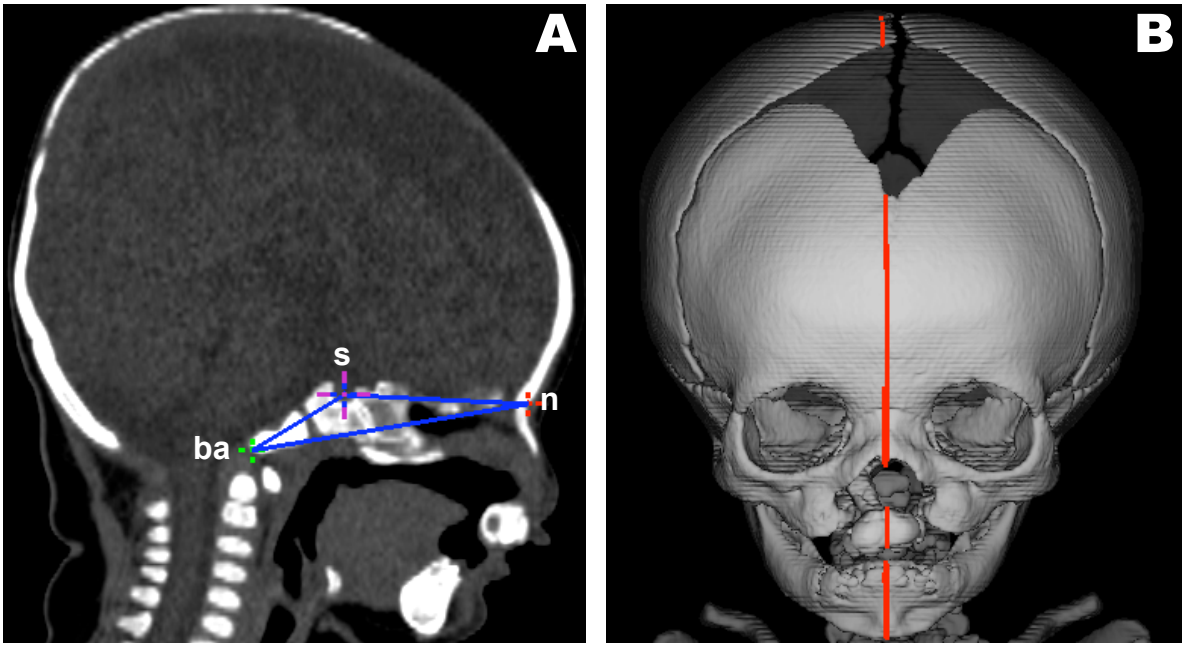

Figure 2: Computed tomography images of the reference plane constructed from 3 osseous landmarks: nasion (n), sella (s), and basion (ba). (A) Sagittal view of "wire frame" constructed midline reference plane. (B) Frontal view of reference plane bisecting a BCLP patient.

measurements for the ipsilateral side of the cleft were subtracted from those obtained for the contralateral side of the cleft. The results showed that the distances from the ipsilateral zt and the contralateral zt to the midline reference plane were significantly different ($P < 0.05$), with a larger distance measured on the contralateral side of the cleft. Additionally, a significant degree of deviation ($P < 0.001$) was observed for the nasal bone variable na–n, which deviated away from the cleft side. In the NC sample, no significant differences were detected between the left and right sides of the face.

Discussion

Despite several growth theories (19), our understanding of the cellular and molecular control mechanisms involved in human craniofacial development remains incomplete. It is believed that during the course of normal craniofacial development, the histogenesis and functional maturity of muscles, nerves, and vessels may influence one another (19). Abnormal craniofacial development, such as clefting, is also likely to influence the growth and development of adjacent facial and dental structures, which can result in noticeable alterations in facial shape and symmetry. By comparing landmark variables

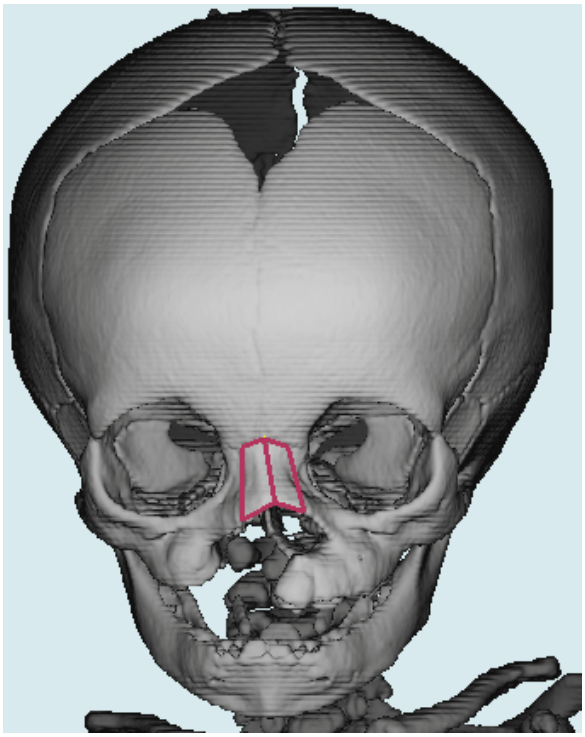

Figure 3: Frontal view of three-dimensional computed tomography image of nasal bone "wire frame".

Table 2: Descriptive statistics for selected variables in male non-cleft (NC) and cleft groups

	Males NC (n = 8)			Males Cleft (n = 17)			
	Mean	SEM	SD	Mean	SEM	SD	P value
Distance from landmark to midline plane, nas–ba (mm)							
Snml (nasal bone)	- 3.64	0.33	0.93	- 4.52	0.28	1.15	NS
Orl	- 16.81	0.64	1.81	- 18.01	0.37	1.53	NS
Gol	- 26.59	1.37	3.87	- 28.52	0.56	2.31	0.04*
Ztl	- 37.15	1.72	4.86	- 39.32	0.63	2.60	0.02*
Ztr	37.44	1.77	5.01	39.47	0.64	2.64	0.02*
Ofl	- 7.90	0.42	1.19	- 8.77	0.23	0.95	NS
Breadth distance (mm)							
Ofl–ofr	16.05	0.72	2.04	17.24	0.43	1.77	NS
Gol–gor	53.05	2.51	7.10	56.58	0.97	4.00	0.03*
Ztl–ztr	74.64	3.49	9.87	78.94	1.24	5.11	0.01*

Positive mean values indicate the right side of the skull, while negative mean values indicate the left side of the skull. *$P < 0.05$ indicates significant difference and NS indicates non-significant difference $(P > 0.05)$ in variances between NC and cleft groups by F test.
Abbreviations: gol = gonion left, gor = gonion right, ofl = optic foramen left, ofr = optic foramen right, orl = orbitale left, snml = superior naso-maxillare left, ztl = zygo-temporale left, ztr = zygo-temporale right.

between cleft and NC individuals by gender, it was possible to explore the dimensional impact of clefting on the adjacent facial structures and to assess whether clefting affects males and females differently. Hence, our findings provide information that is important for practising dentists, who play an important role within the multidisciplinary team of health professionals that manage cleft patients.

Earlier research on sex differences in CLP has demonstrated little variation in the craniofacial morphology of infants (20) or children aged 6 to 10 years (21,22). Our study demonstrated that mean measurements tended to be larger in the cleft sample than the NC sample, for both males and females. There were also some variables that showed significant heterogeneity in variances between cleft and NC samples for both sexes. Mid-facial breadths in the combined cleft sample revealed that an orofacial cleft may influence facial growth away from the immediate cleft location and contribute to asymmetry. Asymmetry in cleft patients has been reported in the orbital, maxillary and nasal regions (23). Similar results were obtained in the present study; the left optic foramen and orbitale in the male sample tended to be further from the midline compared with the contralateral side. There was also some evidence that the zygoma bone in the mid-face region may be affected, which indicates a possible direct influence of the cleft on horizontal mid-facial breadths in comparison with unaffected individuals. We found that regardless of the cleft type, the mandible showed some tendency to be larger in the cleft sample compared with the NC sample, which differs from previous research (24). In general, clefts can influence facial growth away from the immediate cleft location, and these changes in facial morphology may subsequently influence oral function and alignment and growth of the dentition.

Very few studies have reported on the size and orientation of the nasal bones in CLP patients using either radiographs (25) or 3D CT (26), and to our knowledge, no studies have provided results concerning asymmetry. We found that males with clefts tended to show larger superior portions of the left nasal bone and greater left mid-facial breadths compared with the NC group, which suggested potentially left-dominant facial growth. In females, clefts had a somewhat different effect on nasal bone morphology, which tended to be larger superiorly and deviated to the right with a flatter and longer shape. This

morphology suggested a possible effect of the cleft on the prominence of the nasal bridge. Results reported in the literature investigating nasal bone morphology range from reports of considerably shorter nasal bones in subjects with cleft lip compared with subjects with cleft palate (25), to longer nasal bones in cleft patients from 6 years of age through to adulthood compared with non-cleft individuals (26). A combination of the cleft location together with normal lateral growth of the frontal bone and maxilla may explain the increase in nasal bone angulation observed superiorly. It is possible that the inferior dimensions of the nasal bone are less affected by CLP because they form the superior portion of the nasal cavity and are therefore influenced to a lesser degree by the surrounding craniofacial bones. Furthermore, the facial muscles may affect the growth and deviation of facial bones, including the nasal bones (19). It is important to bear in mind that there are differences in craniofacial morphology among ethnic groups and caution is needed in extrapolating findings from one population to another. Nevertheless, head breadth dimensions in Malaysian infants in the 0–1 age group are similar to those reported for Caucasians (15).

Analysis of the UCLP group showed that severe clefts together with dominant lateral growth of the skull resulted in a number of significant differences between the UCLP and the NC groups. These findings are supported by previous research with respect to transverse asymmetry in individuals with UCLP (27,28,29). Nasal bone lengths in UCLP tended to be longer in both vertical and horizontal dimensions compared with the NC group. This result is supported by evidence showing that UCLP individuals have a high frequency of disproportionately wide noses in relation to the nose height both pre- and post-surgical treatment (30), whereas other

Table 3: Descriptive statistics for selected variables in female non-cleft (NC) and cleft groups

	Females NC (n = 4)			Females Cleft (n = 12)			
	Mean	**SEM**	**SD**	**Mean**	**SEM**	**SD**	***P* value**
Distance from landmark to midline plane, na-s-ba (mm)							
Inmr (nasal bone)	4.58	0.42	0.84	5.86	0.37	1.28	NS
Eur	- 51.43	4.57	9.14	- 54.66	1.31	4.54	0.04*
Gor	24.30	1.81	3.62	25.86	0.66	2.29	NS
Mal	- 30.18	3.57	7.14	- 32.40	0.84	2.91	0.01*
Pol	- 31.35	2.42	4.84	- 33.24	0.82	2.84	NS
Ztr	34.20	2.87	5.74	36.47	0.81	2.81	0.03*
Breadth distance (mm)							
Mal-mar	59.83	6.46	12.92	63.42	1.60	5.54	0.02*
Nasal bone distance (mm)							
Na–n	10.03	1.46	2.92	11.41	0.47	1.63	NS
Angle (°)							
Snml–n–snmr	123.03	5.86	11.36	140.50	4.50	15.59	NS
Inml–na–inmr	107.10	7.39	14.78	120.58	2.58	9.80	NS
Nasal bone deviation (°)							
n–na plane	- 4.00	0.65	1.30	2.00	1.52	5.27	<0.001**

Positive mean values indicate the right side of the skull, while negative mean values indicate the left side of the skull. *P < 0.05 indicates significant difference, **P < 0.001 indicates highly significant difference, and NS indicates non-significant difference (P > 0.05) in variances between NC and cleft groups by F test.
Abbreviations: eul = euryon left, gol = gonion left, gor = gonion right, inml = inferior naso-maxillare left, inmr = inferior naso-maxillare right, mal = mastoidale left, mar = mastoidale right, n = nasion, na = nasale, pol = porion left, snml = superior naso-maxillare left, snmr = superior naso-maxillare right, ztr = zygo-temporale right.

researchers have documented that children with UCLP have significant nasal asymmetry that persists after primary surgery (13). In the present study, a significant degree of nasal bone deviation away from the cleft was detected in the UCLP group.

The 3D CT technology employed in this study provides more accurate and reliable measurements compared with earlier methodologies that utilise coronal cephalometric or panoramic radiographs. These methods are limited due to super-imposition; for example, landmarks that are positioned more posteriorly, such as s and ba, may be difficult to locate due to overlap with more anteriorly positioned anatomical structures. Furthermore, cephalometric results rely on positioning the radiographic unit relative to the external auditory meati, which can exhibit intra- and inter-individual variations. Although there are advantages in using the 3D CT methodology, technological advances lead to the loss of some comparability between studies with software

Table 4: Descriptive statistics for nasal bone and facial breadth dimensions in cleft lip and palate (UCLP), without considering the side of the cleft, and non-cleft (NC) control groups

	UCLP (n = 10)			NC (n = 12)			
	Mean	SEM	SD	Mean	SEM	SD	*P* value
Nasal bone distance (mm)							
Na–n	12.58	0.44	1.39	10.66	0.58	2.01	0.02*
Inml–snml	13.43	0.51	1.61	11.70	0.61	2.11	NS
Inmr–snmr	13.27	0.50	1.58	11.90	0.57	1.97	NS
Snml–n	5.52	0.46	1.45	4.48	0.38	1.32	NS
Snmr–n	5.61	0.48	1.52	4.33	0.33	1.14	0.03*
Snml–snmr	10.50	0.91	2.88	7.79	0.62	2.15	0.02*
Breadth distance (mm)							
Orlorr	36.72	1.24	3.92	33.28	0.94	3.26	0.04*
Gol–gor	57.11	1.40	4.43	52.09	2.11	7.31	NS
Mal–mar	68.85	1.28	4.05	63.93	3.19	11.05	NS
Ztl–ztr	80.49	1.60	5.06	72.87	3.01	10.43	0.04*

*P < 0.05 indicates significant difference and NS indicates non-significant difference (P > 0.05) in mean values between NC and UCLP groups by unpaired t test.
Abbreviations: gol = gonion left, gor = gonion right, inml = inferior naso-maxillare left, inmr = inferior naso-maxillare right, mal = mastoidale left, mar = mastoidale right, n = nasion, na = nasale, orl = orbitale left, orr = orbitale right, snml = superior naso-maxillare left, snmr = superior naso-maxillare right, ztl = zygo-temporale left, ztr = zygo-temporale right.

Table 5: Mean distances that demonstrated statistically significant differences between ipsilateral and contralateral sides in the UCLP group (n = 10)

	Mean	SEM	SD	P value
Nasal bone distance (mm)				
Na–n	-7.97	0.88	2.78	<0.001*
Mid-facelandmark distances (mm)				
Zt–zt	0.72	0.31	0.98	0.045*

Positive mean value indicates the ipsilateral side of the cleft, while negative mean value indicates the contralateral side of the cleft. * P < 0.05 indicates significant difference and **P < 0.001 indicates highly significant difference in mean values between ipsilateral and contralateral sides in the UCLP group by paired t test.
Abbreviations: n = nasion, na = nasale, zt = zygo-temporale.

updates, e.g., differences in the definitions and identification of landmarks between different software programs.

A relatively small sample size and pooling of the different types of clefts for some of the analyses present further limitations to the present study. However, considering the difficulties involved in obtaining samples from unoperated CLP patients for whom CT scans are available, we think that the sample size is acceptable. It is expected that infants with an isolated cleft palate ($n = 7$) are more likely to demonstrate facial morphologies that are more symmetric than those of infants with UCLP. Hence, analyses that explored differences in facial asymmetry between cleft and NC groups (Tables 4 and 5) were not based on pooled cleft data and included only UCLP infants from the cleft group. Given that the aims of the present study were to compare craniofacial morphology, including asymmetry, between samples of unoperated infants with CLP (regardless of the cleft type) and a control NC sample of unaffected infants, we consider the aims to have been adequately met by the pooling of cleft types for some but not all of the presented analyses. An additional issue related to the sample is the age distribution in the cleft and NC groups. The age range in the cleft group was slightly greater, i.e., 1.1–12.2 months and a mean of 3.8 (SD 2.5) months, than that in the NC group, i.e., 0.4–11.9 months and a mean of 4.8 (SD 2.8) months. A few older children were included in the cleft group because their primary operation had been postponed due to other health problems, such as upper respiratory tract infection and aspiration pneumonia. Although this represents a limitation of the present study, the cleft and NC groups were age-matched as closely as possible. They demonstrated very similar age distributions, means, and SDs, and all of the presented data were age-adjusted.

