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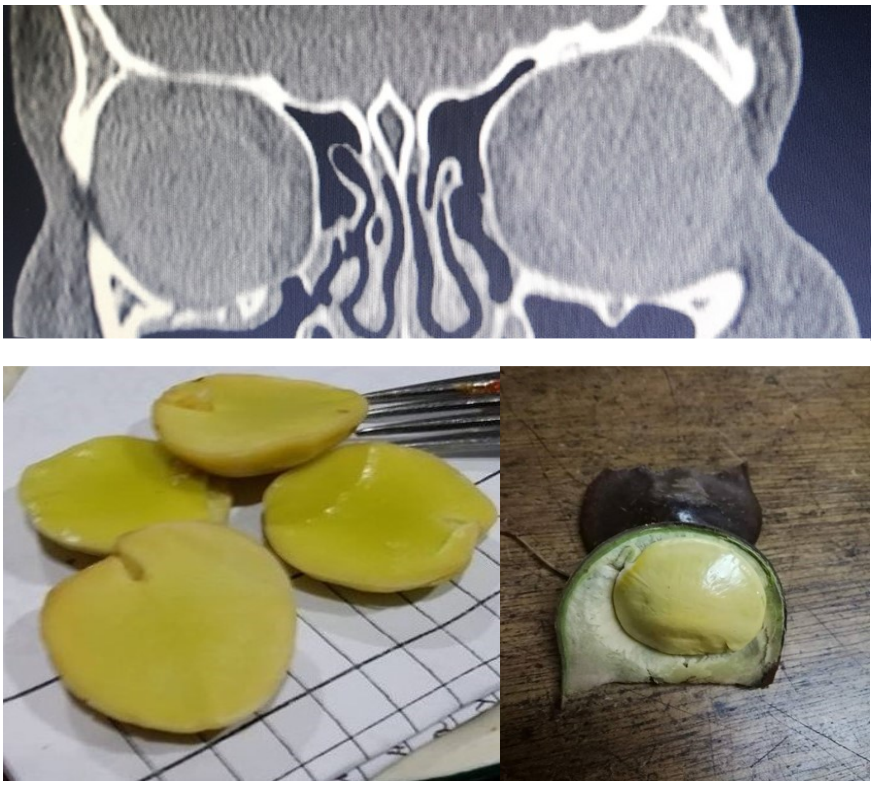

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Original Article **Brunei Int Med J.** *2017; 13 (6): 180-193*

Expression of skin Glyoxalase-I, advanced glycation end products (AGEs) and receptor (RAGE) in patients with long-term type 1 diabetes and diabetic neuropathy.

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ABSTRACT

Background: Certain group of diabetic patients have been shown to remain free of diabetic complications despite having had diabetes for longer periods. Advanced glycation end products (AGEs), their receptor (RAGE) and Glyoxalase-I (GLO-I) have been implicated in the development of diabetic neuropathy. **Objective:** To assess the effect of long-term type 1 diabetes mellitus on skin distribution and expression of AGEs, RAGE and GLO-I and to correlate these expressions with measures of small and large nerve fibre damage. **Methods:** Sixty-seven patients with type 1 diabetes mellitus of shorter (<15 years, n=20), intermediate (15 -40 years, n=25) and longer (>40 years, n=22) duration and 34 non-diabetic controls underwent diabetic neuropathy assessment: Neuropathy disability score (NDS), quantitative sensory testing (QST) including vibration pressure and thermal thresholds, nerve conduction studies (NCS), deep breathing heart rate variability (DB-HRV), corneal confocal microscopy (CCM) and intra-epidermal nerve fibre density (IENFD) and AGEs, RAGE and GLO-I expression in foot skin biopsies. **Results:** Compared to controls, type 1 diabetes mellitus patients showed progressively increased skin expression of AGEs, RAGE but progressively lower GLO-I expression with increasing duration of diabetes. Thus patients with longer-duration diabetes demonstrated significantly higher skin AGEs and RAGE but lower GLO-I expression than both shorter and intermediate-duration diabetic groups. In patients with longer-duration diabetes who developed diabetic neuropathy, the skin expression of AGEs and RAGE were significantly higher but GLO-I were significantly lower than those who did not develop diabetic neuropathy. These expressions also correlated with IENFD, CCM and NCS measures. **Conclusion:** Patients with type 1 diabetes mellitus showed progressively increased skin expression of AGEs, RAGE but progressively lower GLO-I expression with increasing duration of diabetes. Patients with longer-duration diabetes who developed diabetic neuropathy have significantly higher skin AGEs and RAGE and decreased GLO-I expression suggesting a potential role for these macromolecules as aetiological, marker of the disease as well as therapeutic target for diabetic neuropathy.

Keywords: Advanced glycation end products, diabetes mellitus, type 1, diabetic neuropathy, receptor for advanced glycation end products, GLO-I protein, human.

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INTRODUCTION

Higher morbidity and mortality in patients with diabetes mellitus (DM) have been linked to several factors such longer duration of DM,

inadequate glycaemic control, impaired renal function, hypertension, and dyslipidaemia.[1,2] Combination of longer duration of DM with inadequate glycaemic control predisposes patients to development of long-term diabetic complications. Nevertheless, a proportion of diabetic patients have been shown to survive long period of DM and remained free of complications such as diabetic neuropathy (DN).[3–5] These patients constitute a unique group who possesses the endogenous protective factors that mediate longevity, resistance to developing diabetic complications and maintenance of endogenous β-cells function.[6] Studies on patients with long duration of DM are limited and interest in these long survivors of DM founded medal programmes in the US and UK for these survivors.[5]

DN is a common complication of DM, affecting around 50% of diabetic patients and a leading contributing factor for foot ulceration and subsequent amputation. Different questionnaires and tools are available to assess symptoms, signs and neurological deficit of DN. Techniques to evaluate large nerve fibre damage in DN encompass vibration perception threshold (VPT) and nerve conduction studies (NCS).[7] Small nerve fibre damage can be assessed using quantitative sensory testing, nerve biopsy, the less invasive skin biopsy and recently corneal confocal microscopy (CCM) measurements which has been shown to correlate with intra-epidermal nerve fibre (IENFD) loss.[8]

The pathogenesis of DN is complex. Mechanisms that linked hyperglycaemia with neurovascular damage include hexosamine pathway flux, enhancement of polyol pathway, excessive reactive oxygen species formation, protein kinase C, abnormal endothelial nitric oxide (NO) activity, enhanced formation of vascular endothelial factor (VEGF), and advanced glycation end products (AGEs) and their receptor (RAGE).[9,10] AGEs are formed under the influence of sustained hyperglycemia via non-enzymetic condensation reaction called Millard reaction which is initiated by the reaction of reducing sugars with free amino group of proteins. The most widely studied receptor for AGEs is RAGE and AGE-RAGE interaction leads to a series of inflammatory reactions mediated by Phosphatidylinositol-3 Kinase (PI-3K), Ki-Ras, and Mitogen-Activated Protein Kinase.[11] AGEs can be detoxified in vivo by the glyoxalase system, the physiological line of defence against reactive dicarbonyls. The rate-limiting enzyme of the system is Glyoxalase-I (GLO-I) which converts methylglyoxal into D-lactate and reduced glutathione and thus prevents AGEs formation.[12]

There is increasing evidence linking AGEs, RAGE and recently GLO-I to the development and progression of diabetic complications including DN and recently to β-cells apoptosis.[11,13-16] In a study of 216 patients of the Diabetes Control and Complications Trial (DCCT) trial, skin collagen AGEs were lower in the intensive treatment group but not for Carboxymethyllysine (CML) and acid soluble collagen and independently associated with DN, retinopathy and nephropathy.[17] A recent study demonstrated that specific methylglyoxal concentration can differentiate between diabetic patients with painful and painless DN.[18] RAGE has been shown to be upregulated in DN.[15] Very recently, studies have reported that Glyoxalase-I activity was reduced in patients with DN and GLO-I upregulation reduced AGEs and RAGE expression and counteracted accompanying mitochondrial dysfunction.[16,19,20] The influence of DM duration on RAGE and GLO-I in DN is less clear. AGEs and RAGE have been assessed in tissues, plasma and via skin auto-fluorescence. However, plasma levels do not necessarily reflect tissue levels and skin auto-fluorescence has limitations.[21,22] This paper assessed the distribution and expression of skin AGEs, RAGE and GLO-I in the unique group of patients with more than 40 years of

DM with and without DN and correlated these expressions with small and larger nerve fibre damage measures.

METHODS

Patients

This prospective cross-sectional study recruited type 1 DM patients with duration of DM from <15 year to >40 years, from the Manchester Diabetes Centre, UK. Patients were excluded if they have non-diabetic causes of peripheral neuropathy, systemic disease like cancer, Addison's disease, heart failure, history of previous corneal trauma or surgery. A total of 67 type 1 DM patients were recruited and divided into 3 study groups based on duration of DM: shorter-duration of <15 years (n=20), intermediate-duration of 15-40 years (n=25) and longer-duration of >40 years (n=22). Thirty-four healthy non-diabetic volunteers were recruited as controls. These controls were either relative of patients or recruited from the same centre. The study was approved by Central Manchester Ethics Committee (2011/06/22, 354CMEC), and all participants gave informed consent upon recruitment.

Assessment of Neuropathy

DN diagnosis was based on the Toronto consensus as the presence of abnormal personal motor nerve conduction velocity (<42m/sec) and the presence of abnormal symptoms and signs of DN (Neuropathy Disability Score (NDS score) >2).[7] Symptoms of DN were evaluated in study participants using neuropathy symptom profile (NSP) and McGill visual analogue pain scale (McGill VAS).[23,24] Neurological functional impairments were assessed using NDS, quantitative sensory testing (QST) in form of warm (WT) and cold (CT) thresholds assessed by Medoc Neuro Sensory Analyser TSA-II (Medoc Ltd., Ramat, UK).[25] Vibration perception threshold (VPT) was evaluated by using a Neuroaesthesiometer (Horwell, Scientific Laboratory, Wilford, UK).[25] Autonomic nervous system function was quantified by obtaining deep breathing heart rate variability (DB-HRV) using a CASE IV machine (WR Medical Electronics, Inc, MN, USA). Nerve conduction studies (NCS) were undertaken by a consultant neurophysiologist using a Medtronic Keypoint™ EMG system and limb temperature was maintained constantly in a range of 32-35°C. Left Peroneal motor and Sural sensory nerves amplitudes (LSA and LPA) and conduction velocities (LPV and PMNCV) were quantified using silver-silver chloride electrodes according to the approved protocol. All participants were scanned with a laser CCM (HRT III-RCM Heidelberg Engineering GmBH, Heidelberg, Germany) using previously published method.[25] Three parameters were quantified using CCM: Corneal Nerve Fibre Density (CNFD) - total number of major nerves/mm^2; Corneal Nerve Fibre Length (CNFL) - total length of all nerve fibres and branches (mm/mm^2) and Corneal Nerve Branch Density (CNBD) - number of branches emanating from major nerve trunks/mm^2.