Our assumption that the midline points (n–s–ba) can reliably represent a mid-facial plane that divides the face into two equal halves has been drawn from the literature (27,31); however, this may be debatable. For example, the spatial position of n could be affected by the type of cleft, and those of ba and s may be affected in subjects with hydrocephalus. Therefore, preliminary analyses were conducted and revealed that the positions of the 3 landmarks were apparently not significantly affected by abnormalities in craniofacial morphology in either the cleft or NC groups. A number of investigators have reported significant differences in the size and shape of the cranial base of individuals with CLP compared with NC individuals. In contrast, very few differences in post-natal cranial base morphology and growth have been noted between individuals with isolated cleft lip and NC individuals. In the present study, the cranial base values (n–ba and n–s–ba) did not differ significantly between the groups. Although not demonstrated in our study, a trend toward a greater cranial base length in NC individuals compared to cleft individuals has been reported previously (32).

Conclusion

Differences in mid-facial breadths and nasal bone dimensions were associated with clefting (UCLP versus NC). The nasal bones of individuals in the UCLP group deviated away from the cleft. It is important for members of the multidisciplinary team that manages cleft patients to have an understanding of how clefts affect not only dental and oral structures but also other surrounding anatomical structures. This study shows that CLP affects the size and orientation of the nasal bones and is also associated with alterations in the morphology of other facial bones at positions distant from the region of the cleft.

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Authors' contributions

Conception and design: NT, ZR, AY, GT
Analysis and interpretation of the data: NT, SM, GT
Drafting of the article: NT, SM, PJA, GT
Critical revision of the article: SM, PJA, GT
Final approval of the article: NT, SM, ZR, AY, PJA, GT
Provision of patients, collection and assembly of data: ZR, AY
Statistical expertise: GT
Obtaining of funding: NT, GT

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Original Article

Risk Factors and Phenytoin Prophylaxis for Early Post-Traumatic Seizures among Patients with Traumatic Brain Injury

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Abstract

Background: **Post-traumatic seizure is a well-known and serious complication of traumatic brain injury (TBI). The incidence and risk factors vary among study populations. Very little data have been published concerning this in the Malaysian population. The aim of this study was to ascertain the risk factors for the development of early post-traumatic seizures among patients with TBI.**

Methods: **This was a prospective observational study, carried out in Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, under the Department of Neurosciences. A total of 157 patients, from all age groups, who were diagnosed with TBI were enrolled from June 2007 to December 2007. They were followed-up for 12 months until death or their first post-traumatic seizure. Survival analysis with Kaplan–Meier curves and Cox proportional hazards regression was performed.**

Results: **A total of 11 (7.0%) of the patients developed early post-traumatic seizures. The risk factors for early post-traumatic seizures were young age (P = 0.021, 95% CI 0.806 to 0.982) and intubated patients (P = 0.029, 95% CI 1.194 to 25.913). The incidence of early post-traumatic seizures in the local population was 7.0%.**

Conclusion: **The incidence of early post-traumatic seizures in the local population of Kelantan and Terengganu is comparable to the incidences reported elsewhere. Younger as well as intubated patients were at a higher risk of developing this condition. It may be necessary to give antiepileptic prophylaxis because any seizure could adversely affect morbidity and mortality. However, the study showed that antiepileptic drug was not beneficial in preventing late post-traumatic seizures, but may have a role in preventing early seizures.**

Keywords: *post-traumatic epilepsy, traumatic brain injury, head injuries, incidence, risk factors, phenytoin*

Introduction

Traumatic brain injury (TBI) poses a major health and socioeconomic problem throughout the world today (1). One of the important but poorly understood sequelae of TBI is post-traumatic seizures. Post-traumatic seizures can happen either early (occurring within 1 week of the injury) or late (occurring from 1 week to years after the injury). Recurring late seizures make up the clinical syndrome of post-traumatic epilepsy (2–4). The significance of an early post-traumatic seizure lies in the fact that a seizure attack within the acute stage may result in cerebral hypoxia, increased intracranial pressure (ICP) and metabolic demand, an increased release of neurotransmitters, and, thereby, a higher incidence of mortality and morbidity due to secondary brain damage (5,6). Reported risk factors for the development of seizures in the first week after injury include acute intracerebral haematoma (especially subdural haematoma), younger age, increased severity of injury and chronic alcoholism (4).

Although early post-traumatic seizure is an important sequelae of TBI with potentially

deleterious effects to life and recovery, to date, there are very little data concerning incidence and risk factors among the local population. Ong et al. (7) reported an incidence of 5.5% of early seizures in the paediatric population of the Kuala Lumpur General Hospital. The objectives of this study were to determine the incidence and the relevant clinical and patient-related parameters that may contribute to the time in the development of early post-traumatic seizures.

Materials and Methods

This was a prospective observational study carried out in Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan, Malaysia. The study participants were patients who were admitted with a diagnosis of head injury from June 2007 to November 2007, except for those who met any of the following exclusion criteria:

1. Patients who were known epileptics
2. Patients with a prior history of head trauma
3. Patients with severe ischaemic heart disease, chronic renal failure, uncontrolled diabetes, hypertension, or other serious medical problems that theresearchers deemed would adversely affect the results of the study
4. Patients who were not expected to survive 1 year due to the severity of their injuries
5. Pregnant patients
6. Patients with a previous history of neurosurgical procedures
7. Patients with other non-TBI neurological diagnoses, such as brain tumours, stroke, aneurysms, or diabetic coma

TBI was defined as an injury from external causes resulting in either a transient or a prolonged reduced level of consciousness, post-traumatic amnesia, or abnormal findings on computed tomography (CT) of the head. All of the patients who were included in the study were initially treated with the standard treatment protocols for managing head injuries as practised in HUSM.

The relevant clinical data for the patients on admission and their subsequent progress were recorded. The CT scan findings were taken as those that were reported by the admitting neurosurgical resident and confirmed by senior lecturers. The site or multiplicity of intracranial haematomas or contusions were not differentiated. A baseline electroencephalogram (EEG) was scheduled for each patient during the course of the admission. The occurrence of any surgical procedure was also recorded. Surgical procedures were subdivided into craniotomy and craniectomy, insertion of ICP monitoring device or extraventricular drainage (EVD) catheter, and other non-neurosurgical procedures. For those who had more than 1 procedure, the more major procedure was recorded; for example, if a patient had both decompressive craniectomy and ICP monitor insertion, it was recorded under decompressive craniectomy. Patients were monitored continuously during admission for any seizure activity.

Data analysis was completed using SPSS version 12.1 (SPSS Inc., Chicago, IL). The significance level was set at $P < 0.05$. For the analysis of the risk factors, Cox proportional hazards regression was used to obtain the hazards ratio. This model was used because of the multiple covariates that were involved in the analysis. Simple Cox regression analysis was first used to determine which variables were significant, and these variables were then included in the multivariate analysis. The variables analysed were as follows: age; gender; severity of head injury on admission; clinical conditions on admission, such as respiratory distress, intubation, unequal pupils, basal skull fracture, other systemic injuries, as well as open or closed skull fractures; mean arterial pressure MAP on admission; haemoglobin, serum sodium and potassium levels; surgical intervention; phenytoin treatment; and CT scan findings, including diffuse axonal injury, traumatic subarachnoid haemorrhage, linear or depressed skull fracture, subdural haemorrhage (SDH), contusion, and extradural haemorrhage (EDH). Following the simple Cox proportional hazard regression analysis for single variables, the variables were selected for multiple regression analysis based on a P value of less than 0.05. The covariates were then entered together, with the first category as baseline, a 95% confidence interval for the Hazard Ratio, and the Backward Likelihood (LR) test statistic as the automatic model selection method.

Results

A total of 157 patients were recruited in the study: 137 were male (87.3%), 20 were female (12.7%), and the male to female ratio was 6.85:1. The demographic and clinical characteristics of the patients are summarised in Table 1. The age of the patients ranged 2–87 years, with a mean (SD)

Table 1: Demographic and clinical characteristics of the patients (n = 157)

Demographic and clinical characteristics	*n*	%
Age group (years)		
0–12	23	14.6
12–20	67	42.7
20–40	41	26.1
40–65	24	15.3
>65	2	1.3
Sex		
Male	137	87.3
Female	20	12.7
Severity of head injury		
Mild	59	37.6
Moderate	35	22.3
Severe	63	40.1
Type of surgical procedures		
Craniotomy/craniectomy	60	78.9
EVD/ICP*	2	2.6
Non-neurosurgical procedure	14	18.4
Clinical conditions on admission		
Respiratory distress	11	7.0
Intubated	69	43.9
Unequal pupils	22	14.0
Basal skull fracture	25	15.9
Other systemic injuries	29	18.9
Open skull fracture	21	13.4
Closed skull fracture	36	22.9
Computed tomography finding		
Normal	1	0.6
SDH	24	15.3
DAI	40	25.5
Linear skull fracture	47	29.9
tSAH	21	13.4
EDH	15	9.6
Contusion > 1 cm	42	26.8
Depressed skull fracture**	21	13.4
Midline shift > 1 cm	15	9.6
Basal cisterns effaced	30	20.4
Type of post-traumatic seizures		
Focal	1	0.9
Generalised tonic–clonic	10	90.9

EVD/ICP group refers to patients with pure EVD and ICP monitor insertion. Those who had EVD or ICP in addition to craniotomies or craniectomies were included in the former category. ** Patients with depressed fractures more than 1 table thickness were not differentiated. Abbreviation: DAI = diffuse axonal injury, EDH = extradural hemorrhage, EVD = external ventricular drainage, ICP = intracranial pressure , tSAH traumatic subarachnoid haemorrhage

age of 24.5(16.1) years. The majority of patients (42.7%) were in the 12–20 years age group. The most frequent admission Glasgow Coma Scale (GCS) was 7, in (17.8%) of patients. The patients were then divided into different categories of mild (GCS 13–15), moderate (GCS 9–12) and severe (GCS 3–8) head injury. The majority of the patients (n = 63, 40.1%) had severe head injury, while of those remaining, 59 (37.6%) had mild head injury and 35 (22.3%) had moderate head injury.

The number of patients who underwent surgery was roughly the same as those who had non-surgical treatment, 81 (51.6 %) for no surgery versus 76 (48.4 %) for surgery. The patients who underwent surgical procedures were further categorised into types of surgical procedures. Of the 76 patients, 60 (78.9%) had either a craniotomy or craniectomy; 2 (2.6%) had EVD or ICP monitor insertion, while the remaining 14 (18.4%) had a non-neurosurgical procedure performed (such as plating of a fractured limb and maxillofacial procedures). Several other clinical conditions on admission were examined and recorded, namely presence of respiratory distress, status of intubation, unequal pupils, basal skull fractures, other systemic injuries, and open or closed skull fractures. CT scans of brain were done on all patients. The following characteristics were looked for: normal, subdural hemorrhage (SDH), diffuse axonal injury DAI, linear skull fracture, traumatic subarachnoid hemorrhage tSAH, extradural hemorrhage (EDH), contusion (more than 1 cm), depressed skull fracture, midline shift (more than 1 cm), and basal cisterns effaced.

Of the 157 patients recruited in the study, 3 patients died before completing 1 year of follow-up: 1 each at 3 weeks after admission, after 2 months, and 3 months after the trauma. Initial baseline EEGs were performed in 80 cases, but no significant positive finding was observed. Of the 157 patients in the study, 11 (7.0%) developed early post-traumatic seizures (diagnosed based on clinical symptomatology and features): 10 (90.9%) had generalised tonic-clonic seizures, whereas 1 (0.9%) had focal seizures. Only 1 (9.1%) of these 11 patients went on to develop late post-traumatic seizures.

The range and mean values of the patients' mean arterial pressure (MAP) on admission, interval between trauma and admission to HUSM, lowest haemoglobin (Hb), serum sodium, and serum potassium are summarised in Table 2. For the analysis of the prognostic factors, the variables analysed were age, gender, severity of head injury on admission, clinical condition(s) on admission (such as respiratory distress, intubation, unequal pupils, basal skull fracture, other systemic injuries, and open or closed skull fractures), MAP on admission, haemoglobin, serum sodium and potassium levels, surgical intervention, phenytoin treatment, and CT scan findings, (including diffuse axonal injury, traumatic subarachnoid haemorrhage, linear or depressed skull fracture, SDH, contusion, and EDH). The P values for all of these individual covariates as predictors of late post-traumatic seizures are summarised in Table 3.

Following the simple Cox proportional hazard regression analysis for the single variables, 4 variables (age, severity of head injury, MAP on admission, and intubated on admission) were selected for the multiple regression analysis based on a P values of less than 0.05. Based on this final model, there were 2 significant risk factors in the development of early post-traumatic seizures: age (P = 0.021) and intubated patients (P = 0.029), as shown in Table 4.

Discussion

TBI is a major cause of morbidity and mortality worldwide, with an ever-rising trend. Severe head injury remains one of the leading causes of death and permanent disability in the

Table 2: The range and mean values for some of the patients' clinical parameters (n = 157)

Parameters	Range	Mean (SD)
Admission MAP (mmHg)	46.00–133.00	90.43 (14.73)
Interval between trauma and admission (hours)	0.25–24.00	5.31 (5.32)
Lowest haemoglobin (g/dL)	7.10–19.70	11.00 (2.36)
Serum sodium (mmol/L)	114.00–148.00	135.80 (4.59)
Serum potassium (mmol/L)	1.60–5.60	3.38 (0.57)

Abbreviation: MAP = mean arterial pressure

young and productive age group in Malaysia, and causes a great burden and great economic loss to both the family and the country. The total number of road traffic accidents in Malaysia exceeded 223 000 in 1999 with an average of 16 deaths per day (8). In the Hospital Universiti Sains Malaysia, a total of 204 cases of head injury were admitted to the Neurosurgical Department in 2003, rising to 226 cases in 2004 and 231 cases in 2005. There were only 187 cases of head injury in 2006. However, the total rose to 189 cases in 2007 (9).

Post-traumatic seizures can either be early, occurring within 1 week of the injury, or late, occurring from 1 week to years after the injury. Immediate seizures, occurring less than 24 hours after injury, is a third category (10,11). Recurring late seizures make up the clinical syndrome of post-traumatic epilepsy (2–4). Immediate and early post-traumatic seizures are important complications of head injury that need to be detected and treated, as seizure activity in this early period after a head injury can cause secondary brain damage due to increased metabolic demands, increased ICP, and excess neurotransmitter release (12).

The incidence of early seizures ranges from 4%–25%, according to different references (3,5,13,14). In a study of 966 children, Ong et al. found the incidence of early post-traumatic seizures to be 5.5% (7). The 7.0% incidence of early post-traumatic seizures seen in this study is comparable with that from most series. While the study of Ong et al. focused mainly on the paediatric population (7), this study included all age groups, although its finding was that younger patients are more susceptible to early post-traumatic seizures.

The risk factors for developing early post-traumatic seizures have also been studied in many case series. Early post-traumatic seizures could also be related to age, with Black et al. reporting a higher incidence in patients in the 2–14 years age group (3). Other risk factors reported include age greater than 65, chronic alcoholism, fractures at the base of the skull, and intracranial operations (13,15,16). In Ong et al.'s study, the significant risk factors were age less than 2 years, female sex, loss of consciousness for more than 24 hours, and acute subdural haematoma (7). The results from this study show that for early post-traumatic seizures, the risk factors are younger age and intubated patients. In our results, for every increase of 1 year in age, the hazard of developing an early post-traumatic seizure was 0.89. However, no age group or age limit was found for this risk. The other group of patients at higher risk for early post-traumatic seizures was those who were intubated at admission. The main indications for intubation were a GCS of 8 and below (48 of 69) and respiratory distress (10 of 69) and chest injury (1 of 69). This group of patients were found to be more severely injured (81.0% had a GCS score of 3–8). About half (53.4%) of the patients who were intubated also underwent surgery, with craniotomies and craniectomies being the most common operations (74.1%). This result could indicate that the severity of the head injury and open cranial surgery could be related to the development of early seizures. Furthermore, about 20.0% of these patients were intubated due to respiratory distress or severe chest injury. This may indicate that hypoxia could also play a role in the development of early post-traumatic seizures. However, as pre-intubation arterial blood gases were not available, this relationship could not be ascertained and could be included in future studies. Other conditions (such as subdural haematomas, contusions, skull fractures, or contusions) were not significant in this study.

As mentioned earlier, treatment of early and late post-traumatic seizures can have an important bearing on outcome. Adequate control of early seizures can help prevent secondary brain damage, while control of recurring late seizures can improve a patient's rehabilitation and reintegration into society. Numerous regimens and drugs have been studied and proposed in this respect, with some contrasting results. Temkin et al. found that phenytoin and carbamazepine are effective in preventing early seizures, but not effective in preventing late seizures (12). Pechadre et al. treated 34 out of 86 patients with severe head injuries with a loading dose of intravenous phenytoin followed by oral phenytoin administration for at least 3 months (17). After 2 years follow-up, there was a significant difference between the treated and untreated patients (17). Murri et al. found that phenobarbital at 1.5 mg/kg/day had an efficient prophylactic effect against late post-traumatic seizures (18). Schierhout and Roberts did a systematic review of randomised controlled trials on post-traumatic seizure prophylaxis and concluded that prophylactic antiepileptic drugs are effective in reducing early seizures, but have no benefit against late seizures or death and neurological disability (19). In 2003, the American Academy of Neurology issued a practice parameter for the use of antiepileptic drug prophylaxis in severe TBI. The conclusions were that in adult patients with severe head injury, phenytoin prophylaxis is effective in decreasing the risk of early post-traumatic seizures. However, antiepileptic drug prophylaxis is probably not

Table 3: Analysis of the prognostic factors for early post-traumatic seizures (n = 157)

Variable		*P* values
Age		0.014*
Gender	Female	-
	Male	0.769
Severity of head injury	Mild	-
	Moderate	0.150
	Severe	0.052
Mean arterial pressure		0.038*
Respiratory distress	No	-
	Yes	0.791
Intubated	No	-
	Yes	0.018*
Basal skull fracture	No	-
	Yes	0.637
Other systemic injuries	No	-
	Yes	0.442
Unequal pupils	No	-
	Yes	0.190
Open skull fracture	No	-
	Yes	0.432
Closed skull fracture	No	-
	Yes	0.840
Anaemia	No	-
	Yes	0.231
Hyponatraemia	No	-
	Yes	0.312
Hypokalaemia	No	-
	Yes	0.942
Subdural haematoma	No	-
	Yes	0.599
Diffuse axonal injury	No	-
	Yes	0.621
Linear skull fracture	No	-
	Yes	0.753
Traumatic subarachnoid haemorrhage	No	-
	Yes	0.432
Extradural haematoma	No	-
	Yes	0.174
Traumatic contusion	No	-
	Yes	0.525
Depressed skull fracture	No	-
	Yes	0.769
Midline shift > 1 cm	No	-
	Yes	0.939
Basal cisterns effaced	No	-
	Yes	0.392
Surgical intervention	No	-
	Yes	0.215

*P < 0.05 indicates significance by simple Cox proportional hazards regression

Table 4: Prognostic factors for early post-traumatic seizures (n = 157)

Variable	Range	Mean (SD)
Age	0.89 (0.81, 0.98)	0.021*
Intubated		
No	1.00	-
Yes	5.56 (1.19, 25.91)	0.029*

* P < 0.05 indicates significance by multiple Cox proportional hazard regression

effective in decreasing late post-traumatic seizures (20). The results of this study are comparable to the above-mentioned recommendations, in that phenytoin was not found to be an effective as a prophylaxis against late post-traumatic seizures.

Conclusion

This study showed that the incidence of early post-traumatic seizures in the local population of Kelantan and Terengganu was 7.0%, which is comparable to incidence rates elsewhere. Younger and intubated patients were at a higher risk of developing early post-traumatic seizures. Because any seizure event could have an adverse effect on morbidity and mortality, it may be necessary to give antiepileptic prophylaxis in this group of patients. This study, even though in a small cohort, suggests that administration of antiepileptic drug was not beneficial in preventing late post-traumatic seizures but may have a role in preventing early seizures. Future studies on a larger number of patients with a longer follow-up period may be able to answer these doubts.

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Authors' Contributions

Conception and design: CKH, JT
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Original Article

Mastoid Abscess in Acute and Chronic Otitis Media

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Abstract

Background: **Mastoid abscess remains a recognised complication of otitis media despite the advent of antibiotics. The objectives of this study were to describe the risk factors in patients with mastoid abscess following acute and chronic otitis media and discuss the management of this infection.**

Method: **A retrospective analysis was done on all patients who underwent mastoidectomy for mastoid abscess from January 2002 to December 2007. Data on the patients' presentation, associated complications, management, and follow-up were analysed.**

Results: **A total of 12 patients were enrolled in this study population. Group A consisted of patients with mastoid abscess preceded by acute otitis media, while Group B consisted of patients with mastoid abscess and chronic otitis media. In Group A (*n* = 7), 4 patients had a pre-morbid immunocompromised condition, but they did not have cholesteatoma. None of the patients in Group B (*n* = 5) had any pre-morbid illnesses. Out of 12 patients, 7 patients had associated extracranial complications, and 1 patient had intracranial complications. Most patients recovered well after mastoidectomy. Recurrence was noted in 1 patient who had acute lymphoblastic leukaemia.**

Conclusion: **Mastoid abscess is still a recognised complication of acute otitis media, especially in patients who are immunocompromised. Immunocompetent patients may also develop mastoid abscess following chronic otitis media associated with cholesteatoma. Thus, early treatment of otitis media and close vigilant follow-up are advocated to ensure prompt detection of mastoid abscess complications.**

Keywords: *abscess, cholesteatoma, complications, immunocompromised patient, mastoiditis, otitis media, otolyngology; head neck*

Introduction

In the era of antibiotics, mastoid abscess is an uncommon complication of otitis media. This has resulted in a decline in the incidence of mastoidectomy performed for mastoid abscess. Nevertheless, there are still a number of patients who develop mastoid abscess, which requires prompt diagnosis and management. Records of patients who underwent mastoidectomy for mastoid abscess at Universiti Kebangsaan Malaysia Medical Centre (UKMMC) were reviewed. The objective of this review was to study the characteristics of patients who may have a higher risk of developing mastoid abscess following acute or chronic otitis media (COM).

Materials and Methods

This is a retrospective analysis of patients who underwent mastoidectomy for mastoid abscess in UKMMC from 2002 to 2007. The operative census was reviewed to identify patients who underwent mastoidectomy for mastoid abscess. The medical records of these patients were reviewed to confirm the diagnosis of mastoid abscess intra-operatively. The diagnosis of mastoid abscess was defined by findings of pus within the coalescent mastoid air cells. This study was approved by the Research and Ethics Committee of UKMMC (FF-242-2008).

Results

A total of 13 patients were identified in this study, and their ages ranged 3–70 years old with a mean of 30.4 years old. Further data of 1 patient could not be traced and had to be omitted, which left a total of 12 cases. The patients were classified into 2 groups: Group A consisted of patients with mastoid abscess preceded by acute otitis media (AOM), and Group B consisted of patients with mastoid abscess and underlying COM. AOM was defined as having symptoms for duration of less than 12 weeks, and cases were classified as COM when symptoms persisted for 12 weeks or longer. All patients in this series presented with unilateral ear infection.

Group A: Patients diagnosed with AOM with mastoid abscess

There were 7 patients categorised into Group A (Table 1). All paediatric patients (n = 3, age less than 12 years old) in this study were in this group. These patients had aural symptoms between 3 and 28 days prior to presentation. Post-auricular swelling was present in 3 patients, mastoid pain was present in 4 patients, and otorrhoea was present in 2 patients. Otoscopic examination revealed perforated tympanic membrane in 2 patients.

There were 5 out of 7 patients who had other associated complications. There were also 4 out of 7 patients in this group who had pre-morbid conditions leading to a relatively immunocompromised state compared to the other subjects. Cholesteatoma, however, was not noted in any of these patients.

Group B: Patients diagnosed with mastoid abscess and underlying COM

There were 5 patients categorised into Group B (Table 2). In this group, the patients had chronic aural symptoms for 3 to 12 months and acute (new) symptoms for 2 to 6 weeks prior to presentation. Post-auricular swelling was present in 3 patients, mastoid pain was present in 4 patients, and otorrhoea was present in 3 patients. Otoscopic examination revealed that all patients had a perforated tympanic membrane. It was noted that only 3 out of the 5 patients had other associated complications. All 3 patients had underlying cholesteatoma, but none of these patients had any pre-morbid illnesses.

Associated complications of otitis media

Out of 12 patients, 8 (66.7%) had complications of mastoiditis. These were mainly extracranial complications, in 7 out of 8 patients: facial nerve palsy, in 3 patients, Bezold's abscess, in 3 patients (Figure 1), and zygomatic root abscess in 1 patient (Figure 2). In this series, only 1 patient had an associated intracranial complication of meningitis.

Management and follow-up

All patients were admitted and started on broad-spectrum intravenous antibiotics. Intravenous ceftriaxone was chosen because of its good blood-brain barrier penetration. Ceftriaxone was administered at a dose of 1 g daily, unless patients had intracranial complications, which required a dose of 2 g twice daily. The type of antibiotics was modified according to the culture results. The duration of antibiotic treatment was 2 weeks in all patients.

The bacteria isolated from patients' pus culture were Staphylococcus aureus in 3 patients, Klebsiella pneumoniae in 2 patients, coagulase-negative Staphylococcus spp in 1 patient and Pseudomonas aeruginosa in 1 patient. There were 2 patients with mixed growth, but the cultures contained predominantly Enterococcus spp. The other 5 patients had no growth on operative specimen or swab culture.

All of the patients in this series had mastoid exploration for abscess drainage and eradication of diseased mastoid air cells. Modified radical mastoidectomy was performed in almost half of the patients (5 out of 12 patients). Cortical mastoidectomy with myringotomy and ventilation tube insertion was performed in 4 patients, and 3 of those patients had AOM. Radical mastoidectomy was only performed in 2 patients (1 from each group).

Post-operatively, all patients had a good recovery. The average follow-up period was 24 months (range 8–58 months), and 2 out of 12 patients were lost during the post-operative follow-up. Only 1 patient with acute lymphoblastic leukaemia (ALL) in Group A had a recurrence of mastoid abscess, which occurred 1 month later.

The patient with ALL developed AOM while undergoing chemotherapy; the patient was treated with amoxicillin. Despite treatment compliance, the patient developed lower motor neuron facial nerve palsy 5 days later. Radical mastoidectomy was performed, which showed a bony dehiscent over the horizontal segment of the facial nerve which was covered by granulation tissue. The stapes suprastructure was also absent. Post-operatively, the facial nerve palsy improved from House–Brackmann grade IV to grade II. However, he had another episode of AOM with facial nerve

Table 1: Demographics of patients diagnosed with acute otitis media with mastoid abscess (Group A)

Patient	Age (years)	Pre-morbid condition	Duration of symptoms (days)	Other complications	Type of mastoid surgery	Duration of follow-up (months)
NA	3	Nil	3	None	Cortical	lost to follow-up
LMK	48	DM, HT	7	Meningitis	MRM	58
MS	6	ALL	5	Facial nerve palsy	Radical	lost to follow-up
WQE	7	BTM	14	Zygomatic root abscess	Cortical	14
CKC	46	Nil	30	Facial nerve palsy	Cortical	10
CCW	70	DM	21	Bezold's abscess	MRM	8

Abbreviation: ALL = acute lymphoblastic leukaemia, BTM = beta thalassaemia major, DM = diabetes mellitus, HT = hypertension, MRM = modified radical mastoidectomy

Table 2: Demographics of patients diagnosed with acute otitis media with mastoid abscess (Group B)

Patient	Age (years)	Duration of acute symptoms (days)	Choles-teatoma	Other complications	Type of mastoid surgery	Duration of follow-up (months)
NH	37	4	Yes	None	MRM	50
SN	29	4	Yes	None	MRM	24
AU	22	6	Yes	Facial nerve	Radical	10
AH	43	2	No	Bezold's abscess	MRM	18
WJ	14	4	No	Bezold's abscess	Cortical	8

None of the patients had pre-morbid condition. Abbreviation: MRM = modified radical mastoidectomy

palsy grade V a month later. Unfortunately, due to pancytopenia and a poor general condition, he was deemed unfit for another mastoid exploration. This patient later succumbed to the underlying haematological malignancy.

Discussion

The complications of otitis media are broadly categorised into extracranial and intracranial complications. Extracranial complications (such as mastoiditis, subperiosteal abscess, facial paralysis, and labyrinthitis) and intracranial complications (such as cerebral or extradural abscess, meningitis, focal encephalitis, lateral sinus thrombosis, and otic hydrocephalus) are more likely to be associated with AOM than COM (1–3).

Since the introduction of antibiotics in the 1940s, the incidence of acute mastoiditis and surgical intervention has declined. Recent publications, however, have noted an increase in the incidence of acute mastoiditis following AOM in children (4,5). Conversely, there has been a reduced incidence of COM since the 1990s. However, the rate of extracranial and intracranial complications has remained stable (6). There have been significant socioeconomic improvements in many countries during this time. This is important because the established risk factors associated with COM include low socioeconomic class, malnutrition, and congested living conditions (7). Therefore, these studies seem to suggest an increased incidence of mastoiditis following AOM compared with COM.

Mastoiditis has often been recognised as an extracranial complication of otitis media when patients develop tender post-auricular swelling. The current treatment of mastoiditis is mainly antibiotics with surgery reserved to myringotomy (5,8). Mastoid abscess may develop as a complication of mastoiditis following both AOM and COM (9–11). It occurs when purulent material

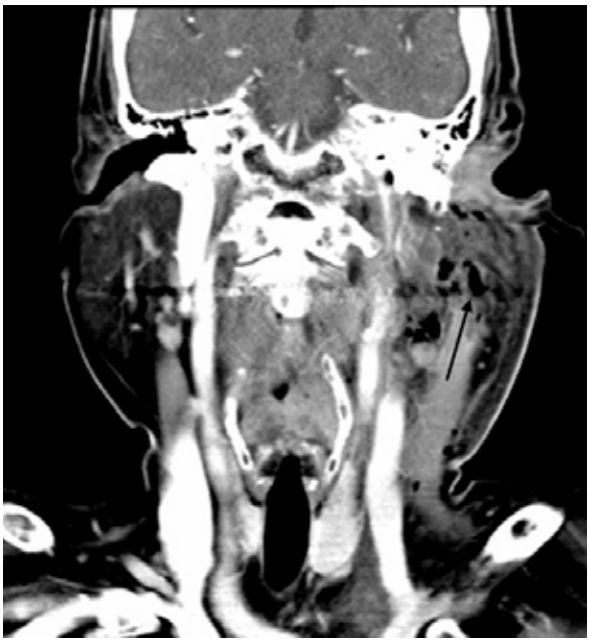

Figure 1: Coronal CT scan of a patient diagnosed with left mastoid abscess and Bezold's abscess (arrow)

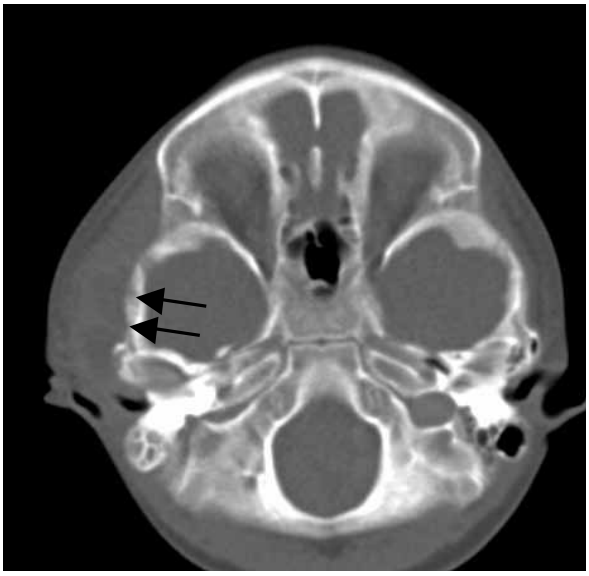

Figure 2: Axial CT scan of temporal bone showing right mastoid abscess and zygomatic root abscess (arrows)

collection accumulates within the middle ear and mastoid air cells, and it is often accompanied by granulation tissue. Surgical intervention is still the most common treatment for mastoid abscess. Therefore, it is important to distinguish mastoid abscess from uncomplicated mastoiditis and manage patients accordingly.