Immunohistochemistry

Skin biopsies were taken using 3-mm punch from the dorsum of the foot, 2cm proximal to the second metatarsal head with the use of 1% lidocaine anaesthesia. The samples were fixed in PBS-buffered (4%) paraformaldehyde for 18-24 hours immediately after collection. One sample was used for IENFD assessment on frozen sections while the second one was routinely processed to paraffin block for immunohistochemistry evaluation. Following washing in TBS buffer and graded solutions of sucrose for 2-4 h for cryoprotection, the specimen was frozen in liquid nitrogen, stored at -80°C and cut into 50 µm sections on a cryostat microtome (Microm HM450, Microm Int GmbH, Germany). Four floating sections from each patient were subjected to further processing. The non-specific staining and endogenous peroxidase activity were blocked by incubation in 5% goat serum and 0.3% hy-

drogen peroxide respectively. The sections were incubated with polyclonal rabbit anti-human PGP 9.5 neuronal marker IgG (Serotec Ltd, Oxford, England) followed by goat anti-rabbit secondary antibody and then by Horse-radish Peroxidase (HRP)-Streptavidin (both from Vector Laboratories, Peterborough, UK). Immunoreactivity was revealed by using SG chromogen (Vector Laboratories, Peterborough, UK). IENFD was calculated as the number of nerve fibres crossing the basement membrane of the epidermis per millimetre length of the epidermis.

Formalin-fixed paraffin-embedded tissue blocks were cut at 5μm thickness on a microtome (Leica Biosystems, Peterborough, UK) for AGEs, RAGE and GLO-I assessment. Following deparaffinisation of sections with xylene and rehydration in graded alcohols, antigens were unmasked with 0.1M citrate buffer pH 6.0 for antigen retrieval. To quench endogenous peroxidase activity, sections were immersed in Dako Peroxidase-Blocking Solution (Dako Ltd, Denmark) and nonspecific binding was blocked by the incubation of sections in 5% Normal Horse Serum (NHS) for AGEs and GLO-I or normal goat serum (NGS) for RAGE. Sections were incubated with primary antibodies goat IgG anti-RAGE (Millipore, CA, USA), rabbit polyclonal anti-AGEs IgG (Abcam, Cambridge, USA) or rabbit polyclonal anti-GLO-I IgG (GeneTex, CA, USA), all diluted in 5% respective sera at 4°C overnight in a humidified chamber. Sections were then incubated with secondary antibodies: biotinylated horse anti-goat IgG antibody (BA-9500, Vector Laboratories, Inc., CA, USA) for RAGE and biotinylated horse anti-rabbit IgG (Vector Laboratories, Inc., CA, USA) for AGEs and GLO-I (all diluted in 5% respective sera). Samples were incubated with HRP-Avidin (Vector Laboratories, Inc., CA, USA) in a humidified chamber. Immunoreactivity was revealed by incubation of sections with SG Chromogen (Vector Laboratories, Inc., CA, USA).

Negative controls comprised substituting the primary antibody with non-immune immunoglobulin at a concentration parallel to that of the primary antibody (DakoCytomation) which showed lack of immunostaining. In each experiment, five new slides from five cases immunostained in the previous experiment were re-stained and compared with the current experiment's staining. Only when the five pairs of sections showed identical staining then the latest experiment was accepted, otherwise the entire series was repeated. A semi-quantitative method was applied to quantify AGEs, RAGE and GLO-I expression using a light microscope under 400x magnification and identical light intensity. A score form 0-5 for staining intensity was used in which 0 represents lack of immunostaining and 5 represents 80-100% immunostaining. The best representation of scores in different anatomical locations was used as a visual aid. Immunostaining of AGEs, RAGE and GLO-I was assessed in skin epithelium, microvessels and extracellular matrix (ECM). Before commencing the semi-quantitative assessment, all sections were reviewed and the best representation of scores for each antigen in different skin structures was selected and used as a visual aid for the final assessment. All sections underwent blind assessment three times in random sequence by the investigator (ATA) to estimate intra-observer repeatability. The sections were also subsequently assessed blindly by an expert pathologist (MJ) to obtain inter-observer repeatability. The final results used for statistical assessment were reconciled scores between the two observers (ATA and MJ).

Statistical analysis. StatsDirect Version 2.7.8 (StatsDirect Ltd., Cheshire, UK) and SPSS 22.0 for Windows (SPSS, Chicago, IL) software were used to perform statistical analysis. Normality of data was assessed with Shapiro-Wilk test and relevant histograms. Normally distributed data were expressed as

Table 1: Demographics and clinical neuropathy assessment.

Variables	Control (n=34)	Type 1 DM		
		Shorter-duration (n=20)	Intermediate-duration (n=25)	Longer-duration (n=22)
Age (years) ‡	39.17±14.25	22.41±3.85¶§	35.51±8.16§	50.07±4.16¶
Duration of DM (years)		9.59±3.12§	27.78±6.07§	45.11±2.12
With DN No. (%)		5 (25%)	9 (34.4%)	12 (54.5%)
HbA1c (Mean ± SD) †	5.32±1.29	8.68±2.37¶	8.19±1.16¶	8.12±1.11¶
NSP(0-37) †	0.10±0.41	2.88±6.2	4.32±6.12¶	5.25±5.28¶
McGill VAS (0-10) *	0.15±0.49	2.25±3.41	2.7±3.62¶	2.29±2.91
NDS(0-10))‡	0(0-1)	0(0-0) §	4(2-5) ¶§	6.5(5.5-8.5) ¶
VPT R(V))‡	3.5(3-5)	4.35(3-8.25) §	8.83(6-13.5) ¶§	25.16(22.83-29.5)¶
CT(°C) ‡	28.75(27.9-29.7)	28.1(23.9-29.7) §	26.2(24.4-28.3)¶§	18.91(7.4-22.1) ¶
WT(°C) ‡	36.55(35.1-37.8)	39.2(37-42.4) §	38.9(37.3-42) ¶§	44.31(41.3-45.8) ¶
DB-HRV (beats per min) ‡	25.73±8.17	28.16±13.44§	20.24±12.01¶§	11.3±6.79¶
LSA (uV) ‡	18.52±7.26	13.24±5.34§	9.37±6.58¶§	7.82±3.74¶
LSV (m/s) ‡	50.5±4.07	44.86±2.03§	43±4.94¶§	35.19±6.92¶
LPA (m/s) ‡	5.82±2.07	5.29±2.43§	3.15±1.84¶§	1.24±1.32¶
PMNCV (m/s) ‡	49.0±3.92	41.11±3.43¶§	39.54±7.59¶§	34.26±9.56¶
CNFD (no/mm^2) ‡	37.27±6.06	28.45±5.71§	23.85±8.7¶§	16.24±9.58¶
CNBD (no/mm^2) ‡	94.44±3.98	58.93±32.5¶§	57.31±32.35¶§	42.63±33.23¶
CNFL(mm/mm^2) ‡	37.27±6.06	19.95±4.08§	18.77±6.36¶§	13.64±7.79¶
IENFD(no/mm^2) (mm) ‡	10.33±2.31	6.04±2.01¶§	4.05±1.81¶§	2.75±1.42¶
Cholesterol (mmol/l)	5.04±0.78	4.41±1.1	4.45±0.94	4.34±1.12
HDL(mmol/l) †	1.56±0.37	1.46±0.34§	1.41±0.44	1.7±0.95¶
TRIG(mmol/l)	1.43±0.68	1.64±0.79	1.01±0.44	1.7±0.43
LDL(mmol/l)	2.4±0.92	2.22±1.09	2.2±0.82	2.17±1.1

Results are expressed as mean ± SD or median (interquartile range). Statistically significant differences using ANOVA or Kruskal Wallis test: * P<0.05, † P<0.01, ‡ P<0.001, ¶ Post hoc (Tukey or Conover Inman test) results significantly different from control subjects, § Post hoc results significantly different from long duration group. DPN, diabetic peripheral neuropathy; NSP, Neuropathy Symptom Profile; McGill VAS, McGill Visual Analogue Scale; NDS, Neuropathy Disability Score; VPT, Vibration Perception Threshold; WT, Warm Threshold; CT, Cold Threshold; CIP, Cold Induced Pain; HRV, Hear Rate Variability; HRV-DB, Heart Rate Variability to Deep Breathing; LSA, Left Sural Amplitude; LSV, Left Sural Velocity; LPA; Left Peroneal Amplitude; PMNCV; Peroneal Motor Nerve Conduction Velocity; HDL, High-Density Lipoproteins; TRIG, Triglycerides; LDL, Low-Density Lipoproteins; CNFD (Corneal Nerve Fibre Density); CNBD (Corneal Nerve Branch Density); CNFL (Corneal Nerve Fibre Length); IENFD (Intra-Epidermal Nerve Fibre Density).

mean ± SD. Analysis of variance (ANOVA) was used to compare the means among the groups with Tukey test as a post hoc test. Non-normally distributed data were presented as median and interquartile range with Kruskal-Wallis test to compare groups and Conover-Inman test as a post hoc test. Correlations between variables were performed using Pearson correlation coefficient. Intra-observer and inter-observer repeatability was estimated using repeatability coefficient. P<0.05 was considered as statistically significant.