The most common clinical presentation of mastoid abscess in this series was a tender, fluctuant post-auricular swelling, which was similar to other cases in the literature (11,12). Otorrhoea was less common, and facial asymmetry, neck swelling, and meningism were even rarer. All of the patients with cholesteatoma had a history of chronic otorrhoea since childhood.

Complications following COM were more prevalent in subjects with cholesteatoma (13). Mustafa et al. showed that 15% of patients with COM had associated cholesteatoma, and one-third of them presented with complications. In COM without cholesteatoma, only 6.7% presented with complications. In the current series, the numbers were too small to make any significant comparison; however, 3 out of 5 patients with mastoid abscess following COM had cholesteatoma. Interestingly, the incidence of multiple complications can occur between 11% and 58% of cases and appears to be more prevalent in patients with intracranial complications (13–15).

Not surprisingly, the complication rate following COM has been reported to be higher than that following AOM (14,15), but caution should be exercised in young children with AOM because intracranial complications may occur relatively rapidly in the course of the disease (16). In this series, there was only 1 patient with meningitis as a complication of mastoid abscess. However, patients with mastoiditis or mastoid abscess who did not undergo mastoid surgery was excluded; therefore, the series may not have captured these cases.

In our centre, patients with suspected mastoid abscess following mastoiditis were promptly admitted and commenced on broad-spectrum intravenous antibiotics. A high-resolution CT of the temporal bone and contrast-enhanced CT of the brain were also performed in all patients. Mastoidectomy with abscess drainage was indicated when there was purulent collection clinically, evidence on the CT scan or in patients with cholesteatoma.

The predominant organisms cultured in this series were Staphylococcus aureus and Klebsiella pneumoniae; however, there was no single predominant organism in AOM or COM. There were 5 (42%) patients' samples that exhibited no

growth on routine cultures. Previous antibiotic treatment may have resulted in the absence of bacterial growth (4). In addition, tests for anaerobic cultures were not routinely performed in our institution when anaerobes are expected to be prevalent in COM. Previous studies have shown that common organisms in AOM include Streptococcus pneumoniae and Haemophilus spp. whereas common organisms in COM include Proteus mirabilis, Enterococcus spp., and Pseudomonas aeruginosa (5,13–15,17).

Mastoidectomy was performed expediently once the patient's medical condition was stabilised, and the decision to bring down the posterior canal wall or to perform radical mastoidectomy was depended on the intra-operative findings. Generally, intra-operative findings of intact ossicles with no cholesteatoma indicated cortical mastoidectomy with myringotomy (if the tympanic membrane was intact). In this series, intra-operative findings of ossicular erosion, including erosion of the stapes suprastructure, led to radical mastoidectomy in two patients.

Interestingly, serious co-morbidities were noted to be present in patients who developed mastoid abscess following AOM. The only 4 (25%) patients with pre-morbid illness were those who developed mastoid abscess following AOM. These pre-morbid conditions included ALL in 1 patient, BTM in 1 patient, and diabetes mellitus in 2 patients. It is postulated that an immunocompromised state due to illness may make a patient susceptible to developing mastoid abscess following AOM.

Factors that have been shown to influence the spread of infection include the type and virulence of the infecting organism, host resistance, and the adequacy of treatment (15). Patients with haematological malignancy, such as ALL, may present with leukaemic infiltration of the temporal bone; however, this is uncommon. Moreover, surgical findings often revealed greenish soft tissue mass with gelatinous fluid within the middle ear (18), which was not evident in our patient. In this case, it is postulated that enhanced organism virulence might explain the extensive ossicular destruction despite the acute presentation. Interestingly, a study showed that patients with BTM were prone to infection due to impaired phagocytic action and anaemia (19). They are also prone to recurrent upper respiratory tract infections because of generalised lymphoid hyperplasia and expanding marrow of facial bones, which results in nasal obstruction. Patients with diabetes mellitus may present with masked symptoms due to neuropathy. Both patients with diabetes mellitus developed mastoid abscess with associated complications of meningitis or Bezold's abscess in the absence of otorrhoea symptoms. The immunocompromised condition of these 4 patients could have resulted in the dissemination of infection before any apparent ear symptoms.

Therefore, early adequate treatment of AOM and close vigilant follow-up are important, especially in immunocompromised patients. Antibiotic treatment, however, does not provide absolute protection against the development of complications and, at worst, may mask the symptoms and signs of complications (4,16). Increasing antibiotic resistance behaviours by organisms in biofilms (demonstrated in Streptococcus pneumonia and Haemophilus influenza) may explain why antibiotic treatment does not provide absolute protection (17).

Facial nerve palsy occurred in 4 out of 12 patients in this series; 3 were patients with AOM had facial nerve palsy grade IV to VI (House–Brackmann), which improved after surgery (to grade II at best). The other patient had COM and grade II facial palsy, which had improved to grade I on the second post-operative day. These observations were in contrast to previous studies, which reported total recovery in all AOM patients with facial paralysis (20,21). They were also in contrast to another study, which suggested that facial paralysis in COM had a poor prognosis (22).

A literature review by White and McCans (23) suggested that several potential processes were involved in facial palsy secondary to otitis media: 1) direct involvement of the facial nerve by bacterial invasion, 2) mechanical compression on the vascular supply of the nerve by the purulent exudates or granulation tissue, 3) acute toxic neuritis with venous thrombosis resulting in ischaemia, and 4) bacterial toxins that lead to facial nerve demyelination. More than one of these processes may be involved in the pathophysiology of facial palsy.

Therefore, it is postulated that the recovery of facial nerve function may depend on the underlying pathophysiological processes that resulted in the facial nerve palsy. Recent studies using the results of electrophysiological tests have shown that facial nerve palsy secondary to AOM may be treated clinically (24,25). Another study on facial nerve palsy due to non-cholesteatomatous otitis media also showed good recovery without surgical decompression of the nerve (25,26). However, facial nerve palsy associated with cholesteatoma tends to have a poor prognosis, and mastoid surgery is required to create a safe and dry ear.

Conclusion

Despite the advancements in the treatment of otitis media, mastoid abscess is still a recognised complication in both acute and COM. Although mastoid abscess can occur over a wide age spectrum (3–70 years old), it predominantly occurs in adults. Patients who are immunocompromised have a greater risk of developing mastoid abscesses secondary to AOM. They may also present with vague symptoms, severe disease or other associated complications that require vigilance on the part of the physician. Mastoid abscesses may also develop in immunocompetent patients with COM, especially in association with cholesteatoma. In contrast to previously published data, facial palsy secondary to AOM may not recover completely.

Authors' Contributions

Conception and design: MA
Provision of study materials or patients: GBS, AA
Collection and assembly of data: ZZ
Analysis and interpretation of the data: ZZ
Drafting of the article: MA, ZZ
Critical revision of the article: MA, GBS, AA
Final approval of the article: LS

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Brief Communication

Computed Tomography Perfusion Imaging on Traumatic Cerebral Contusion: A Preliminary Report

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Abstract

Background: **Brain ischaemia and infarction are the leading factors in morbidity and mortality of traumatic brain injury. This study aimed to determine the perfusion status of pericontusional hypodense areas in traumatic cerebral contusion**

Methods: **Ten patients involved in motor vehicle accidents were enrolled in this study, and contusions were diagnosed from plain computed tomography scans of the brain. Subsequent computed tomography perfusion (CTP) was performed to analyse the perfusion of pericontusional hypodense areas, which were divided into 4 regions of interest (ROI).**

Results: **Most ischaemic perfusion was found in ROI 6 (affecting 60% of patients), although the mean of the perfusion parameters were normal. A significant positive correlation was found between the perfusion status in the pericontusional area nearest to the skull vault (ROI 3) and its distance/thickness to the skull vault (r = 0.698, P = 0.025). Two adjacent pericontusional hypodense areas (ROI 4 and ROI 5) showed a significant positive correlation with each other (r = 0.667, P = 0.035) in terms of perfusion status. The presence of a hypodense pericontusional area is suggestive of oedema and perfusion disturbances.**

Conclusion: **CTP is a useful, fast, and appropriate method in evaluating perfusion of pericontusional hypodensity area that may help the treating physician to provide an appropriate treatment to the patient.**

Keywords: *brain contusion, emission-computed tomography, medical imaging, oedema, perfusion, trauma*

Introduction

Brain injury is the leading factor in morbidity and mortality following head trauma/injury. The devastating personal, social, and financial consequences of traumatic brain injury (TBI) are compounded by the fact that most people with TBI are young and otherwise healthy. Advances in the current management of TBI, including brain imaging, have led to increased survival rates in cases that would have previously been fatal (1). Because brain function is exceedingly complex, brain injury and recovery are also complex (2). Therefore, diagnostic imaging is extremely important in TBI patients to understand the clinical implications. To make matters even more complex, an early computed tomography (CT) scan does not identify which patients will develop neurological deficits, even after minor head injury. Although there is no consensus regarding which patients should be scanned, many authors agree that an abnormal result has a major impact on a patient's management (3). CT scan is a sensitive diagnostic tool for the evaluation of acute head injury; however, the prognostic ability of conventional CT scan has limited value.

Cerebral contusions are characterised by mixed densities of lesions, which are commonly surrounded by perilesional hypodense areas in close contact with the internal surface of the skull. Cerebral contusions have a tendency to enlarge over time and become significant space-occupying lesions, which exert a mass effect to surrounding brain parenchyma. This leads to an increased intracranial pressure with subsequent clinical deterioration or worsening neurological condition. A survey of 729 patients with TBI by the TBI European Brain Injury Consortium found that cerebral contusions alone (44%) or in association with subdural haematoma (29%) were the most frequent causes for delayed surgical intervention (4). In addition, ultrastructural studies have provided evidence that progressive neuronal damage leads to a growing area of

necrosis, which enhances the role of cerebral contusions as a vector of secondary brain damage (5). Therefore, cerebral contusions can become a major therapeutic challenge because they have the potential to become growing masses, mixed with presumably viable tissue, which may be of critical functional importance whenever surgical removal of the lesion is contemplated in neurologically eloquent areas, i.e., areas involved in critical functions (6). Several studies on ischaemic stroke have revealed that hypodense areas in plain brain CT scans have regional alterations in perfusion. Most CTP studies have been performed in ischaemic stroke patients, and only a few studies have examined CTP in patients with TBI (7). Therefore, the purpose of this study was to ascertain the perfusion status of the hypodense pericontusional area.

Materials and Methods

All experimental protocols were approved by the Ethical Committee (Human) Universiti Sains Malaysia (USMKK/PPK/JKEP(M)-191 USM) and institutional informed consent guidelines were followed regarding the consent of the patient or his/her legal representative. All adult patients with trauma were prospectively identified in the emergency room of the Hospital Universiti Sains Malaysia (HUSM) as candidates for enrolment from July 2007 to November 2008. Inclusion criteria were in-patients, with minimum age of 15 years, who had a traumatic brain contusion observed on a plain CT scan of the brain and cervical C1–C2. Patients were excluded if they were pregnant, did not give their informed consent, sustained an infratentorial brain contusion, had subarachnoid haemorrhage, had extradural haemorrhage, or were required to undergo any surgical or endovascular intervention post-TBI.

The series consisted of 10 patients (8 men and 2 women) with a median age of 25 years, age range of 16–48 years, and interquartile age range of 17–33 years. A multidetector CT scanner (Light speed; General Electric Medical Systems, Milwaukee, WI) to obtain a helical plain CT scan of a 3.75 mm thick basal section and a 7.5 mm thick supratentorial axial section, and the sections containing the largest contusion/intraparenchymal haematoma were selected.

Computed tomography perfusion (CTP) is initiated by injecting 50 mL (320 mg/mL) iodinated contrast media (iodixanol) into each patient's peripheral vein through a 20-gauge cannula at 4 mL/s using an angiographic power injector. The scan was delayed for 4 seconds, and the total scan time was 45 seconds. We obtained 4 sections per second with a thickness of 5.0 mm/section and an image matrix of 512 x 512. The scan was set at 80 kV with a current of 190–200 mA. A total of 180–200 images were obtained and sent to a workstation for review and post-processing. All CTP examinations were well-tolerated, and there were no reported side effects (e.g., allergic reaction to the contrast media or extravasation). The CTP software in the CT workstation (AW 3.1) was used for the analysis of perfusion parameters: cerebral blood flow (CBF), cerebral blood volume (CBV), and mean transit time (MTT). We selected images of the largest diameter of the contusion. A small region of interest (ROI) was drawn in an artery (ROI 1) and vein (ROI 2). The artery nearest to the contusion was selected, and sagittal or transverse sinuses were selected for the ROI placement in the vein. The arterial curve should be displayed before the venous curve because the venous height was higher and later than the arterial curve. The pericontusional hypodense area was drawn with cursor and divided into 4 quadrants named ROI 3, ROI 4, ROI 5, and ROI 6. A correlation of pericontusional hypodensity area was not matched with rainbow colour changes of post-processing generated CTP images. ROI 3 was the closest to the skull vault, and it was followed by ROI 4, ROI 5, and ROI 6 in a clock-wise fashion if the contusion was on the left side (Figure 1) and a counter-clock-wise fashion if the contusion was on the right side. After drawing the ROIs, the cursor was placed and the function algorithm was selected. The CBF, CBV, and MTT values of each ROI were calculated. CBF of less than 20 mL/100 g/min, CBV of less than 2.0 mL/100 g, and MTT of more than 8 seconds were considered as abnormal (8).

Results

The median size of the contusion was 52.00 mm^2 (range, 14.00–493.00 mm^2, interquartile range, 34.75–196.50 mm^2). The mean (SD) distance of the contusion from the nearest skull vault was 5.35 (6.73) mm; median, 2.35 mm; range 0.80–18.10 mm; and interquartile range, 1.37–7.25 mm. Ischaemic perfusion, which affected 60% of the patients, was mostly seen in ROI 6. ROI 4 displayed the largest hypodense pericontusional area, 114.00 (SD 212.00) mm^2, and ROI 6 showed the smallest area, 48.80 (SD 44.28) mm^2. Mean values of CBV, CBF, and MTT were within normal limits in all ROIs (Table 1).

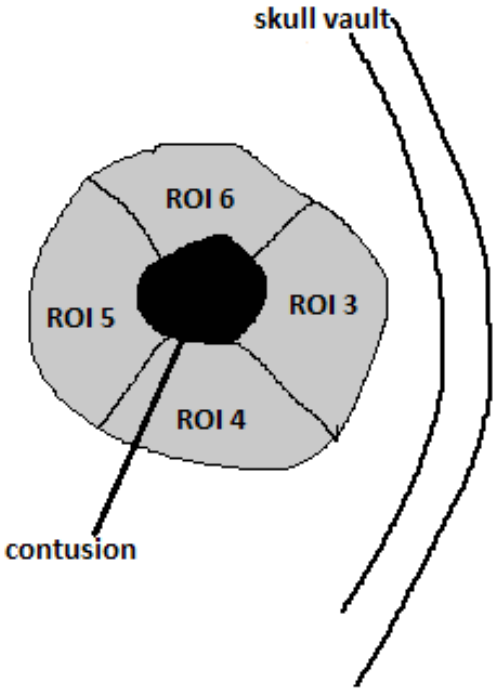

Figure 1: Diagrammatic presentation of the hypodense pericontusional area in each region of interest (ROI), if the contusion was on the left side.

The mean values of the perfusion parameters of the hypodense pericontusional area in ROI 6 were 4.13 (SD 6.19) mL/100 g, 21.44 (SD 16.39) mL/100 g/min, and 3.58 (SD 1.98) seconds for CBV, CBF, and MTT, respectively.

Because the data were not normally distributed, the Spearman correlation statistical test was used to evaluate the relationship of the parameters (Tables 2 and 3). A significant positive correlation (r = 0.667, P = 0.035) was observed between the perfusion of the hypodense pericontusional areas of ROI 4 and ROI 5 (Table 2). Significant positive correlations were also observed between the distance of the contusion to the nearest skull vault and the perfusion of the hypodense area in ROI 3 (r = 0.698, P = 0.025) and ROI 6 (r = 0.642, P = 0.046). Interestingly, a significant negative correlation was observed between the perfusion of the hypodense pericontusional area of ROI 3 and the size of the hypodense area of ROI 4 (r =-0.698, P = 0.025). In addition, there are significant positive correlation between size of contusion and size of pericontusional hypodensity area in each ROI 3 (r = 0.839, P = 0.002), ROI 4 (r = 0.723, P = 0.018), ROI 5 (r = 0.842, P = 0.002), and ROI 6 (r = 0.717, P = 0.020) (Table 3).

Discussion

CTP has been introduced as a simple imaging technique that can be used in routine clinical practice. It has gained recognition in management cases of acute stroke and other cerebrovascular disorders because it is able to provide information about the cerebral perfusion status. Most CTP studies have been performed on ischaemic stroke cases; only a few studies have been performed on TBI. The results of the present study showed that there were significant perfusion changes in cases of TBI, which expands the use of CTP technique in the management of TBI.

The values of the perfusion parameters of the hypodense pericontusional area in ROI 6, which was the region that showed the most ischaemic perfusion, were comparable to a study by Soustiel et al. (6), which reported values of 2.9 (SD 1.3) mL/100 g for CBV, 26.2 (SD 11.9) mL/100 g/min for CBF, and 6.7 (SD 2.9) seconds for MTT. Another study of pericontusional areas also revealed a normal CBF value of 42.5 (SD 15.8) mL/100 g/min despite all of their patients having a Glasgow Coma Scale (GCS) score of less than 10 (9). Our study consisted of an initial GCS score of more than 12 without any endovascular or surgical interventions. CBF is the initial indicator of CTP parameters that denotes perfusion disturbances (10). However, a study by Schroder et al. (11) found that oedematous pericontusional areas showed a CBF value of 17.5 (SD 4.0) mL/100 g/min. Interestingly, all 11 patients in the Schroder et al.'s study had severe head injuries (GCS of 8 or less), which likely accounted for the CBF value. Compared with this study, the GCS scores of all 10 of the patients in the present study were above 12, and none of the patients underwent any surgical interventions that would have affected the GCS scores.

We observed significant positive correlations between the distance of the contusion and perfusion of the hypodense pericontusional area in ROI 3 and ROI 6. A related study by Rosand et al. (12) revealed that the perihaematoma perfusion parameter was increased as a function of the distance from the skull. This meant that an increase in the distance of the contusion from the nearest skull vault increased/improved the perfusion. In the present study, this distance–perfusion interaction was stronger in ROI 3 compared with ROI 6.

There was a significant positive correlation between the perfusion of the hypodense pericontusional areas in ROI 4 and ROI 5. This correlation suggested that both of these areas

Table 1: Perfusion parameters of the pericontusional hypodensity in each region of interest (ROI)

Perfusion parameter	ROI 3	ROI 4	ROI 5	ROI 6
CBV (mL/100 g)	6.19 (6.94)	3.32 (3.65)	2.99 (3.76)	4.13 (6.19)
CBF (mL/100 g/min)	40.07 (25.33)	25.82 (22.11)	28.68 (33.05)	21.44 (16.39)
MTT (s)	5.34 (4.28)	5.53 (4.84)	4.07 (2.55)	3.58 (1.98)

Data are expressed as mean (SD).
Abbreviation: CBF = cerebral blood flow, CBV = cerebral blood volume, MTT = mean transit time

Table 2: Spearman correlations between the perfusion, the distance of the contusion from the nearest skull vault and the size of the hypodense pericontusional area of ROI 3, ROI 4, ROI 5, and ROI 6 (n = 10).

	PROI 3 r (P value)	PROI 4 r (P value)	PROI 5 r (P value)	PROI 6 r (P value)
PROI 3	1.000	0.102 (0.779)	-0.102 (0.779)	0.408 (0.242)
PROI 4	0.102 (0.779)	1.000	0.667 (0.035)*	0.250 (0.486)
PROI 5	-0.102 (0.779)	0.667 (0.035)*	1.000	0.583 (0.077)
PROI 6	0.408 (0.242)	0.250 (0.486)	0.583 (0.077)	1.000
Distance of contusion#	0.698 (0.025)*	0.178 (0.622)	0.285 (0.425)	0.642 (0.046)*
Size of ROI 3	-0.0175 (0.629)	0.071 (0.845)	0.249 (0.487)	0.321 (0.366)
Size of ROI 4	-0.698 (0.025)*	0.214 (0.553)	0.356 (0.312)	-0.071 (0.845)
Size of ROI 5	-0.522 (0.122)	0.213 (0.554)	0.569 (0.086)	0.284 (0.426)
Size of ROI 6	-0.393 (0.261)	0.428 (0.218)	0.428 (0.218)	0.107 (0.769)

Distance was measured from site of contusion to the nearest skull vault. * $P < 0.05$ indicates significance by Spearman correlation test.
Abbreviation: ROI = region of interest, PROI = perfusion of ROI

have a similar regional blood supply. We also observed a significant negative correlation between the size of the hypodense pericontusional area in ROI 4 and the perfusion of the hypodense pericontusional area in ROI 3. Thus, when the size of the hypodense pericontusional area increased in ROI 4, a reduction of perfusion in ROI 3 is expected. This finding also suggested the possibility of both ROI 3 and ROI 4 sharing the same branches of regional blood supply. A study by Schroder et al. (11) revealed that microvascular complications in the hypodense pericontusional area were due to external compression from the swelling of podocytic processes. Additionally, some vascular occlusion occurred because of stasis of erythrocytes and leucocytes.

In this study, we observed a strong association between the size of the contusion and the size of the hypodense pericontusional area in each ROI. These data suggested that any changes in the size of the contusion would significantly influence the size of the hypodense pericontusional area in each ROI. In this study, ROI 4 displayed the largest hypodense pericontusional area, 114.00 (SD 212.00) mm², and ROI 6 showed the smallest area, 48.80 (SD 44.28) mm². A study of CTP parameters haemorrhagic hypertensive stroke by Abdullah et al. (13), which had divided similar perihaematoma hypodensity area to ROI 4 to ROI 6, also found that ROI 4 had the largest perihaematoma area, 194.10 (SD 155.21) mm²; however, they observed the smallest area in ROI 3. The relative size of the ROI in the pericontusional area was smaller than the perihaematoma area. This might be due to a smaller surrounding mass effect by the contusion compared with the intracerebral haematoma because the contusion was smaller than intracerebral haematoma caused by haemorrhagic hypertensive stroke.

The size of hypodense pericontusional areas of each ROI were strongly related to each other, and any changes of the size of the hypodense pericontusional area in one ROI would affect the neighbouring ROIs. This could result from

Table 3: Spearman correlations between the size of the hypodense pericontusional areas of ROI 3, ROI 4, ROI 5, and ROI 6 (n = 10).

	Size of contusion r (P value)	Size of ROI 3 r (P value)	Size of ROI 4 r (P value)	Size of ROI 5 r (P value)	Size of ROI 6 r (P value)
Size of ROI 3	0.839 (0.002) **	1.000	0.716 (0.020) *	0.760 (0.011) *	0.720 (0.019) *
Size of ROI 4	0.723 (0.018) *	0.716 (0.020) *	1.000	0.827 (0.003) **	0.817 (0.004) **
Size of ROI 5	0.842 (0.002) **	0.760 (0.011) *	0.827 (0.003) **	1.000	0.875 (0.001) **
Size of ROI 6	0.717 (0.020) *	0.720 (0.019) *	0.817 (0.004) **	0.875 (0.001) **	1.000

*P < 0.05 and ** P < 0.01 indicate significance by Spearman correlation test.
Abbreviation: ROI = region of interest

a significant correlation between the size of the contusion and the size of the hypodense pericontusional area in each ROI, which might be attributed to the mass effect by the contusion that lead to hypodensity changes of pericontusional area .

The major limitation of this study was the small sample size. Some of the potential subjects had to be excluded because we were unable to analyse their CTP results due to movement during images acquisition, and some patients refused consent. A technical limitation of the present study was that our machine could only encompass a 20.0 mm thickness of the contusion. Our CTP results may have been affected because part of the contusion could not be included. In the future, a similar study with a larger sample size should be performed for better statistical analysis in order to yield results that are more significant.

Conclusion

This study showed abnormal perfusion was found in the pericontusional hypodensity area that mostly affected in ROI 6 with reduced value of CBF and CBV with normal MTT. In conclusion, this study highlighted that hypodense pericontusional areas not only reflect oedema but it also have an ischaemic component.

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Case Report

Chronic Sclerosing Sialadenitis (Küttner's tumour) of the Parotid Gland

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Abstract

Chronic sclerosing sialadenitis is a chronic inflammatory salivary gland disease. Küttner reported 4 cases of submandibular gland lesions for the first time in 1896. Chronic sclerosing sialadenitis is a very rare inflammatory lesion of the parotid gland and cannot be easily distinguished from salivary malignant masses. We reported a 28-year-old male with a painful parotid tumour, which grew slowly for 4 years.

Keywords: *chronic illness, inflammation, oral surgery, parotid gland, sclerosis, sialadenitis*

Introduction

A series of patients with unilateral, hard, tumour-like masses of the submandibular gland were diagnosed with chronic sclerosing sialadenitis by Küttner in 1896 (1). This disease is clinically similar to salivary gland neoplasms and is classified as a tumour-like lesion of the salivary glands by the World Health Organization (2).

Chronic sclerosing sialadenitis is clinically characterised by a firm, relatively painful swelling of one of the submandibular glands. This disorder is characterised by plasmocytic and lymphocytic periductal infiltrate eventually leading to encasement of ducts with thick fibrous tissue (3).

Histologically, chronic sclerosing sialadenitis is characterised by periductal sclerosis, acinar atrophy, and infiltration of the gland by lymphocytes, which some studies have recognised as predominantly activated B cells with a subpopulation of helper-inducer T cells. The distribution pattern of these lymphocytes suggested that the response was immunological. However, sialoliths and mucous plugs were found in 29% to 83% of the lesions. This association was meaningful for some authors, such that it was considered in favour of a cause and result relationship. Other possible aetiologies of chronic sclerosing sialadenitis are ascending bacterial infections of the oral cavity and duct obstruction by foreign bodies.

Case Report

A 28-year-old male was investigated at the Otolaryngology Outpatient Department. He first noted the mass 4 years earlier. Physical examination revealed a tender, hard and fixed, 3 x 2 cm mass at the angle of the left maxillary arch, and it seemed to be attached to underlying structures. No other masses or adenopathy were noted in the head or neck. No related events were present in the patient's medical history. He reported no other symptoms or complaints. His facial nerve function was intact. A sonographically guided fine-needle biopsy was performed. Cytology revealed only lymphocytes and other blood elements; however, no glandular epithelial cells were present. Magnetic resonance imaging detected a 3 x 2.5 x 2 cm, smooth-surfaced, multilobular mass in the left parotid gland. This mass had a heterogenic opaque appearance after intravenous contrast injection. No pathologic nodes were identified (Figures 1 and 2). Left total parotidectomy was conducted with mass excision and preservation of the facial nerve.

The specimen collected for pathological examination measured 2.5 cm at its longest diameter. It was a yellow-white in colour, and its cut surface demonstrated a firm consistency. The whole tissue sample was submitted for pathological examination. Pathology revealed chronic sclerosing sialadenitis. The microscopic examination revealed a collagenised fibrous tissue in all sections. Residual salivary gland tissue seemed to be embedded in the fibrous tissue in

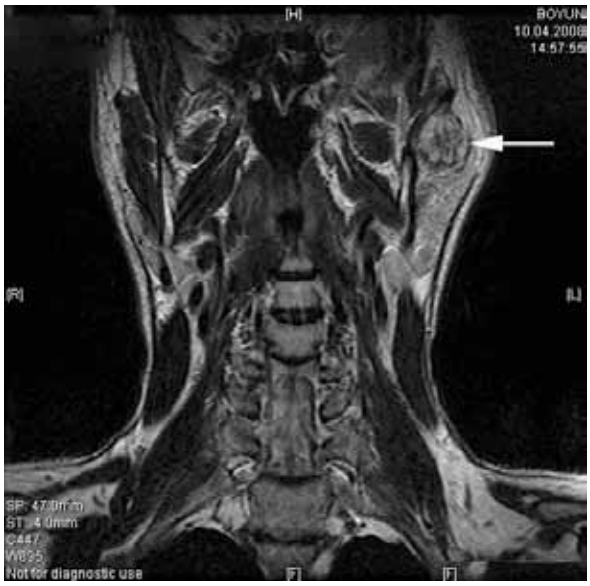

Figure 1: Magnetic resonance image (coronal view) of the head showed chronic sclerosing sialadenitis located on the zygomatic arch (white arrows).

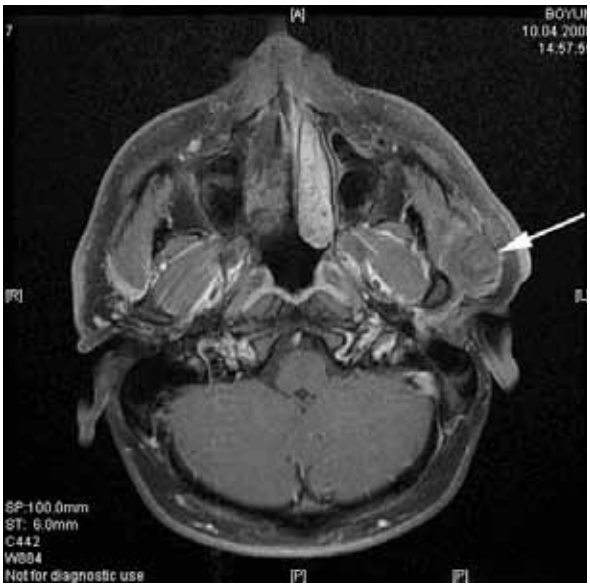

Figure 2: Magnetic resonance image (transverse view) of the head showed chronic sclerosing sialadenitis located within the left cheek (white arrows).

some areas (Figure 3). In the salivary gland tissue, mononuclear cells, mostly lymphocytes, were also observed (Figure 4). Mononuclear cells were also observed in the fibrous stroma. No evidence of malignancy was observed, and the final diagnosis was chronic sclerosing sialadenitis. Post-operative complications and recurrence were not encountered in the 2-year follow-up.

Discussion

Chronic sclerosing sialadenitis is an inflammatory process that primarily affects the submandibular gland and presents clinically as a painful swelling. Histologically, chronic sclerosing sialadenitis demonstrates a loss in acinar tissue, dense fibrosis, which is mainly periductal, and lymphocytic infiltration with lymphoid follicle formation in some cases (4).