RESULTS

The prevalence of DN was higher in patients with longer-duration DM (54.5%) than those with shorter-duration (25%) and intermediate -duration (34.3%) DM groups (Table 1). The levels of HbA1c were comparable between groups of different duration of diabetes but higher than controls. DM patients with longer-duration diabetes demonstrated significantly higher NDS, VPT (P<0.001, P<0.01, P<0.001), WT (P<0.001, P<0.01, P<0.01) and lower CT (P<0.001, P<0.01, P<0.001) as compared to controls, shorter-duration and intermediate-duration DM groups respectively. NCS findings were consistent with small fibres measures and patients with longer-duration DM have significantly lower sural and peroneal

Table 2: Skin AGEs, RAGE and GLO-I in controls and patients with short to long duration of diabetes.

Variables		Control (n=34)	Type 1 DM		
			Shorter-duration (n=20)	Intermediate-duration (n=25)	Longer-duration (n=22)
AGEs	Epidermis ‡	2.17±0.38	2.40±0.75§	2.82±0.98¶§	3.58±1.40¶
	Microvessels ‡	2.20±0.55	2.72±0.70¶§	2.86±0.57¶§	3.51±1.18¶
	Endothelium ‡	1.26±0.44	2.00±0.78¶§	2.20±0.84¶§	3.09±0.14¶
	Basement Membrane ‡	2.05±0.54	2.13±0.64§	3.03±0.71¶§	3.71±1.22¶
	Papillary ECM ‡	1.87±0.84	1.70±0.53§	2.03±0.61§	3.00±1.21¶
	Reticular ECM ‡	1.93±0.59	2.57±0.9¶§	2.60±0.76¶§	3.63±1.18¶
RAGE	Epidermis ‡	2.07±0.72	2.93±0.74¶§	3.32±1.1¶§	4.09±0.94¶
	Microvessels ‡	2.15±0.57	2.73±0.78¶§	2.87±0.67¶§	3.85±1.28¶
	Endothelium ‡	2.09±0.59	2.40±0.84§	2.97±0.93¶	3.71±1.27¶
	Basement Membrane ‡	1.92±0.73	2.50±0.67¶§	2.62±0.65¶§	3.54±0.96¶
	Papillary ECM ‡	2.02±0.28	2.24±0.78§	2.61±1.24¶§	3.28±1.08¶
	Reticular ECM ‡	1.99±0.64	2.68±1.06¶§	3.10±0.72¶§	4.00±0.81¶
GLO-I	Epidermis ‡	3.64±1.22	2.90±1.08§	2.45±0.68¶	1.62±0.69¶
	Microvessels ‡	3.50±1.37	2.79±0.68¶§	2.57±0.75¶§	1.69±0.54¶
	Endothelium ‡	3.41±1.33	2.89±0.93§	2.31±0.75¶	2.08±0.56¶
	Basement Membrane ‡	3.25±1.06	2.29±0.62¶§	2.40±0.63¶§	1.58±0.7¶
	Papillary ECM ‡	2.33±1.40	1.91±1.12	1.29±0.61¶	1.50±0.90
	Reticular ECM ‡	2.94±1.57	2.53±0.84§	2.00±0.96	1.42±0.64¶

Results are expressed as mean ± SD or median (interquartile range). Statistically significant differences using ANOVA or
Results are expressed as mean ± SD or median (interquartile range). Statistically significant differences using ANOVA or
Kruskal Wallis test: † P<0.01, ‡ P<0.001, ¶ Post hoc (Tukey or Conover Inman test) results significantly different from control subjects, § Post hoc results significantly different from long duration group. ECM; Extracellular Matrix.

nerves amplitudes (P<0.001, P<0.001, P<0.01) and nerve conduction velocities (P<0.001, P<0.001, P<0.01) as compared to controls, short and intermediate duration groups respectively.

Lipid profile values were comparable between study groups apart from HDL, which was significantly higher in the longer-duration group than controls (P<0.01). As for IENFD, there was progressive reduction in IENFD as compared to controls (P<0.001), shorter-duration (P<0.01) and intermediate-duration (P<0.01) DM groups with increasing duration of DM and these differences were statistically significant. CM metrics namely CNFD, CNBD and CNFL also decreased significantly with increasing duration of DM (P<0.001, P<0.01, P<0.01 as compared to controls, short and intermediate duration groups respectively).

Intra-observer repeatability coefficients for immunohistochemical scores of AGE, RAGE and GLO-I in epidermis were 0.90, 0.87 and 0.85 respectively. Inter-observer repeatability coefficients for immunohistochemical scores of AGE, RAGE and GLO-I in epidermis were 0.88, 0.86 and 0.82 respectively.

Skin AGEs expression was significantly higher (P<0.001) in all patients compared to control in all skin structures assessed (epidermis, microvessels, endothelium, basement membrane and reticular ECM) apart from papillary ECM (Table 2). There was progressive increase of skin AGEs with increasing duration of DM in the epidermis (P<0.01, P<0.05), microvessels (P<0.05,P<0.05), endothelium (P<0.01,P<0.01), basement membrane (P<0.001, P<0.01), papillary ECM (P<0.001,P<0.01) and reticular ECM (P<0.001,P<0.01) in longer-duration DM group compared to shorter-duration and inter-

mediate-duration DM groups.

Similarly, skin RAGE expression was higher in patients than controls in all skin structures (P<0.001) (Table 2). There was progressive increase of skin RAGE with increasing duration of DM in epidermis (P<0.001, P<0.01), microvessels (P<0.001, P<0.001), basement membrane (P<0.001, P<0.01), papillary ECM (P<0.001, P<0.01) and reticular ECM (P<0.001, P<0.01) in longer-duration DM group compared to shorter-duration and intermediate-duration DM groups.

Skin expression of GLO-I was the inverse of those seen with AGEs and RAGE expression. Skin expression of GLO-I was significantly lower in patients than controls (P<0.001) (Table 2). Skin GLO-I progressively decreased with increasing duration of DM in epidermis (P<0.001) in longer-duration DM group compared to shorter-duration DM group, microvessels (P<0.01, P<0.01) and BM (P<0.01, P<0.01) in longer-duration group compared to both shorter-duration and intermediate-duration DM groups.

Patients with longer-duration DM were further stratified into those with and without DN and analysed (Table 3). Patients with longer-duration DM without DN exhibited significantly lower NSP, NDS, VPT, WT, DB-HRV and higher CT as compared to those with DN. Sural and peroneal nerves amplitudes and conduction velocities were also higher in patients without DN as compared to those with DN. CCM metrics were also consistent with other small nerve fibres measure. Scores of IENFD, CNFD, CNBD and CNFL were higher in patients without DN as compared to those with DN.

Skin expression of AGEs, RAGE and GLO-I in controls and Patients with longer-duration DM with and without DN are summarized in table 4 and figure 1. Patients with longer-duration DM without DN had significantly lower skin AGEs expression in the epidermis (P<0.01), microvessels (P<0.05), endothelium (P<0.01), basement membrane (P<0.01), papillary ECM (P<0.05) and reticular ECM (P<0.01) as compare to those with DN. Skin RAGE expression in patients without DN was significantly lower in epidermis (P<0.05), microvessels (P<0.01), endothelium (P<0.01), and basement membrane (P<0.01). Skin GLO-I expression was higher in patients without DN in epidermis (P<0.01), microvessels (P<0.01) and basement membrane (P<0.05) as compared to those with DN.

Skin AGEs and RAGE expression in multiple skin structures correlated directly (Supplementary page: Appendix I and II) and skin GLO-I expression correlated inversely (Supplementary page: Appendix III) with IENFD, CCM metrics and NCS findings.

DISCUSSION

In the current study, Patients with longer-duration (>40 years) DM exhibited more advanced large fibre damage as evidenced by their NDS, VPT and NCS findings as well as more advanced small fibre damage as indicated by their CT, WT, CCM and DB-HRV results. Interestingly, HDL-cholesterol was higher in patients with longer-duration DM which is in agreement with another study and this could provide one explanation for the long survival of these patients.[5]

This study is the first to report skin distribution and expression of the combined set of AGEs, RAGE and GLO-I in patients with longer-duration type 1 DM who has undergone detailed assessment of neuropathy. Skin AGEs expression was higher in patients with longer-duration DM as compared to control subjects and shorter-duration or intermediate-duration DM. There was progressive increase of AGEs expression with increasing DM dura-

Table 3: Demographics and clinical neuropathy assessment in longer-duration (>40 years) DM patients with or without DN.

Variables	Control (n=34)	Longer-duration (>40 years) Type 1 DM	
		NO NP (n=10)	NP (n=12)
Age (years) ‡	39.17±14.25	48.15±3.82¶	51.87±4.24¶
Duration of DM (years)		44.11±2.32	46.01±2.98
HbA1c (Mean ± SD) †	5.32±1.29	8.22±0.98¶	46.01±2.98
NSP(0-37) †	0.10±0.41	2.23±1.11¶	5.86±5.65¶§
McGill VAS (0-10) *	0.15±0.49	1.50±1.75¶	2.63±2.86¶
NDS(0-10))‡	0(0-1)	2(0-3)	7(4.5-8.5) ¶§
VPT R(V))‡	3.5(3-5)	7.33(5-11)¶	30.16(13.85-38.1)¶§
CT(°C) ‡	28.75(27.9-29.7)	26(22.6-28.5)	14.11(8.4-26.7) ¶§
WT(°C) ‡	36.55(35.1-37.8)	38.2(36.41-39.73)	46.5(40.5-49.4)¶§
DB-HRV (beats per min) ‡	25.73±8.17	18.3±3.79¶	8.25±3.46¶§
LSA (uV) ‡	18.52±7.26	10.66±3.82¶	4.47±2.22¶§
LSV (m/s) ‡	50.5±4.07	43.7±4.72¶	32.6±6.27 ¶§
LPA (m/s) ‡	5.82±2.07	2.72±1.55¶	0.68±0.78¶§
PMNCV (m/s) ‡	49.0±3.92	44.5±3.98¶	31.6±7.70¶§
CNFD (no/mm^2) ‡	37.27±6.06	27.74±8.02¶	15.67±8.94¶§
CNBD (no/mm^2) ‡	94.44±3.98	63.47 31.22¶	31.59±22.04¶§
CNFL(mm/mm^2) ‡	37.27±6.06	20.81±5.31¶	12.36±6.70¶§
IENFD(no/mm^2) (mm) ‡	10.33±2.31	6.73±3.38¶	3.38±4.79¶§
Cholesterol (mmol/l)	5.04±0.78	4.67±1.08	4.26±0.97
HDL(mmol/l) †	1.56±0.37	1.98±0.46¶	1.69±0.55§
TRIG(mmol/l)	1.43±0.68	1.63±0.43	1.71±0.55
LDL(mmol/l)	2.4±0.92	2.01± 1.06	2.20±0.92