According to Seifert (5), chronic sclerosing sialadenitis may progress through 4 different histological stages:

Stage 1. Focal chronic inflammation with aggregates of lymphocytes around moderately dilated salivary ducts containing inspissated secretion is present.

Stage 2. Diffuse lymphocytic infiltration and severe periductal fibrosis are more marked. The ductal system shows inspissated secretion and focal metaplasia with proliferation of ductal epithelium. Periductal lymphoid follicles are well developed. There is fibrosis in the centres of the lobules accompanied by atrophy of acini.

Stage 3. Even more prominent lymphocytic infiltration with lymphoid follicle formation, parenchymal atrophy, periductal hyalinisation, and sclerosis as well as quamous and goblet cell metaplasia in the ductal system.

Stage 4. Cirrhosis-like with marked parenchymal to loss and sclerosis (the "burnt-out-phase).

The origin of chronic sclerosing sialadenitis has been attributed to many etiologic agents. Sialoliths and mucous plugs are found in 29% to 83% of cases, but it is not clear whether the sialoliths are the causes or results of the inflammatory process (2,6,7). In this case, no sialoliths were found. Anything that causes an obstruction of salivary flow or stasis of secretions can lead to acinar cell swelling, necrosis, ductal dilation, and retention of salivary secretions accompanying oedema and inflammatory cell infiltration (8). Salivary gland stones may cause obstruction of salivary flow or stasis of secretions. Seifert and Donath (5) proposed a theory of obstructive electrolyte sialadenitis in which a secretion disorder produces inspissated secretion that obstructs the small ducts, which leads to

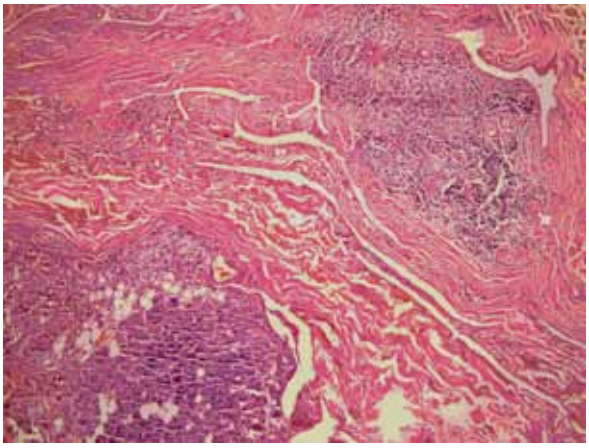

Figure 3: Histology of the salivary gland tissue showed chronic sclerosing sialadenitis (Küttner's tumor) with only a few residual ducts persisting, marked fibrosis and foci of lymphocytic infiltrate often with germinal centers. (Hematoxylin and eosin, 200x magnification)

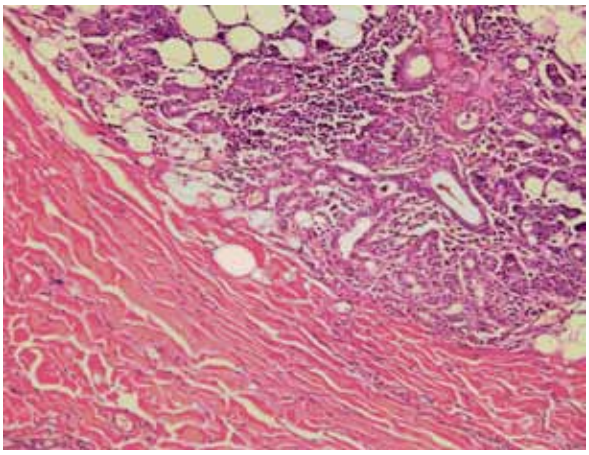

Figure 4: Histology showed the salivary gland residue embedded in collagenised fibrous tissue. On the upper right, a focus of mononuclear cells is also shown. (Haematoxylin and eosin, 100x magnification)

inflammation, fibrosis, parenchymal atrophy, and an immune reaction of the duct system.

Immunohistochemical studies of the lymphoid population in the chronic sclerosing sialadenitis revealed abundant cytotoxic T cells especially near ducts and acini. The B cell reaction was less pronounced and largely restricted to lymph follicles. There was an intimate relationship between the T-cell-dominated inflammatory infiltrate and acinar and duct cells. The monoclonal and oligoclonal populations of cytotoxic T cells and their histopathological behaviours suggested that chronic sclerosing sialadenitis may be the result of an immune process triggered by intraductal epithelial agents. The lymphocytes that infiltrate the epithelial component of chronic sclerosing sialadenitis are mainly B cells and are often characterised by a lack of Bcl-2 expression (9,10).

The duration of symptoms before the patient seeks treatment is variable, from 1 month to about 3 decades, and the induration and enlargement often lead clinicians to diagnose chronic sclerosing sialadenitis as a salivary gland neoplasm (7). Possible differential diagnoses of this entity include other benign inflammatory lesions of salivary glands, such as simple chronic sialadenitis, granulomatous sialadenitis, necrotising sialometaplasia, sialolithiasis, radiation effects, an inflammatory pseudotumour, and benign lymphoepithelial lesions. One of the most common causes of chronic inflammation of the salivary glands is related to rheumatoid arthritis, which is consistent with immune pathogenesis (11).

Usually, these cases do not demonstrate symptoms related to sialadenitis. However, sialolithiasis is the most common cause of sialadenitis in symptomatic patients. Histologically, various degrees of atrophy, fibrosis, and chronic inflammation are seen, but lymphoplasmocytic periductal infiltrate and fibrous encasement of ducts, which are typical of chronic sclerosing sialadenitis, are not present. The causes of granulomatous sialadenitis range from infections to duct obstruction caused by calculi or malignancies. Granulomas of different types can be present, and a xanthogranulomatous variant has been described (12).

Necrotising sialometaplasia occurs mainly in the minor salivary glands of the palate and may be confused with carcinoma. In necrotising sialometaplasia, an ischemic aetiology is thought to produce ulcerating lesions with partial necrosis of glands associated with regeneration and squamous metaplasia of the adjacent ducts. Its localisation and lack of lymphoplasmocytic infiltration differentiates necrotising sialometaplasia from chronic sclerosing sialadenitis. Sialolithiasis can be observed in all major salivary glands, although it is more common in the submandibular gland. Ductal obstruction induces inflammation of the surrounding tissue and acinar atrophy ensures. Radiographic examination may demonstrate a radiopaque mass. Histological examination shows dilated ducts and variable destruction of salivary tissue. The characteristic histological symptoms of chronic sclerosing sialadenitis are not seen.

Radiation effects are more frequently seen in the submandibular glands because they are located in the irradiation fields for tumours of the oral cavity. Acinar atrophy, chronic inflammation, and squamous metaplasia are commonly seen. The lymphocyte population is mainly formed by B cells with sparse T cells without the peculiar topographic distribution seen in chronic sclerosing sialadenitis.

Inflammatory myofibroblastic tumours are rare inflammatory processes that may occur in the salivary glands primarily as a result of injury. They are composed of lymphocytes, plasma cells, histiocytes, and myofibroblasts, which is in sharp contrast with the monotonous lymphoplasmocytic infiltrate of chronic sclerosing sialadenitis. Benign lymphoepithelial lesions are seen in Mikulicz syndrome, which is a bilateral enlargement of the salivary and lacrimal glands (13). The swelling of the salivary glands is usually symmetric. Mikulicz disease is part of a more complex syndrome known as Sjogren's syndrome, which also includes keratoconjunctivitis, xerostomia, and rheumatoid arthritis. The main histological features are lymphoid infiltration and epimyoepithelial islands, which consist of solid nests of prominent myoepithelial proliferation infiltrated by a mixed population of B and T lymphocytes. Therefore, the presentation and histological features of Mikulicz disease are different from chronic sclerosing sialadenitis.

Chronic sclerosing sialadenitis is a totally benign inflammatory lesion, and until now, there have been no reports of malignancy. In fact, chronic sclerosing sialadenitis has often been removed, and no additional treatment has been required (14). This disease is rare and most commonly occurs in the submandibular gland (15,16). We present a very rare case of chronic sclerosing sialadenitis, which affected the parotid gland as a solitary mass.

Authors' Contributions

Provision of patient, collection and assembly of data: OA
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Critical revision of the article: SK

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Case Report

Perineal Myxoid Liposarcomas: A Case Report and Literature Review

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Abstract

Myxoid liposarcoma is the major subtype of liposarcoma and commonly presents in the extremities, particularly in the thigh. We introduce an unusual case of a myxoid liposarcoma presenting as a large perineal swelling occupying the para-rectal and para-anal spaces in a 49-year-old male patient. The diagnosis, management, and prognosis of myxoid liposarcoma are discussed. A literature review is performed for myxoid liposarcoma.

Keywords: *: myxoid liposarcoma, oncology, perineum, soft tissue sarcoma, surgery, tomography*

Introduction

Liposarcomas are rare soft tissue tumours, with an incidence of 30 cases per 1 million individuals. Myxoid liposarcoma (MLS) is a major subtype of these liposarcomas (1). It represents approximately 30%–50% of all the liposarcomas. Most patients present between the ages of 18 and 67 years, with a mean age of 42 years (2–4). MLS has the potential for overt malignant behaviour, and therefore determination of clinical behaviour and pathological subtype is invaluable to the management of these patients. We report a rare presentation of perineal MLS. The diagnosis, management, and prognosis will be discussed.

Case Report

A 49-year-old Malay man presented with a swelling in the right perineal region of 1 year's duration. When initially noticed, the swelling was thumb-sized but had increased gradually over the next few months to the size of a fist. The swelling was associated with a mild, dull aching pain. He gave no history of bowel or other constitutional symptoms.

Clinical examination revealed a swelling extending from the right perineum laterally to the right gluteal region, with extension anteriorly to the base of the penis and posteriorly to the tip of the coccyx. It measured approximately 16 x 10 cm. The clinical impression was that the tumour was in the subcutaneous plane and had the consistency of a lipoma. Digital rectal examination revealed the presence of an extra luminal compression over the right and posterior aspect of the lower rectum.

The patient was claustrophobic and therefore refused a magnetic resonance imaging (MRI) examination. A computed tomography (CT) scanning of the abdomen and pelvis was performed. It revealed a well-defined, multi-loculated mass arising posterior to the right seminal vesicle. The mass extended inferiorly into the mesorectum and right ischioanal space. It compressed and displaced the rectum to its left. The mass extended towards the symphysis pubis inferior to the shaft of the penis. The maximum cranio-caudal width and anterior–posterior dimensions were 16 x 8 x 14 cm. The mass appeared predominantly cystic with areas of septation (Figure 1). Based on these findings, the differential diagnosis of lymphangioma or a tailgut cyst was considered.

In view of the radiological diagnosis, a biopsy was not taken. After consultation with the patient, a decision was made to operate. Wide excision of the tumour was performed via a perineal approach by the colorectal surgeon. Intra-operative findings revealed a well-encapsulated, multi-loculated tumour with areas of admixed fatty, cystic, and solid components.

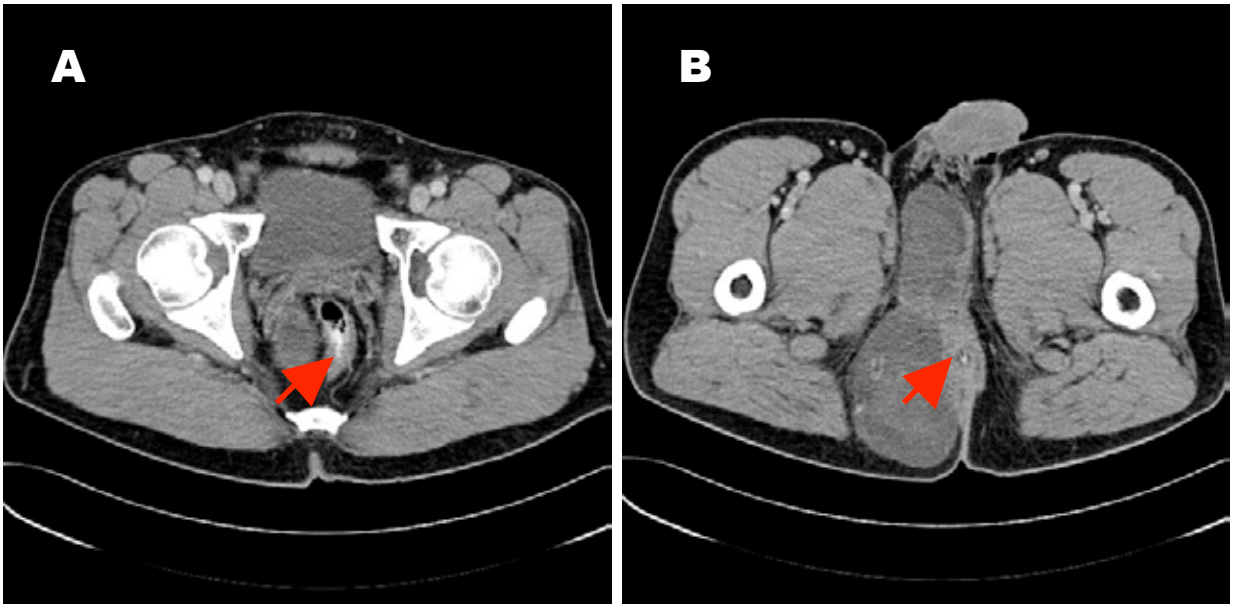

Figure 1: Computed tomography images showing a well-defined, multi-loculated tumour which appeared predominantly cystic with areas of septation. (A) Tumour compressing the rectum (arrow) and occupying the mesorectal space. (B) Tumour displacing and compressing the anus (arrow).

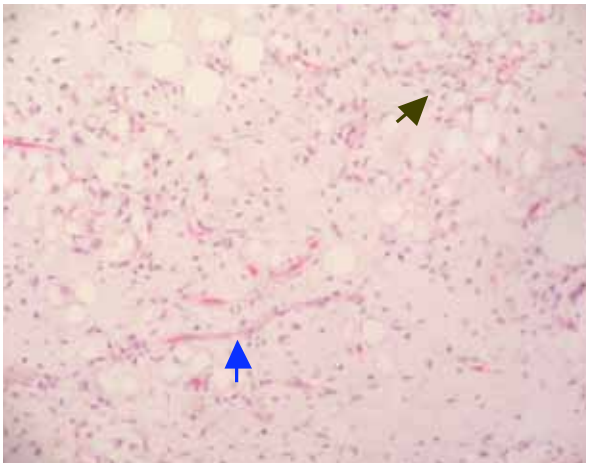

Figure 2: Low-power view of myxoid liposarcoma showing non-lipogenic mesenchymal cells and signet ring lipoblasts (black arrow) in myxoid stroma, with delicate arborizing "chicken wire" capillary vasculature(blue arrow). (Haematoxylin and eosin, 10x magnification)

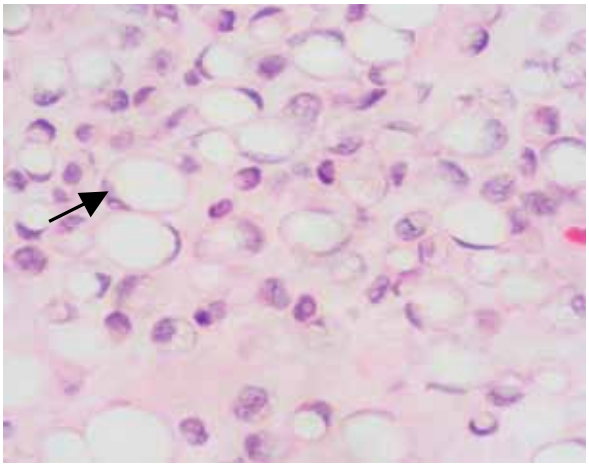

Figure 3: Signet ring lipoblasts (black arrow). (Haematoxylin and eosin, 40x magnification)

The histological examination revealed round to oval mesenchymal cells and signet ring lipoblasts in a myxoid stroma (Figure 2). The cells had hyperchromatic nuclei with conspicuous nucleoli and scanty cytoplasm (Figure 3). The stroma was rich in delicate, arborizing capillary vasculature, which is also described as a "chicken wire" appearance (Figure 2). The tumour, with predominantly myxoid matrix and lipoblasts, conformed to the diagnosis of MLS. The round cell component comprised 10% of the cell field. The resected margins were close.

The patient was subsequently referred to the oncologist for adjuvant therapy. He was given radiotherapy consisting of a total of 65 Gy over 45 fractions to the tumour bed and 10 Gy to the operative scar. The patient was followed up every 6 weeks for the first 6 months and every 3 months thereafter. On his first 6-month follow up after

surgery, clinical examination and CT scan showed no local recurrence or distant metastasis.

Discussion

Liposarcoma is the second most common soft tissue tumour, and MLS is a major subtype, consisting of 30%–55% of these tumours (2,3). MLS commonly involves the thigh and retroperitoneum, but it is relatively rare in the perineum or pararectal spaces (1,2). Clinically, these tumours present as painless swellings that progressively increase in size. They usually grow to a considerable size before they become symptomatic. In the case of our patient, he remained asymptomatic even though the tumour occupied his right pararectal and perianal spaces with the displacement of the anus.

A multi-imaging technique approach is often necessary for an accurate pre-operative evaluation, diagnosis, and staging of MLS. This approach helps to ensure curative resection with adequate surgical margins, thereby minimizing the risk of local recurrence. MLS presents a unique radiological challenge. The tumours exhibit a spectrum of MRI signal intensities and CT attenuation. A major contributing factor is the low fat content (less than 10%–25% of the tumour volume), while other contributors are the quantity of mucinous and myxoid materials, vascularity, and necrosis within the tumour (4). These tumour characteristics explain why the signal is close to that of water on non-enhanced CT and MRI scans (4). As encountered with this patient, the administration of contrast produced little enhancement on CT. Hence, the images mimic those of a cyst (2,4).

Gadolinium-enhanced MRI is the imaging of choice for MLS, where cystic or necrotic lesions are distinguishable from solid or cellular lesions (1,2). Sung et al. reported that 96% of MLS appeared as solid masses with varying patterns of enhancement using gadolinium-enhanced MRI (4). On MRI, the enhancement patterns are classified into homogeneous, heterogeneous, and no enhancement (4). Unfortunately, due to the patient's claustrophobia, the MRI scan was not performed. In his case, the use of ultrasonography, especially endoanal ultrasonography, might have been useful to differentiate the solid from cystic components (2). Despite the shortcomings in imaging this patient, adequate pre-operative assessment of the tumour extent was obtained. This assessment allowed the planning and positioning of the incision and resection of the tumour with the preservation of the pelvic organs and continence.

Surgery remains the mainstay of treatment for MLS (1,2). An adequate margin is required to prevent local recurrence, as it is associated with a 2.8-fold risk of disease mortality (5). A wide surgical margin should always be attempted, unless there are vital structures in proximity; in these circumstances, post-operative radiotherapy is recommended for adequate local disease control (1,5). In the present case, the tumour proximity to the anus prevented a wide surgical margin.

The classical histological appearance of MLS is characterized by the presence of myxoid matrix with a signet-ring type lipoblast and a capillary network that creates the appearance of "chicken wire" (7). When MLS is associated with a round cell component that exceeds 5% of the tumour, it is considered a round cell liposarcoma, RCLS (5). Currently, the pure MLS and RCLS are generally considered to be of the same subtype; pure MLS is described as being of low grade with low metastatic potential, whereas the RCLS represents the opposite end of the spectrum (5,7). The demonstration of chromosome translocation t(12;16)(ql3;pl1) in both MLS and RCLS serves as genetic evidence for the hypothesis that they are of the same subtype (5).

There is no specific grading or staging system for liposarcomas; they are broadly graded and staged as soft tissue sarcomas. Among the various scoring systems available are the American Joint Committee on Cancer (AJCC) and Enneking staging systems (6,9). The AJCC system (Table 1) is commonly used on account of its reproducibility and reliability (8). The factors considered in the grading of these tumours in the AJCC system are cytological atypia (differentiation), tumour necrosis, and mitotic counts. In this system, the grading is considered low (I, II) or high (III, IV) only (8). This reduces intra- and inter-observer variations of grading and its effects on the tumour staging.

The rates of local recurrence for MLS are relatively high at about 40%–50% of the cases. Pure MLS has a 20% rate of metastasis, which compares to its round cell subtype that metastasizes in almost 35%–70% of the cases. Common metastatic sites include lungs, bones, and occasionally, the serosal surfaces of the pleura, pericardium, and peritoneum (1,2). The 5 and 10 year survival rates are 80% and 50%, respectively (3,5). A major predicting factor for local recurrence is the surgical margins; for metastasis, the factors are the histological grade and the percentage of round cells (1,5).

Table 1: American Joint Committee on Cancer (AJCC) staging of soft tissue sarcoma (8)

Stage	Grade	Tumour Size and Location	Nodal Involvement	Metastasis
IA	Low	≤ 5cm	-	-
IB	Low	>5cm, superficial	-	-
IIA	Low	≥5cm, deep	-	-
IIB	High	≤ 5cm	-	-
IIC	High	>5cm, superficial	-	-
III	High	>5cm,deep	-	-
IVA	Any	Any	+	+
IVB	Any	Any		

There is no consensus on the use of neo-adjuvant and adjuvant therapies in the form of chemotherapy or radiotherapy for non-metastatic soft tissue sarcomas (9). Surgery is therefore followed by an adjuvant radiation therapy only if the resection margins are deemed inadequate, as was the case in this patient. Chemotherapy with doxorubicin and ifosfamide is usually reserved for treatment of metastatic disease (7,10),. The use of chemotherapy for high-grade tumours and local recurrence is controversial (9). MLS is relatively more chemo-sensitive when compared to other soft t issue sarcomas as a group (7). After the completion of treatment, a scheduled follow-up should be commenced in the form of clinical examinations and imaging (preferably MRI) performed every 6 months for 5 years and then annually for at least 5 years after (1).

Conclusion

MLSs are rare soft tissue sarcomas that have benign clinical presentation. Pre-operative radiological diagnosis is crucial for staging and surgical planning. Surgery remains the mainstay of treatment for both non-metastatic and metastatic disease. The quality of the surgical margins greatly affects the patient's prognosis. Proper histological typing and grading will help determine the metastatic potential of this sarcoma. These factors will further aid in detection of local recurrence and distant metastasis.

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Case Report

Fatal Airbag-Mediated Atlanto-Occipital Dislocation in a Child

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Abstract

An atlanto-occipital dislocation is a rare airbag-induced injury in trauma patients. We report a case of an atlanto-occipital dislocation in a 6-year-old patient who was an unrestrained passenger in the front seat of a vehicle involved in a low-speed motor vehicle accident. This case illustrates the fatal threat of airbag deployment to the child passenger travelling in the vehicle front seat even in a low-speed collision, and supports the recommendation that children under 12 years of age travelling in vehicles with dual airbag systems should be seated in the back.

Keywords: *air bags, atlanto-occipital joint, child, dislocation, traffic accidents, trauma*

Introduction

The dual-airbag system introduced in passenger vehicles has been proven to be effective in preventing fatalities among front-seat adults involved in motor vehicle accidents (1). However, this same system poses a potential threat to the front-seat child passenger even in the event of a low-speed collision (2). The bag inflates quickly from a chemical explosion, which has been likened to a collision at 300 km/h (3). This explosion can cause fatal injuries to the head and cervical spine. Atlanto-occipital dislocation is a serious and often fatal type of injury that can be caused by airbag deployment (4).

Case Report

A previously healthy 6-year-old girl was travelling with her family in a car. She was unrestrained and seated in the front passenger seat. The family was trapped in a traffic jam and the car was moving slowly, closely tailing a car in front of it. The car struck the rear end of the vehicle in front when it braked suddenly. The child was thrown forward towards the dashboard, and the impact deployed the airbag, which hit her on the face, causing her to immediately be knocked unconscious. The driver, who was her father, was not sure whether she actually hit the dashboard or not. No other people were hurt in the accident, including a 6-month-old baby who was on the mother's lap seated in the rear seat. The 6-year-old was sent to the nearest private hospital about 15 minutes later. Upon arrival in the emergency department, clinical assessment revealed a Glasgow Coma score of 3. She was hypotensive, apnoeic, and flaccid with bilaterally dilated and sluggish pupils. She also had a large laceration wound on her chin. Otherwise, there were no external injuries. Her abdomen was distended. Cardio-pulmonary resuscitation was performed for about 45 minutes. A bedside ultrasound of the abdomen showed the presence of free fluid. She was clinically diagnosed with an intra-abdominal injury with hypovolemic shock and hypoxic encephalopathy. She was rushed to the operation theatre and an emergency laparotomy was performed. Intraoperatively, there was a splenic laceration, which involved the splenic hilum. This laceration resulted in an estimated blood loss of 2 litres. The rest of the intraperitoneal structures were intact. The laceration wound at the tongue and chin was also sutured during the operation. Post-operatively, her condition remained the same, and she was referred to Hospital Tengku Ampuan Afzan for further management 2 days later. Computed tomography (CT) of the brain and cervical spine was done in our hospital. The head CT showed extensive subarachnoid haemorrhages in both cerebral hemispheres with generalised effacement of the cerebral sulci, a loss of grey and white matter differentiation and obliteration of the lateral ventricles and suprasellar cistern (images not shown). The cervical CT revealed an atlanto-occipital dislocation with a basion dental distance (BDI) measuring 25 mm and a basion

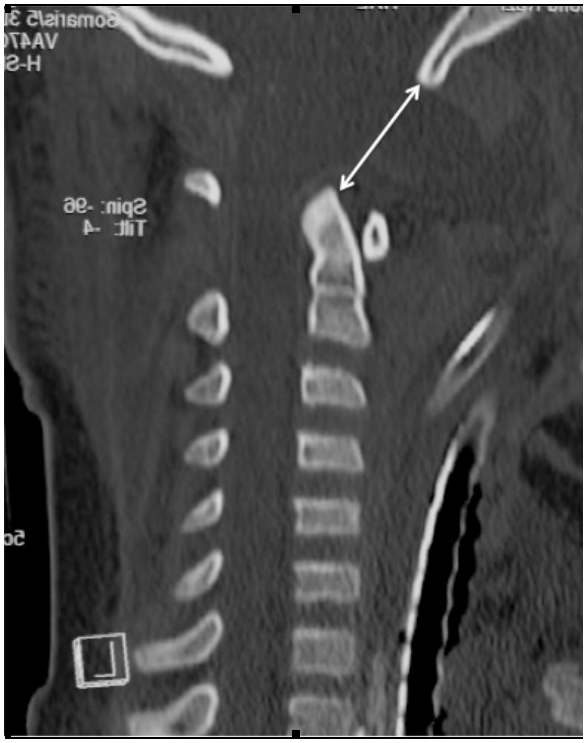

Figure 1: Mid-sagittal reformatted computed tomography scan of the cervical spine in the bone window setting demonstrates widening of the atlanto-occipital joint with a basion-dens interval (arrow) measuring 25 mm.

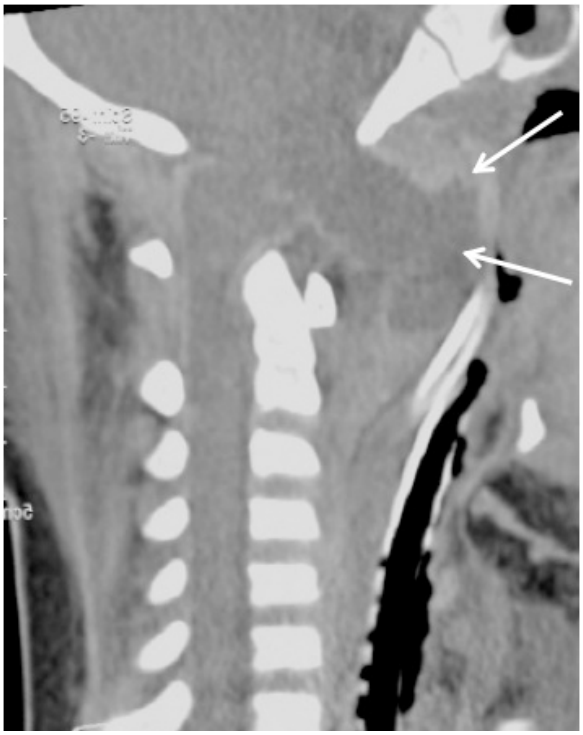

Figure 2: Mid-sagittal reformatted computed tomography scan of the cervical spine in the soft tissue window showing pre-vertebral soft tissue swelling. The arrows show the fluid density area within this pre-vertebral swelling, which was suggestive of cerebrospinal fluid leakage.

axial interval (BAI) measuring 22 mm (Figures 1 and 2). There was no fracture of the cervical spine. The patient died 5 days after the accident due to multiple injuries.

Discussion

Airbag deployment systems in passenger vehicles were introduced for extra protection in addition to seatbelt use. Airbags are designed to cushion the adult's head and chest in a crash event. Numerous studies have confirmed that airbags reduce fatalities in frontal car crashes among the 13 years and older age group (1). However, they pose a potential threat to the front-seat child passenger even in the event of a low-speed collision involving a child properly restrained with a safety device (2).

When there is a frontal crash, airbag deployment can occur even during a low-speed collision ranging 13–62 km/h with an average of 27 km/h (4). The airbags inflates quickly from a chemical explosion—within 0.05 seconds or less—at speeds likened to a collision at 300 km/h (3). This forward-facing front-seat child victim was propelled forward toward the dashboard at the time of collision. If unbelted, the child may achieve a near-standing posture and will be closer to the airbag when it deploys (4). The face and frontal cranium receive the first impact, followed by violent hyperextension of the head and neck as the bag inflates and propels the child toward the rear end of the vehicle. The child may also be subjected to upward-directed forces, depending on the angle of deployment from the dashboard, and this might explain the wide separation of the atlanto-occipital joint and the associated soft tissue injuries to the chin and tongue seen in this patient. This severe form of cervico-cranial injury in paediatric patients as a direct result of a passenger airbag deployment occurring in a low-speed collision deemed otherwise survivable has been reported previously (4,5). However, the

mechanism of injury causing splenic laceration in this patient was unclear. It might have occurred from the impact of blunt trauma to her body from any parts of the vehicle before or after the airbag deployment.

An atlanto-occipital dislocation is a rare airbag-induced injury. The exact epidemiology of atlanto-occipital dislocation is difficult to establish because lethal cases are not autopsied and reported in the medical literature (5). It is normally fatal when it occurs; however, increasing numbers of survivors have been reported. This increase is attributed to the improvements in on-site resuscitation, rapid transportation to the hospital with head support, and increasing awareness of this injury. The injury has been noted to be three times more common in children than in adults (5). Children are more vulnerable to this type of injury. The immature spine is hyper mobile because of ligamentous laxity, shallow and angled facet joints, underdeveloped spinous processes, and physiologic anterior wedging of vertebral bodies. Incomplete ossification of the odontoid process, a relatively large head, and weak neck muscles are other factors that predispose the joint to instability in this age group (6). In adults, this dislocation is frequently accompanied by a cervical bone fracture. However, in children, as in this case, vertebral body fracture is uncommon because of the inherent elasticity of the juvenile spine.

The diagnosis of the atlanto-occipital dislocation is often difficult because inadequate initial neurological assessments are made due to concomitant head injuries, blunt thoracic, and abdominal trauma and fractures of the limbs. A high degree of suspicion is mandatory in children who have experienced airbag deployment (5).