Results are expressed as mean ± SD or median (interquartile range). Statistically significant differences using ANOVA or Kruskal Wallis test: * P<0.05, † P<0.01, ‡ P<0.001, ¶ Post hoc (Tukey or Conover Inman test) results significantly different from control subjects, § Post hoc results significantly different from no neuropathy group. DN, diabetic neuropathy; NSP, Neuropathy Symptom Profile; McGill VAS, McGill Visual Analogue Scale; NDS, Neuropathy Disability Score; VPT, Vibration Perception Threshold; WT, Warm Threshold; CT, Cold Threshold; CIP, Cold Induced Pain; HRV, Hear Rate Variability; HRV-DB, Heart Rate Variability to Deep Breathing; LSA, Left Sural Amplitude; LSV, Left Sural Velocity; LPA; Left Peroneal Amplitude; PMNCV; Peroneal Motor Nerve Conduction Velocity; HDL, High-Density Lipoproteins; TRIG, Triglycerides; LDL, Low-Density Lipoproteins; CNFD (Corneal Nerve Fibre Density); CNBD (Corneal Nerve Branch Density); CNFL (Corneal Nerve Fibre Length); IENFD (Intra-Epidermal Nerve Fibre Density).

tion in epidermis, micro-vessels, endothelium, basement membrane and ECM. Evidence shows that AGEs have been previously localized in skin, skin collagen, epidermal and peripheral nerves, dorsal root ganglia, blood vessels, heart, renal and retinal tissues and serum in both animal models of diabetes and patients.[13,15, 18,26,27] Our findings are also in keeping with those of previous studies which reported an association between AGEs expression and DM duration in patients with DN.[13,28] However, recent studies have reported independent association between AGEs and DN even after adjustments for age, DM duration and HbA1c. [13,17,26,29 30]

We have detected higher RAGE expression in longer-duration DM group as compared with control subjects and patients with shorter-duration or intermediate-duration DM groups in the same skin structures, which exhibited higher AGEs expression. Furthermore, there was a progressive increase in RAGE expression in these structures with increasing duration of DM. These results are consistent with reports of previous studies that observed higher RAGE expression in epidermal nerves, peripheral nerves, renal and retinal tissues,

Table 4: Skin AGEs, RAGE and GLO-I expression in patients with 40 years of diabetes with and without diabetic neuropathy.

Variables		Control (n=34)	Longer-duration (>40 years) Type 1 DM	
			NO NP (n=10)	NP (n=12)
AGEs	Epidermis ‡	2.17±0.38	3.11±1.21¶	3.95±1.55¶§
	Microvessels ‡	2.20±0.55	3.14±1.23¶	3.86±1.38¶§
	Endothelium ‡	1.26±0.44	1.81±0.20	3.90±0.44¶§
	Basement Membrane ‡	2.05±0.54	3.24±1.11¶	4.11±1.34¶§
	Papillary ECM ‡	1.87±0.84	2.46±1.35	2.46±1.35
	Reticular ECM ‡	1.93±0.59	3.10±1.18¶	4.22±1.15¶§
RAGE	Epidermis ‡	2.07±0.72	3.61±0.76¶	4.28±1.04¶§
	Microvessels ‡	2.15±0.57	2.45±1.43	4.06±1.62¶§
	Endothelium ‡	2.09±0.59	3.02±1.37¶	4.36±1.18¶§
	Basement Membrane ‡	1.92±0.73	2.51±0.74	3.87±0.96¶§
	Papillary ECM ‡	2.02±0.28	3.04±1.21¶	3.42±1.18¶
	Reticular ECM ‡	1.99±0.64	2.48±0.65	3.85±0.44¶
GLO-I	Epidermis ‡	3.64±1.22	2.26±0.86¶	1.11±0.52¶§
	Microvessels ‡	3.50±1.37	2.38±0.78¶	1.28±0.43¶§
	Endothelium ‡	3.41±1.33	2.31±0.46¶	1.56±0.68¶
	Basement Membrane ‡	3.25±1.06	1.93±0.82¶	1.25±0.74¶§
	Papillary ECM ‡	2.33±1.40	1.80±1.11	1.38±0.61¶
	Reticular ECM ‡	2.94±1.57	1.36±0.66¶	1.42±0.42¶

Results are expressed as mean ± SD or median (interquartile range). Statistically significant differences using ANOVA or Kruskal Wallis test: † P<0.01, ‡ P<0.001, ¶ Post hoc (Tukey or Conover Inman test) results significantly different from control subjects, § Post hoc results significantly different from no neuropathy group. ECM; Extracellular Matrix.

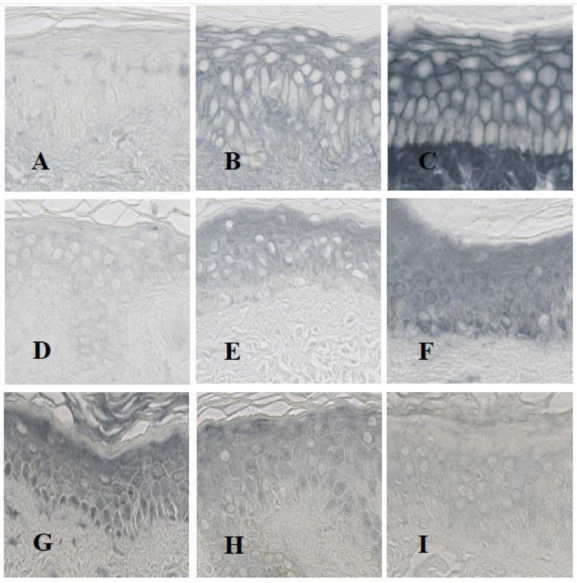

Figure 1: Immuno-localization of AGEs, RAGE and GLO-I in controls and diabetic patients with 40 years of diabetes mellitus. Upper row: Immuno-localization of AGEs in the epidermis (A-C) in control, diabetic patient without neuropathy and diabetic patient with neuropathy. Middle row: Immuno-localization of RAGE in the epidermis (D-F) in control, diabetic patient without neuropathy and diabetic patient with neuropathy. Lower row: Immuno-localization of GLO-I in the epidermis (G-I) in control, diabetic patient without neuropathy and diabetic patient with neuropathy.400x Magnification. (Click to enlarge)

blood vessels and serum in DM patients.[9,15,29,31,32,33] Higher RAGE expression could be explained by upregulation of RAGE in response to increased AGEs expression and this process is more enhanced in longer-duration DM patients. However, the relationship between the duration of DM and RAGE expression have not been fully investigated and whether this upregulation is a response to increased AGEs expression in these sites, other ligands, reactive oxygen species or a response to combined factors remains to be investigated.

In combination with increased AGEs and RAGE expression, we have detected decreased GLO-I expression in longer-duration DM group as compared with control subjects and with shorter-duration DM patients in the epidermis, micro-vessels, endothelium and basement membrane. Moreover, GLO-I expression gradually decrease with increasing

duration of DM. GLO-I is a key enzyme that detoxifies the precursor of AGEs and limits AGEs production. Thus, lower GLO-I expression in our longer-duration DM group could partially explains the increased AGEs expression detected in these patients. These findings are in keeping with the findings of more recent studies which demonstrated reduced GLO-I activity in blood and peripheral nerves and dorsal root ganglia in experimental DM and in patients.[16,34,35] The reason for reduced GLO-I activity in DM patients, however, is unknown although genetic variability of the enzyme activity have been reported in murine models and a recent study reported an association between single nucleotide polymorphism (SNP) of minor alleles rs1130534 and rs1049346 and decreased GLO-I activity in type 1 and type 2 DM patients.[34,36]. Our results indicate that AGEs, RAGE and GLO-I levels cannot explain how these longer-duration DM group have survived long duration of the disease therefore genetic or other factor like higher HDL may play a role in their longevity.

Longer-duration (>40 years) DM patients without DN demonstrated lower skin AGEs expression in the epidermis, micro-vessels, endothelium, basement membrane and reticular ECM in comparison with those with DN. A mechanistic role for AGEs in the development and progression of diabetic microvascular complications including DN has been proposed with a link to neuronal structural changes of DN.[14,18,37,38,39] Moreover, AGEs have been reported recently as a marker of DN and linked to β-cell apoptosis, decreased insulin synthesis.[11,13] Evidence shows that the association between AGEs and DN remained significant even after controlling for HbA1c.[17,30] AGEs were shown to correlate with age and diabetes duration but recent studies showed significant association between certain AGEs and DN after adjustment for these factors.[13,28,29,40] Data about AGEs expression in this unique group with longer-duration DM are rare but one recent study which have assessed serum AGEs in 351 type-1 DM patients with longer-duration of DM and interestingly have reported a dual predisposing and protective effect for AGEs combinations in relation to DN and other complications.[1] Yet, in that study, AGEs were assessed in the serum and serum AGEs levels does not necessarily reflect tissues levels [21].

Skin expression of RAGE in the unique protected group was also lower in the same skin structures that showed low AGEs expression apart from ECM as compared to patients withlonger-duration of DM and DN. The increased AGEs and RAGE expression in longer-duration DM group in the same skin structures adjacent to the small epidermal fibres together with higher prevalence of DN in this group points to a potential mechanistic role of AGEs and RAGE interaction in DN. Our observations are in agreement with previous observations of similar co-localization of these macromolecules in the target tissues for DM microvascular complications and link with these complications including DN.[15,31] The exact mechanism of RAGE-mediated neural damage, however, remains to be identified

As for skin expression of GLO-I, DM patients who did not develop DN demonstrated higher levels in epidermis, micro-vessels and basement membrane. These data and in combination with higher AGEs and RAGE expression and high prevalence of DN also suggest that GLO-I under expression induces detrimental effects that may lead to DN in these patients. Emerging reports are also linking reduced GLO-I to the development DN and painful DN.[12,16,36] A study investigated different GLO-I expressions in STZ-induced diabetic mice on DN revealed that lower GLO-I expression is associated with the behavioural changes of neuropathy, reduced IENFD and reduced mitochondrial oxidative phosphorylation while GLO-I overexpressing mice were protected against neuropathy and showed opposite changes.[35] A more recent study in 108 type-

1 and 109 type-2 DM patients demonstrated lower blood GLO-I in patients with painful DN as compared to non-painful DN.[16]

Skin AGEs and RAGE expression in multiple skin structures correlated directly and skin GLO-I expression correlated inversely with measures of small (IENFD and CCM measures) and large nerve fibre (NCS measures) damage in our study and these correlations were significant. These findings suggest that AGE, RAGE and GLO-I axis contribute to both structural and functional neuronal damage in DM patients which ultimately lead to DN. In a small study in DM patients with DN, AGEs were detected in 90% of sural and femoral nerve biopsies and correlated with morphological alterations of nerve damage including reduced numbers of nerve fibres.[39] Similarly, lower GLO-I expression was shown to reduce IENFD and higher expression to increase IENFD in animal models of DN.[35] We demonstrated very significant correlations between these three macromolecules and IENFD and CCM measures, adding to the data supporting the notion that CCM is a robust surrogate marker of DN.

There are some limitations in our study. We cannot provide measures for the variability of AGE, RAGE and GLO-I over time due to the cross-sectional nature of the study. Another limitation is the semi-quantitative nature of the scoring system albeit we undertook rigorous blinded assessment with excellent reproducibility. Of course other techniques such as liquid chromatography/ mass spectrometry (LC/MS) and polymerase chain reaction could be utilized to measure the levels of these molecules but these techniques cannot localize AGE, RAGE and GLO-I in their natural anatomical locations. Furthermore, the sample size of patients with >40 years of DM is small because it was challenging to recruit these longer-duration DM patients and to obtain skin biopsy from them.

CONCLUSION

Findings from our study provide further supporting evidence regarding the progressive increased skin expression of AGEs and RAGE, and a progressive decrease in GLO-I expression with increasing duration of DM. Our study also showed here for the first time in patients with >40 years of DM but without DN, the expression of skin AGEs and RAGE, although in general significantly higher than patients with <40 years of DM, the levels are still significantly lower than those with DN. This is also true of GLO-I expression which was significantly higher in patients with >40 years DM without DN compared with those with DN. Skin AGEs, RAGE and GLO-I expression also correlated significantly with small and large fibre damage measures which augments the notion that these macromolecules induce detrimental structural and functional effects that culminate in the development of DN which suggests potential role as a marker of the disease and therapeutic target. Further studies are required to consolidate the findings of this study.

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This work was carried out in collaboration between all authors. ATA researched and analysed data and wrote manuscript. ION, MF, WJ, HV, SA, UA, OA, AM, AK, GP, AM, AJMB and MT researched data. MJ and RAM designed the study and reviewed work.

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Conflict of interest

The authors declare that he has no conflict of interest.

Human and Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed Consent

Informed consent was obtained from all patients for being included in the study

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Appendix I: Relationship between skin AGEs and (IENFD, CCM metrics, LSA and LSV).

SKIN AGEs Structure		IENFD	CNFD	CNBD	CNFL	LSA	LSV
EP	r	***-0.634***	***-0.582***	-0.209	***-0.616***	-0.205	***-0.476***
	p	***0.000***	***0.000***	0.119	***0.000***	0.149	***0.000***
MVO	r	***-0.593***	***-0.546***	***-0.468***	***-0.666***	***-0.412***	***-0.525***
	p	***0.000***	***0.000***	***0.000***	***0.000***	***0.003***	***0.000***
EN	r	***-0.561***	***-0.523***	***-0.609***	***-0.551***	***-0.402***	***-0.501***
	p	***0.000***	***0.000***	***0.000***	***0.000***	***0.003***	***0.000***
BM	r	***-0.630***	***-0.434***	***-0.468***	***-0.527***	***-0.426***	***-0.542***
	p	***0.000***	***0.001***	***0.000***	***0.000***	***0.002***	***0.000***
PECM	r	***-0.467***	***-0.458***	-0.161	-0.210	***-0.408***	-0.216
	p	***0.000***	***0.000***	0.231	0.117	***0.003***	0.116
RECM	r	***-0.509***	***-0.442***	***-0.337***	***-0.504***	***-0.431***	-0.168
	p	***0.000***	***0.001***	***0.010***	***0.000***	***0.002***	0.224

Appendix II: Relationship between skin RAGE and (IENFD, CCM metrics, LSA and LSV).

SKIN AGEs Structure		IENFD	CNFD	CNBD	CNFL	LSA	LSV
EP	r	***-0.525***	***-0.591***	***-.0490***	***-0.560***	-0.264	***-0.508***
	p	***0.000***	***0.000***	***0.000***	***0.000***	0.061	***0.000***
MVO	r	***-0.568***	***-0.484***	***-0.517***	***-0.537***	***-0.474***	***-0.494***
	p	***0.000***	***0.000***	***0.000***	***0.000***	***0.000***	***0.000***
EN	r	***-0.444***	***-0.547***	***-0.425***	***-0.528***	-0.244	***-0.516***
	p	***0.001***	***0.000***	***0.001***	***0.000***	0.084	***0.000***
BM	r	***-0.504***	***-0.441***	***-0.267***	***-0.426***	***-0.494***	***-0.468***
	p	***0.000***	***0.001***	***0.044***	***0.001***	***0.000***	***0.000***
PECM	r	***-0.363***	***-0.425***	-0.120	-0.140	***-0.394***	-0.217
	p	***0.006***	***0.001***	0.375	0.299	***0.004***	0.114
RECM	r	***-0.408***	-0.214	-0.020	***-0.426***	-0.186	***-0.368***
	p	***0.002***	0.121	0.882	***0.001***	0.190	***0.006***

Appendix III: Relationship between skin GLO-I and (IENFD, CCM metrics, LSA and LSV).

SKIN AGEs Structure		IENFD	CNFD	CNBD	CNFL	LSA	LSV
EP	r	***0.464***	***0.482***	0.252	***0.503***	***0.408***	***0.472***
	p	***0.000***	***0.000***	0.059	***0.000***	***0.003***	***0.000***
MVO	r	***0.498***	***0.446***	***0.397***	***0.489***	***0.363***	***0.445***
	p	***0.000***	***0.000***	***0.002***	***0.000***	***0.009***	***0.000***
EN	r	***0.442***	***0.464***	***0.356***	***0.502***	***0.311***	***0.462***
	p	***0.000***	***0.000***	***0.006***	***0.000***	***0.026***	***0.000***
BM	r	***0.464***	***0.431***	***0.331***	***0.460***	***0.354***	***0.388***
	p	***0.000***	***0.001***	***0.012***	***0.000***	***0.011***	***0.003***
PECM	r	0.228	0.238	0.119	***0.351***	0.145	***0.367***
	p	0.094	0.082	0.375	***0.007***	0.309	***0.006***
RECM	r	***0.390***	***0.358***	0.055	***0.417***	***0.374***	***0.326***
	p	***0.003***	***0.007***	0.684	***0.001***	***0.007***	***0.015***

Footnote: r- Pearson's correlation coefficient, significant correlations ***p*** are in **bold italic**. EP, Epidermis; MVO, Microvessels overall; EN, Endothelium; BM, Basement Membrane; PECM, Papillary Extracellular Matrix; RECM, Reticular Extracellular Matrix; ; LSA, Left Sural Amplitude; LSV, Left Sural Velocity; CNFD (Corneal Nerve Fibre Density); CNBD (Corneal Nerve Branch Density); CNFL (Corneal Nerve Fibre Length); IENFD (Intra-Epidermal Nerve Fibre Density).

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Knowledge and perceptions of eating disorders among young adult university students in Brunei Darussalam: A qualitative study.

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ABSTRACT

Introduction: Eating disorders are very well reported in western societies. However, prevalence of eating disorders among Asians is on the rise. This study aims to explore the knowledge and perceptions of young adults regarding eating disorders in Brunei Darussalam. **Materials & Methods:** A qualitative study through four focus groups was conducted with 23 young adults from Universiti Brunei Darussalam. Participants were recruited through open meetings. Transcribed audio data were analysed under the guidance of thematic analysis. **Results:** Five key themes were identified. 1) Brunei university students have some 'understanding about eating disorders' however it is most associated with obesity; 2) 'awareness about eating disorders' explores the participant's knowledge on the psychological causes and complications; 3)'maintaining body image' describes the vulnerability of women and teenagers to eating disorders; 4) 'affluent Brunei Society' describes how living in abundance can influence individual's perspectives on eating disorders; and 5)'taking stock on services' illustrates the services and facilities available in Brunei. **Conclusions:** Young adult university students, living in an affluent society like Brunei, have limited awareness on eating disorders and the services available. Community based mental health interventions should be increased, as there is a possibility of eating disorder being a serious health issue in the near future.

Keywords: Anorexia, Bulimia, Brunei Darussalam, Obesity, Qualitative study

INTRODUCTION

Emerging evidence indicates eating disorders occur in a wide range of ethnicity, cultures, and socioeconomic groups.[1–3] Currently majority of studies exploring the views of people with eating disorders, such as bulimia and anorexia are conducted among Western population.[4–8] Yet there has been a recent increase in eating disorders among Asian population and this is now widely acknowledged.[9,10] When taken at a scientific basis, eating disorders in Singapore can be considered similar in terms of clinical presentation and pathology, to eating disorders in the West.[11] Yet, despite the increasing incidence of eating disorders, there has been very few publications on South East Asian perspectives on eating disorders.[12,13]

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The discovery of abundant petroleum resource in numerous third world developing countries have led to rapid economy growth

and combined with globalization of trade, have shifted many of these countries to become affluent societies.[14] Brunei Darussalam is one of the oil and natural gas rich countries situated in South East Asia. The economic and political stability with grace of monarchial government enabled the residents to lead a high quality of life with world's top fourth GDP per capita and top ten richest economies in purchasing power.[15] To date, there is no published literature on eating disorders in Brunei Darussalam, other than a local newspaper reporting on six cases of eating disorders in Brunei Darussalam from 2010 to 2014.[16] Thus, the aim of this paper is to explore the perspectives and knowledge of eating disorders among young adults in Brunei Darussalam.