There are a variety of measurements proposed for the diagnosis of atlanto-occipital dislocation on radiograph and computed tomography, each with different specificities and sensitivities (7–9). The basion-axial interval (BAI) and basion-dental interval (BDI) method proposed by Harris et al. is recommended. The BAI is the distance, either anterior or posterior, between the basion and the rostral extension of the posterior cortical margin of the body of the axis (known as the posterior axial line). The normal BAI extends from 12 mm anterior to 4 mm posterior to the posterior axial line. The BDI is the distance between the basion and the tip of the dens and normally does not exceed 12 mm (7). Pre-vertebral soft tissue swelling at the occipitocervical junction is a consistent finding on lateral radiograph (10). However, many cases of atlanto-occipital dislocation are missed using only plain lateral cervical spine X-rays. The shortcomings of this approach are related to the magnification factors, variations in the position of the neck, and concomitant fractures of the atlas and axis. In paediatric patients, this is more challenging, and the identification of landmarks is more difficult due to the variability of bone ossification in the craniocervical junction (6). High-resolution CT with reformatted coronal and sagittal images is the imaging study of choice when there is suspicion of atlanto-occipital dislocation. Subarachnoid haemorrhage at the craniocervical junction is often associated with atlanto-occipital dislocation and should raise the suspicion of severe craniocervical ligamentous injury (11).

There is insufficient evidence to support treatment standards and guidelines for the management of atlanto-occipital dislocations (12). Without treatment, nearly all patients develop neurological worsening, and some did not recover. Traction and external immobilisation have been used successfully in some patients; however, transient or permanent neurological worsening and late instability have been reported more often with these techniques compared to surgical treatment. Craniocervical fusion with internal fixation is recommended for the treatment of patients with acute traumatic atlanto-occipital dislocation.

This case demonstrates the perils of sitting a young patient less than 12 years old in the front passenger seat of a vehicle with dual airbag systems. Fatal injuries can occur even in the event of a low-speed impact.

Authors' Contributions

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Case Report

Multiple Metastatic Deposits in the Head and Neck Region from a Renal Cell Carcinoma

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Abstract

Metastatic renal cell carcinoma (RCC) presenting with multiple deposits in the head and neck region is unusual. It is not uncommon for a RCC to metastasise to a distant site after years of a tumour-free period, but most of it would be expected to have a single site of deposit. We report a rare case of a patient who had a nephrectomy 10 years earlier for RCC and presented with tumours in the frontal sinus and posterior pharyngeal wall. Radiological imaging and histology confirmed metastatic RCC at both sites.

Keywords: *cancer of the head and neck, diagnostic imaging, neoplasm metastases, oncology, renal cell carcinoma, signs and symptoms*

Introduction

Renal cell carcinoma (RCC) is the most frequent urological malignancy in adults and has a male preponderance. It accounts for approximately 3% of adult malignancies and 90%–95% of neoplasms arising from the kidney. Metastases have been reported to develop 17 years or more after the primary lesion is removed (1). RCC usually metastasises to lungs, bones, and regional lymph nodes, but very rarely to the head and neck region (2). We report a very rare presentation of a male patient, previously diagnosed with RCC, with metastasis in the head and neck region.

Case Report

This case was of an 80-year-old man diagnosed with RCC at stage T3N0M0 in 1998. He underwent a left radical nephrectomy and was well during the 10-year follow-up, with no evidence of recurrence. In July 2008, he presented with a swelling at the left frontal region, which had persisted for 9 months and was progressively increasing in size and associated with left proptosis. However, there was no reduced vision, diplopia, persistent headache, nasal obstruction, or epistaxis. In addition, he complained of a foreign-body sensation in the throat for the previous month, but without odynophagia, dysphagia, or hoarseness. Physical examination revealed a large left frontal mass, measuring 6 x 5 cm, soft to firm in consistency, non-tender, and with normal overlying skin. It caused displacement of the left eye inferolaterally. Nasal endoscopy showed a mass at the left frontal recess pushing the middle turbinate medially and uncinate process laterally. Another mass was found at the posterior pharyngeal wall. A biopsy was taken from the nasal mass during the clinic visit. This resulted in profuse epistaxis requiring a nasal packing. The histological report demonstrated clear cell carcinoma showing malignant cells that exhibited large pleomorphic nuclei with conspicuous nucleoli and abundant, clear cytoplasm. Immunohistochemistry showed that the tumour cells expressed cytokeratin (CK), CD10, EMA, and vimentin. Magnetic resonance imaging (MRI) of the brain showed a large frontonasal tumour involving the ethmoid sinus and both frontal sinuses, with intraorbital and intracranial extension (Figure 1). Computed tomography (CT) of the neck showed a contrast-enhanced 1 x 1 cm mass at the posterior pharyngeal wall (Figure 2). The patient underwent an endoscopic-assisted craniofacial resection of the tumour with pre-operative embolisation because of the high vascularity of the tumour on imaging. Intraoperative findings revealed a large, vascular, soft tumour involving the left frontal and both

frontal sinuses. It had eroded the lamina papyracea on both sides and invaded the dura. Because of the extensive involvement of the surrounding tissues, a tumour-debulking surgery was performed. The tumour was removed piecemeal from its posterior extension via craniofacial resection to its inferior extent via endoscopic approach. Orbital exenteration was not performed because the patient did not consent

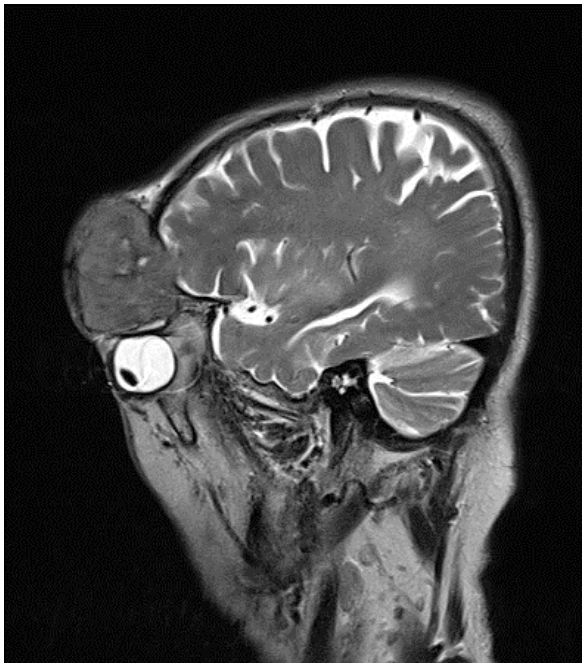

Figure 1: Brain T2-weighted magnetic resonance image (sagittal view), showing the frontonasal tumour (arrow) with intraorbital and intracranial extension.

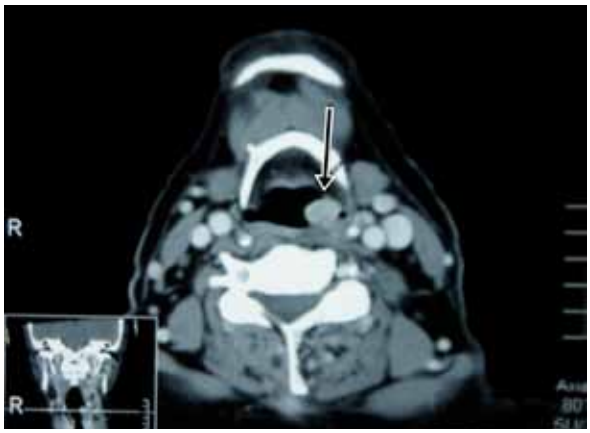

Figure 2: Neck computed tomogram image showing a laryngeal mass at the posterior pharyngeal wall (arrow).

to it. A direct laryngoscopy showed a vascular tumour measuring 1.0 x 1.5 cm with a pedicle arising from the posterior pharyngeal wall. It was excised completely. Post-operative recovery was uneventful, and the patient was discharged on the seventh post-operative day. Histopathological examination from the frontonasal and the posterior pharyngeal tumours confirmed the same histology (Figure 3) and immunophenotype (Figure 4) as in the earlier biopsy. On follow-up, there was residual tumour at the left frontal recess. Surgery was recommended for excision of the residual tumour. However, the patient refused further treatment.

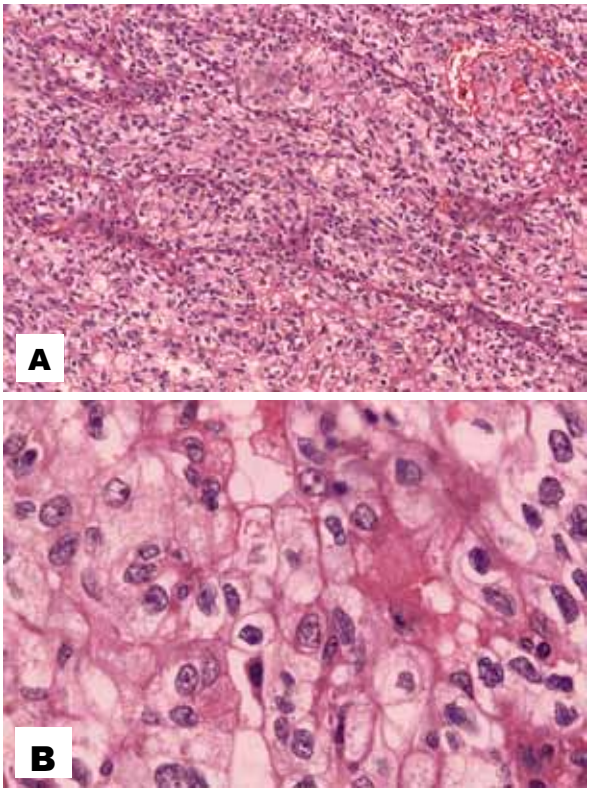

Figure 3: Haematoxylin and eosin stain of the tumour specimen. (A) A low-power view (10x magnification) showed groups of neoplastic cells separated by fibrous septa with intervening blood vessels. (B) The cell morphology is better appreciated in the high-power view (40x magnification), which clearly revealed the neoplastic features of the cells with pleomorphic nucleus, some with conspicuous nucleoli and abundant, clear to eosinophilic cytoplasm.

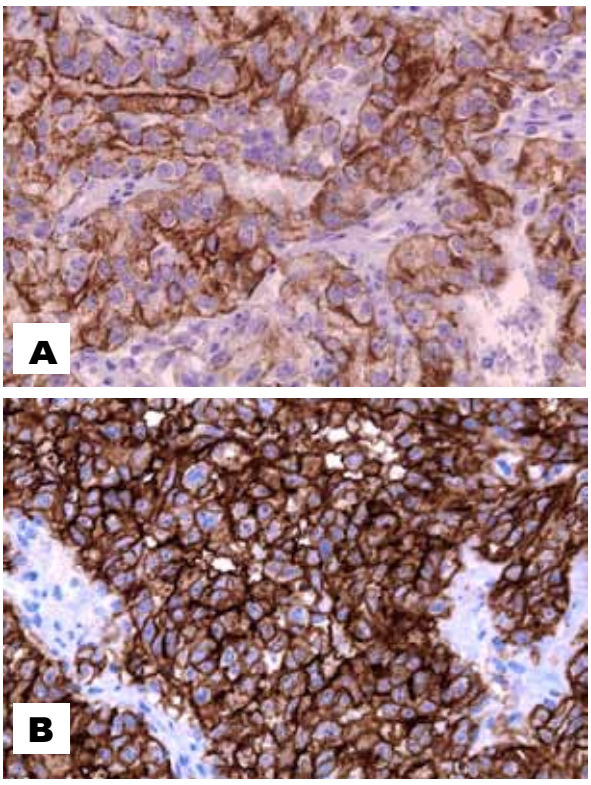

Figure 4: Immunohistochemical stains of the tumour specimen showed that the neoplastic cells were strongly positive for (A) cytokeratin and (B) CD10.

Discussion

In patients with RCC, tumour stage, regional lymph node status, tumour size, nuclear grade, and histological tumour necrosis show statistically significant associations with metastasis (3). RCC has frequently been reported to metastasise to the lung, liver and bone, whereas metastases to the head and neck region constitute only about 15% of cases (2). Common sites for metastasis in the head and neck region include paranasal sinuses, thyroid, larynx, mandible, and parotid glands (1), and they are usually present at a single site. Previously resected RCC that metastasises to multiple sites in the head and neck region is very rare. Extensive searches of the medical literature have, so far, not shown any reported cases of multiple metastases in the head and neck region. Pritchyk et al. (4) reported 4 cases of metastatic RCC, all with single metastatic sites. In addition, Gottlieb and Roland (1) reported cases with single metastatic sites. Other studies have also reported single sites of metastasis in the head and neck region (5,6).

The clinical behaviour of metastatic RCC is unpredictable, and the signs and symptoms depend on the affected organ and the presence of local invasion. Metastatic deposits from RCC are vascular in nature and often bleed. The vascular stroma of metastatic RCC is responsible for the fact that the most common symptom of sinonasal lesions is epistaxis in 70% of cases (7). An office-based biopsy of the lesion could result in uncontrolled haemorrhage, as demonstrated in this patient. A contrast CT scan of the paranasal sinuses would have shown the highly vascular nature of the lesion. Once the vascular nature of the lesion is known, a biopsy should be performed in the operating room.

Clear cell carcinoma is the most common histological variant of RCC. The clear cells are rounded or polygonal with abundant cytoplasm, which contains cholesterol, cholesterol esters, phospholipids, and glycogen. These are dissolved during routine histological processing, creating a clear cytoplasm surrounded by a distinct cell membrane. Histopathological examination commonly shows a network of small, thin-walled blood vessels interlacing between the tumour cells. Immunohistochemical staining typically shows that the tumour is positive for CK, CD10, EMA, and vimentin. Other tumours may also contain clear cells; these include acinic cell carcinoma, mucoepidermoid carcinoma, odontogenic clear cell carcinoma, and metastatic clear cell carcinoma of the thyroid. Histochemical and morphological analysis is useful in the differential diagnosis of these lesions and the clear cell carcinoma of the head and neck.

Treatment should be individualised according to the tumour location and general health of the patient. Our patient was subjected to craniofacial surgery mainly to debulk the tumour mass and relieve compression to the brain and orbit. Historically, the role of surgery in metastatic RCC has been to diagnose and debulk the disease. A review of the literature revealed that excision of solitary metastatic lesions of RCC following nephrectomy results in 41% survival at 2 years and 13% survival at 5 years, regardless of the time interval between nephrectomy and diagnosis of the metastatic lesion (2). For small, localised disease at the nasal and paranasal region, endoscopic resection is recommended to decrease post-operative morbidity. However, for more extensive disease with local spread, such as intracranial or intraorbital extension, we believe that endoscopic-assisted craniofacial debulking is the treatment of choice to improve quality of life. This, however, is technically challenging and may result in an inadequate resection for palliation of

symptoms. Therefore, an open approach is more appropriate. Regardless of the approach, the goal of therapy should be an adequate local resection that allows for appropriate short-term palliation of symptoms, despite the poor long-term prognosis of the disease (4). This offers improved quality of life, may provide a chance for a cure for the head and neck metastasis, and is warranted based on the associated morbidity that may occur if the lesion is left untreated.

Other forms of treatment have been advocated for metastatic RCC. Immunotherapy with interferon (IFN)-α and interleukin (IL)-2 has been the mainstay of treatment for people with advanced and metastatic RCC. However, this treatment is frequently associated with unpleasant side effects and confers only modest benefits. Recent advances in understanding the biology and genetics of RCC have led to several novel, targeted approaches to treat metastatic RCC, with higher response rates. Treatment with sunitinib (oral multi-targeted tyrosine kinase inhibitor), sorafenib, bevacizumab/erlotinib (recombinant humanised monoclonal antibody), and CCI-779 (temsirolimus) have shown promising results in terms of progression-free survival (8). Previously, hormonal treatment and standard chemotherapies have been described, but they are generally not considered effective due to high levels of drug resistance (9).

In conclusion, metastasis of RCC to multiple sites in the head and neck region is rare. Awareness of other metastatic sites in this region is required, and careful evaluation should be done to identify these metastatic deposits. Although surgery may not be adequate for tumour clearance, it is crucial to palliate symptoms and to reduce the morbidity associated with it.

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