METHODS

Study design

This study uses a qualitative exploratory approach of informed focus group to explore the perspective and knowledge of eating disorders among young adults in Brunei Darussalam. Qualitative studies are useful in exploring sensitive topics, especially unknown territories of knowledge and expanding the horizon of known knowledge.[17,18] Focus groups were chosen as method of data collection as it allows members to share and construct the collective meanings on the phenomenon under investigation.[19] Focus group discussions can nurture different perceptions and are used to gather information for discovery, bench marking, evaluating, feelings, opinions and thoughts.[20]

Sampling and Recruitment

The study population were students from University of Brunei Darussalam (UBD). Students are eligible to participate if they: (1) are aged 18 years or over; (2) could speak English; and (3) are able to give written and informed consent. We excluded students below the age of 18 years as they need parental consent. Prospective students were identified through UBD student registry and contacted via email attached with study invitation flyers. Study flyers were also posted in UBD social media platform such as UBD facebook page and WhatsApp groups. However, both these approaches achieved zero recruitment yield. Finally, lecturers from different university programmes were approached and acted as facilitators to conduct open meetings with their students on the nature and purpose of this study and to hand out participation information sheets. Potential participants volunteered to stay back after their lectures to participate in focus groups. Written consents were obtained before starting focus groups.

Fieldwork

Four focus groups were conducted across faculties of the university from January to April 2016. After obtaining consent, focus groups' discussions were audio recorded. In majority of cases, participants' focus groups' discussions were conducted using a mixture of both English and Malay languages. This reflects their everyday usage of languages. Before starting the focus groups, participants were reminded of the confidentiality and the anonymity of the study. The focus groups' discussion lasted between 45 to 60 minutes. A topic guide was used as prompt to conduct and facilitate discussion among the focus group (Table 1).

Table 1: Focus group Topic guide

1.	**Can you tell me anything you know about eating disorders?**
2.	**Share with us any stories related to people you know of with eating disorders in Brunei Darussalam.**
3.	**Share with us if you have heard or have any myths on eating disorders?**
4.	**Tell us your views on causes of eating disorders**
5.	**How can eating disorders be prevented?**
6.	**What are your thoughts on cultural values of eating disorders?**
7.	**Share with us your knowledge and awareness about availability of health services for eating disorders in Brunei Darussalam**

Data management and analysis

The audio recordings were checked against field notes taken during data collection to improve accuracy and then transcribed by the main researcher. Other researchers then verified the field notes. Transcripts were analysed using thematic analysis allowing us to 'identify, analyse and report patterns (themes) within data'.[21] Transcripts underwent five phases of thematic analysis:(1) familiarising with data, (2) generating initial codes, (3) searching themes, (4) reviewing themes, (5) defining and naming themes, (6) report writing.[22] All researchers (IWHKZ, YCL, MRV) met frequently to review initial coding to apply 'inter coder reliability' and search, review and define the themes. Final report was presented to open meetings with young adults students of the University to clarify and confirm our final themes.

Ethical Considerations

Ethical committee approval was obtained prior to the start of the study (Institute of Health Sciences Research and Ethics committee (IHSREC), UBD). Verbal explanation was given to ensure participants understand the ethical considerations after reading the participant information sheets. Participants were informed there were no right or wrong answers, and all their answers will be kept confidential. As 'eating disorders' is considered a sensitive topic, students were reminded of their rights to refuse to participate. Recorded audio discussions were only used for the purpose of the study and not made available to a third party. The research team included both experienced qualitative researcher, who guided the data analysis and study conduct and an interviewer with local knowledge on cultural and religious issues, who conducted interviews with adequate cultural/religious considerations (including use of sensitive language and religious needs before and after data collection).

RESULTS

Four focus groups of 23 young adults (9 male and 14 female) from UBD participated in this study. Characteristics of each focus group is shown in Table 2.

Data analysis resulted in the construct of five main themes: (i) 'understanding about eating disorders', (ii) 'awareness of risk and complication of eating disorder', (iii) 'maintaining body image', (iv) 'affluent society' and (v) 'taking stock on services'. Table 3 summarises the description of each theme.

Theme 1: Understanding about eating disorders

The interviewees were able to differentiate different types of eating disorders such as anorexia and bulimia. Interviewees mentioned that eating disorders refer to individuals who either refuse to eat anything or goes on dieting to lose weight:

I think the people with this sort eating disorder, like attempt to take bulimia or anorexia because they want to lose weight right so they tend to think, oh! If I'm gonna lose weight I should do like that's so

Table 2: Participant demographic characteristics.

	Study Participants (Focus group)			
	Focus group 1	**Focus group 2**	**Focus group 3**	**Focus group 4**
Age	Between 18 and 25	Between 18 and 25	Between 18 and 25	Between 18 and 25
Gender	3 males 2 females	2 males 4 females	6 females	4 males 2 females
Year of Study	Year 4	Year 2	Year 2	Year 2
Faculty	PAPRSB Institute of Health Sciences	Faculty of Arts & Social Sciences	Faculty of Science and Faculty of Integrated Technology	Faculty of Business and Economic studies

Table 3: Description of each theme.

THEME	Description of Theme
1. 'understanding about eating disorders'	To determine the knowledge and meaning of eating disorders understood by the participants
2. 'awareness of risk and complication of eating disorder'	To gauge participants 'understanding of causes and complications of eating disorders at the end
3. 'maintaining body image'	To describe the societal stereotypical acceptance of certain body image leading to eating disorders
4. 'affluent society'	To assess how living in abundance can influence individual's perspectives on eating disorders
5. 'taking stock on services'	To explore the health services and facilities available to treat eating disorders in Brunei Darussalam

quick in a matter of weeks. (Female, Focus group 2)

I have a close family relative who have an eating disorder. I think she's anorexic. She doesn't eat. Like sometimes in a day she just drink water. (Female, Focus group 1)

Participants also reported imbalanced diet and frequency of eating as having a relation to eating disorders. For example, some respondents reported that sufferers of eating disorders are individuals who do not have a balanced diet. Others also said that they have an abnormal Body Mass Index (BMI):

Not having a balanced diet. (Female, Focus group 3)

Just judging from the BMI of the person itself you can also suspect that they could also be some eating disorders. (Male, Focus group 1)

Most of the participants agreed that an eating disorder meant either excessive or inadequate eating. Others linked eating disorders with obesity and reported that the sufferers eat every type of food:

I strongly believe that eating more than 3 (meals per day) or less than 3 (meals per day) can be considered as eating disorder. (Male, Focus group 1)

What I know about these eating disorders is they eat everything (Male, Focus group 2)

Awareness of risk and complication of eating disorder

Some of the participants had refused the myth that states eating disorder is a disease by choice as they explained that eating disorders have a psychological basis. These include stress, low self-esteem and peer pressure. Most attributed stress as a main contributor to eating disorders as it impacts an individual's appetite. One participant conveyed that eating distracts a person from stressful thoughts:

For me, I would think it's more of a psychological thing. (Female, Focus group 2)

I would say that stress is a big factor. (Male, Focus group 2)

So it's something like that, so when you eat you're distracted by how full you are that you forget how stressed you are. (Female, Focus group 2)

Because when we're stressed like when we're busy we don't have the appetite. Yeah loss of appetite. (Female, Focus group 3)

Another cause of eating disorder mentioned is the individual's 'mindset' such as low self-esteem and insecurity with regards to the individual's body image. Due to low self-esteem and insecurity, these individuals feel the need to lose weight to achieve a supermodel body image in their perceptions:

It's about the mindset basically like it effects how you eat at the same time. (Female, Focus group 2)

Self-esteem. (Female, Focus group 3)

I think, sometimes they have eating disorders because of (feelings of) insecurity. (Female, Focus group 2)

The next cause of eating disorders raised is peer pressure, with the participants stating that the sufferers wanted social acceptance. The thought of wanting to be 'like others' might stem from being bullied for their appearances as mentioned by one of the participants. One participant described that the sufferer does not want to be the fattest among his/her circle of friends for fear of being rejected or isolated due to less than 'ideal' body image:

Yeah, I think as she mentions, peer pressure. She wants to be liked by others, like to be thinner. That's another factor. (Male, Focus group 1)

Because she weighs a lot, she got bullied because of it so she stopped eating to lose a lot of weight. (Female, Focus group 2)

I think its peer pressure. When we ask her why, she doesn't directly answer it because of her friends, but she said that among her friends she's the fattest. (Female, Focus group 1)

When inquired regarding the complications of eating disorders, most participants were quite knowledgeable on the topic. The complications that were mentioned by the participants included malnutrition, mental health deterioration such as suicidal thoughts and depression, fatigue or dizziness, organ failure, and also death:

All I know is they will be malnourished and therefore they will be feeling weaker and weaker (Male, Focus group 1)

They got too stressed from thinking too much and they think there's no point in life so they try to stop it by suicide. (Female, Focus group 3)

Yeah. Organ and your teeth. Yeah, if it's bulimia it's going to affect your teeth. (Female, Focus group 2)

Starving to death. (Female, Focus group 3)

Maintaining body image

Demographic factors such as age and gender have an impact on the risk of eating disorder. Most participants agreed that teenagers are the most likely age group to suffer from eating disorders:

I mean it's more prevalent in teenagers or young adult. I don't really hear kids having eating disorders or elderly. (Female, Focus group 2)

The vulnerability of teenagers to eating disorders is due to their consciousness of their outer appearances at puberty age. They are more prone to being influenced by images of 'ideal' body type portrayed by the media:

Most probably the teenagers, because from what I understand they care about what people think of their looks. That's the period of time where they start trying to be trendy. (Female, Focus group 1)

They're easily influenced by the outside culture. (Male, Focus group 2)

According to participants, females are more vulnerable to eating disorders than males:

There are absolutely males who have eating disorders, but not as many as females. It's not really common among the guys. (Female, Focus group 3)

The portrayal of images of 'ideal' woman by society is a cause of pressure towards female to possess similar image that are considered attractive. In addition, females tend to be more sensitive regarding comments on their looks as compared to males:

Caused by society because how we view female right now that they have to be thin to be attractive. (Female, Focus group 1)

Because women like to criticize women. (Female, Focus group 4)

We don't really care about what other thinks of our image especially concerning our weight. These comments affect males less than females. (Male, Focus group 1)

The Affluent Society

Many participants noted that there are more overweight or obese individuals than bulimics, anorexics or underweight individuals in Brunei. They reported that the higher socio-economic status of the people may lead to excessive eating. These factors promote lifestyle diseases such as obesity:

I think mostly in Brunei case, it's mostly linked to obesity. (Male, Focus group 4)

I think because we can afford it. I don't think there's any problem in trying to buy food. (Female, Focus group 1)

Yeah. I think its Brunei's culture that… makes us eat. (Female, Focus group 3)

Therefore, obesity is much more advocated compared to the counterparts of eating disorders. The poor advocacy of anorexia or bulimia as compared to obesity may be due to the lack of awareness of such disorders. In addition, abnormal eating behaviors might not be dealt with:

Because in Brunei, I think we're not really aware about eating disorders. (Male, Focus group 2)

They never seem to advertise you know eating disorder not even at posters so I think if people don't see it then probably people won't think about it. (Male, Focus group 4)

We observed that some of our participants reported that they felt worried, scared, and sad to see their family members with abnormal eating habits and are concerned about their wellbeing. In a culture of abundance, where food is affordable and easily available, an individual's refusal to eat might be seen as something negative:

My dad usually eats more. Even though when he is full already he keeps eating and eating and eating non-stop like as long as he see got any food on the table he will just keep eat. Like the 2kg langsat and the 2 kg rambutan, he ate it all. …… And it's quite scary because once he ate one whole Durian (local fruit) then he got sick the next day and then he have to go to the hospital. Very scary. (Female, Focus group 1)

It feels sad to see her not eating because she used to be fine then suddenly she became like that. It is kind of sad to see her becoming worse. (Female, Focus group 1)

Others reported how family values influence the sensitivity of discussing eating disorder as a disease within family. For example, a participant reported that as a daughter, she did not dare to approach her 45-year-old father regarding his eating disorder as it is a sensitive subject at home:

We don't dare to ask him about it. Because he is quite sensitive about it. (Female, Focus group 1)

Taking stock on (eating disorder) services

With the lack of awareness on eating disorder, people therefore are not able to identify abnormal eating behaviors. As many participants have pointed out, sufferers of eating disorder might not perceive what they are doing as a problem as they may have been doing it for a long time. Hence, they will not be seeking treatment due to their belief that it is a habit, and they are therefore healthy individuals:

But actually, I think it's normal because I'm always like this. (Female, Focus group 3)

They will only do something about it when problems actually arise. Yeah so that's why they think that since they have been doing it (for) a long time and they do not feel any problem so they will just keep on doing it because they think it's normal to them already. (Male, Focus group 1)

Many reported about the lack of services for management of eating disorders in Brunei. They indicated their lack of awareness and knowledge regarding facilities on treatment of eating disorders available in Brunei:

Not sure actually. (Male, Focus group 4)

I haven't search. (Male, Focus group 4)
I don't think so. I don't know. But do we? (Male, Focus group 1)

Others reported that current services were highly focused on obesity and healthy eating in Brunei since obesity has a higher prevalence than eating disorders. Some recognized that eating disorders have the potential to be a serious disease in the future and should be tackled early to prevent it from wide spreading:

Yeah we have for obesity. But for anorexic and bulimic, don't have. I don't think so. (Female, Focus group 3)
It's a potential serious disease in the future, Brunei should do something before it becomes serious. (Female, Focus group 3)

Participants suggested support groups, awareness campaigns or educational talks, rehabilitation centers, and consultations as intervention avenues:

First a support group. It's just Brunei don't have support group, right? So they have to have that one first so they don't feel ashamed to come forth with their problem. (Female, Focus group 3)
Giving awareness. (Male, Focus group 2)
Make a talk so that people will realize that this thing, too thin or overweight is dangerous for their health. (Female, Focus group 3)
I think this has to be through government initiative to do such facilities or to open such facilities. (Female, Focus group 4)
I think seeing a doctor or get perspective from health professionals would help them to see the problem. (Male, Focus group 1)

There were mixed responses about influence of social media with regards to eating disorder in Brunei. As social media exerts a certain degree of influence, this can be used as an effective platform to increase the awareness of both the disorder itself and the services or facilities that are available in Brunei:

I don't think so in Brunei. I don't think they are influenced. (Female, Focus group 3)
I think that social media is very… very influential in Brunei. (Male, Focus group 1)

DISCUSSION

The aim of this study was to explore the knowledge and understand the perceptions of young adult in Brunei regarding eating disorders. Key findings of this study were that although the participants have some knowledge on eating disorders, this knowledge is mostly associated with obesity. Similar to other studies, these young adults reported on abnormal eating behaviors such as imbalanced diet and frequency of eating as underlying causes for eating disorders.[23,24] Psychological factors such as stress and self-esteem were also reported as causes of eating disorders in this study.[25–28] Social factors, importantly peer pressure, were also perceived to cause body image dissatisfaction that lead to eating disorder.[29–32] Our study reports the perceived link between gender and eating disorder. In particular, females were perceived to be more prone to develop eating disorders than males. Indeed the incidence rate of anorexia nervosa, particularly in young females aged 15-19 years is increasing.[7] Two key reasons were postulated. First, peer pressure among young women to achieve low weight and second, anorexic models are usually portrayed as models in media.[33] Both these reasons are thought to pressurize women to desire to achieve low body weight.[34]

Furthermore, our study shows that unique features of Brunei society influence perspectives on eating disorders. Thus, living in affluent society and presence of higher incidence of lifestyle diseases seem to influence the meaning of eating disorders among young adults in Brunei. Members of 'affluent society' commonly achieve higher level of economic

well-being due to less scarcity of resources.[35] Brunei Darussalam may be considered as an affluent society, with one of the top performing GDP growth.[15] Our findings are congruent with other studies that reported those of the higher socioeconomic status tend to suffer from eating disorders due to the culture of abundance.[36,37] There is indeed evidence that an affluent society tend to have a higher prevalence of obesity.[38] Such high prevalence of obesity seems to influence these young adults to view obesity as eating disorder.

Final theme 'taking stock on services' reports that the young population is unaware of health care services for people with eating disorders in Brunei Darussalam. As this is the first study done on eating disorders in Brunei, there is currently no available literature on the extent of eating disorders in Brunei apart from a local newspaper article that reported six 'clear cut' cases of bulimia and anorexia during 2010 to 2014.[16] It is noteworthy to mention that the problem of eating disorders may be underreported. The Ministry of Health in Brunei urged residents with abnormal eating habits and concerns on their body image to access local clinical psychology services. Eating disorders in developed countries receive cognitive behavior therapy (CBT), and family therapy.[39] Schmidt et al reported that CBT guided self-care has the slight advantage of offering a more rapid reduction of bingeing, lower cost and greater acceptability for adolescents with bulimia or eating disorder not otherwise specified compared to family therapy.[40] Future research should explore evaluation of current health service provision for people with eating disorders in affluent societies such as Brunei Darussalam.

Our study reported on strategies to improve knowledge on eating disorders in the public sector of Brunei. As the use of social media has an impact on eating behaviors, educating the public using social media reduce the negative impact as well as educate the public of healthy eating.[41,42] Other means of educating people about healthy eating include support groups, awareness campaigns or educational talks, rehabilitation centers, and consultations. A study amongst USA university students reported that educational talks and awareness campaigns are effective in reducing the needs for mental health services due to eating disorders.[43]

Limitations

Although our study is the first qualitative study that explored views of young adults in Brunei Darussalam on eating disorders, this scope is still limited. First, our sample consisted of university students only, neglecting young adults from general public. Second, the presence of dominant voices of male participants in a few focus groups cannot be prevented. This may drive the discussion to a biased end. Third, as this is a qualitative study with small sample size, further work to explore the perceptions and attitudes of a larger scale of the Brunei population with diverse study designs would prove more useful.

CONCLUSION

We conclude that meaning of eating disorder in an affluent society like in Brunei Darussalam is often misinterpreted with lifestyle diseases such as obesity. Social factors and womenisation held their role to influence understanding of eating disorders among the community. There is a need for community-based intervention on improving public awareness on eating disorders. Health services need to pilot community friendly eating disorder services to improve care for people with eating disorders.

DECLARATIONS

Competing interests

The authors declare that they have no competing interests.

Authors' Contributions

IWHKZ reviewed the relevant literature to develop

the project research questions and rationale, conducted the interviews and interpreted the results, as well as drafting the paper for publication. YCL contributed to development of the study rationale and methodology, interpretation of results and writing and editing of paper for publication. MRV developed the study methodology and contributed to the interpretation of the results and writing of paper for publication. All authors read and approved the final manuscript.

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Chee Fui CHONG

Figure 1

A 25-year-old man presented to Accident and Emergency Department with a bruise and swelling in his right lower thigh, having been bitten by a dog yesterday evening. On clinical examination, the lower third of his right thigh was swollen, tense and tender as well as pulsatile. There was also two deep punctured wound which have clotted off. A right femoral angiogram was requested and is shown in Figure 1.

What is the diagnosis?

Answer*: refer to page 219*

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Ian BICKLE and Chee Fui CHONG

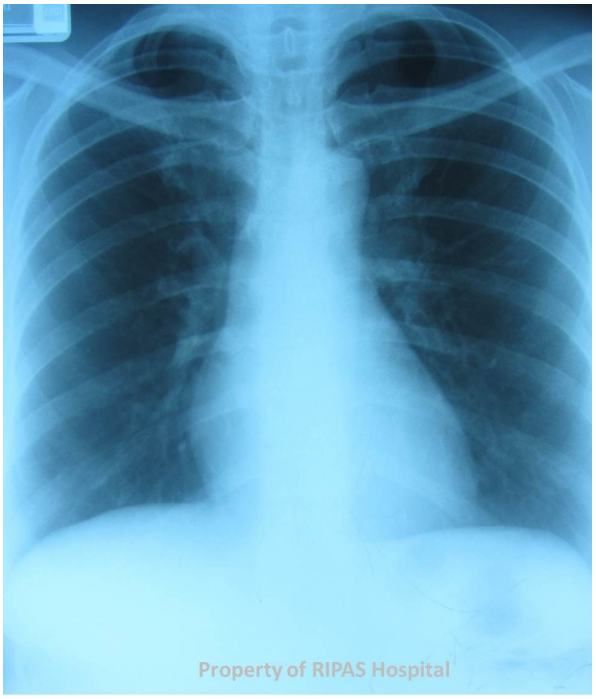

Figure 1

A 35 years old woman was referred to a vascular clinic with recurrent intermittent painful bluish discolouration of her right finger tips which comes about whenever she played tennis vigorously. These usually resolved after resting but lately it seems to last longer and more painful. She decided to visit her GP where she further admitted that she has been getting cramps in her right hand and fingers. The GP decided to take a chest radiograph as shown in Figure 1 and subsequently referred to the Vascular Clinic at the main Tertiary referral centre.

What is the diagnosis?

Answer*: refer to page 220*

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Retained Fragment of Surgical Gauze in Hip Arthroplasty – Case Report and Literature Review.

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ABSTRACT

Cases of retained foreign body are rarely reported due to its medico-legal implications. We present a case of retained surgical gauze fragments after bipolar hemiarthroplasty of hip in a patient who sustained left neck of femur fracture. This complication occurred despite strict adherence to standard operating theater protocols which includes meticulous swab and instrument count. We proposed an additional operating room processes and surgical technique in preventing such complications in future hip arthroplasties in our centre.

Keyword*:* Foreign body*,* femur neck fracture, gossypiboma, hemiarthroplasty, medico-legal

INTRODUCTION

Retained foreign body during surgery does not only cause harm to patients but is also a stressful psychological and emotional event to surgeons and operating room personnel due to its associated medico-legal implications.[1] Cases of retained foreign body continue to be reported in literatures despite implementation of standard practices in operation theaters such as using radio-opaque materials in surgical gauzes, correct swab and instrument count before closure of operation site. We present a case of retained surgical gauze fragment in a patient who underwent a bipolar hemiarthroplasty for a sustained neck of femur fracture and propose an additional operating room processes in our centre, to prevent such complication in hip replacement surgeries in the future.

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CASE REPORT

A 74 years old female lady presented to our center after a fall at home due to slippery floor. Patient was unable to ambulate after the fall due to severe pain over left hip. On examination, the left lower limb was shorter and externally rotated. There were no bruises noted over the left hip and buttock region. No neurovascular deficit was found. Plain radiograph of the pelvis and left hip showed a Garden type III left neck of femur fracture (Figure 1). Initial management includes the application of skin traction and deep vein thrombosis prophylaxis. She was planned for a cemented bipolar hemiarthroplasty.

The bipolar hemiarthroplasty was performed via a direct lateral approach. A surgical gauze was inserted into the acetabulum to

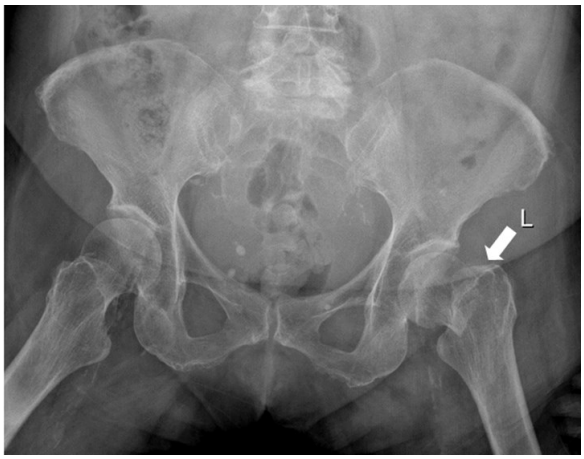

Figure 1: Plain radiograph showing left neck of femur fracture. (Click to enlarge)

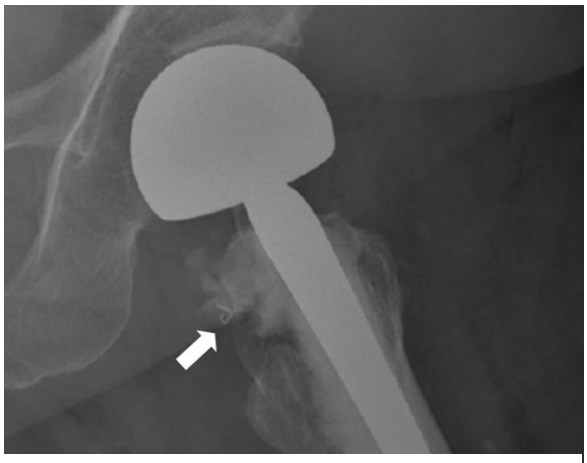

Figure 2: Enlarged image of a plain radiograph (lateral view) showing radio-opaque foreign body. at posterior aspect of the proximal femur. (Click to enlarge)

prevent accumulation of bone cement debris in the joint before proceeding to applying bone cement to the femur. Once this was completed, the femoral component of the prosthesis was inserted. Anteversion of the implant was controlled using the instrument provided. The surgical gauze in the acetabulum was removed after the cement has hardened. Other operative procedures were done as per standard practice.

Post-operative plain radiograph was taken in the ward, which showed a fragment of radio-opaque material retained at the posterior aspect of the neck of femoral, measuring approximately 1cm in length (Figure 2). The patient was informed regarding the findings and a revision surgery for foreign body removal was carried out (Figure 3).

Subsequently she was started on one week of intravenous antibiotics, physiotherapy and was discharged home well. Patient was able to ambulate without aid during a routine post-operative follow up 2 months later and exhibit no signs of infection.

DISCUSSION

The prevalence of retained foreign body in orthopaedic surgeries is unknown. Such cases are rarely reported due to its medicolegal implications. Literature search reveals that most cases of retained foreign body were discovered years later after the initial surgery.[1] A large majority of these cases present as swellings described as gossypiboma near previous operation site mimicking the presentation of soft tissue tumors, hemorrhagic granuloma or aggressive granulomatous lesions.[2,3]

Orosco et al reported that retained foreign body constitutes 6 percent of all the medical malpractice allegations between 1990 till 2006. In addition, they also found that there has been a rise in surgical malpractice claim amount. Patient's outcome was reported to be the strongest predictor in determining payment size.[1]

Moreover, retained foreign body after abdominal surgery has been widely reported.

Figure 3: Picture showing the foreign body removed. Note that the Raytec of the surgical gauze can be visualized in radiograph while the long cotton material was radiolucent.

Sharma et al had documented several types of foreign body found in patients in India such as abdominal gauzes, scissors, scalpels, forceps and needles.[4] Retained surgical gauzes after hip, femur and spine surgeries has also been reported previously.[3,4] Dalbayrak et al presented a case of retained surgical gauze mimicking sacral tumor 31 years after lumbar spine surgery was performed.[3] Sahin et al had also reported similar cases of gossypiboma found in the spine, which was misdiagnosed as tumors.[5] Connelly et al reported a case of retained pulsatile lavage tip after pelvic surgery.[6] He further discussed regarding the process-related error of removal of central filter cap of the irrigation tip, which was perceived to reduce irrigation time and increase outflow of washing solution. Despite that being the standard practice in his center, these findings prove to be crucial in prevention of future occurrence.[6]

Retained foreign body was found to demonstrate either an aseptic fibrinous process leading to granuloma formation, or an exudative inflammatory reaction causing abscess formation.[7] Masatoshi et al had reported a case of microscopic surgical gauze particles causing foreign body granuloma evident by similar microscopic birefringence appearances of the excised granuloma with a non-absorbable braided cotton surgical gauze.[2]

In this case report, the retained surgical gauze could be easily identified due to its radio-opaque Raytec component. We believe that cases of retained foreign body are under-reported as the retained surgical cotton could be radiolucent without the Raytec component and therefore will not be discovered during routine post-operative radiograph. Ghahremani et al had presented a case of retained surgical gauze after a hip replacement surgery diagnosed using 99mTc-labeled Ubiquicin scan.[8] In his report, he successfully demonstrated the use of this non-invasive scan in helping with the diagnosis of a retained foreign body. Takigami et al had reported a similar case in a total hip replacement in 2008.[9]

It is a standard practice in our institution during hip hemiarthroplasty and total hip replacement to insert a surgical gauze into the acetabulum before applying bone cement to prevent spilling of bone cement on the articulating surface of the acetabulum. The surgical gauze is usually removed after insertion of the hip implant and only when the cement has hardened. We suspect a part of the surgical gauze was caught and torn off by the sharp edges of the bone cement formed around the proximal femur. The quantity of swabs and instruments were accounted for before closure of the surgical wound. However, this process is inadequate to detect errors as described by this case report.

CONCLUSION

In conclusion, we advocate additional operating room processes to ensure that the surgical gauze used to cover the acetabulum during application of bone cement be removed before bone cement hardens. Secondly, excessive cement at the proximal femur should be removed before removing the gauze. Thus, by applying these additional processes, we hope that such cases of retained surgical gauze fragments after hip hemiarthroplasty will be prevented in future cases. We also suggest that the posterior aspect of the proximal femur should be carefully inspected by internally rotating the affected lower limb. Lastly, all surgical gauzes removed from the surgical site should be inspected to ensure they are complete and not torned. We strongly believe that incidence of retained foreign body especially surgical swabs will be drastically reduced with implementation of these additional yet vital processes.

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Djenkolism: An uncommon cause for Acute Kidney Injury.

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ABSTRACT

Djenkolism is a condition that results in acute kidney injury and occurs after the consumption of djenkol beans, which are a local delicacy and traditional medicine in some Southeast Asian countries. The pathogenesis of acute kidney injury secondary to djenkolism is not well understood but the current hypothesis is acute tubular necrosis secondary to renal obstruction due to djenkolic acid crystals (principal toxin of djenkol beans). This report describes a previously healthy 28-year-old man experiencing acute kidney injury after the consumption of djenkol beans. He presented with features of acute djenkolism which included suprapubic pain, dysuria and hematuria. With conservative treatment based on the principles of rehydration with normal saline and alkalinisation of urine with sodium bicarbonate, the acute kidney injury resolved. Healthcare practitioners in the Southeast Asian region need to consider this uncommon cause as one of the differentials of acute kidney injury.

Keywords: Acute Kidney Injury, Asia, Southeastern, Haematuria, Hydronephrosis, Hydroureter, Kidney Tubular Necrosis

INTRODUCTION

Djenkol beans are a local delicacy and a form of traditional medicine in some Southeast Asian countries. Djenkolism is a condition that affects a small proportion of people who consumes djenkol beans, resulting in acute kidney injury (AKI). The clinical presentation of djenkolism is varied but generally presents as a spasmodic loin to groin pain and AKI, with evidence of urinary obstruction. The onset of AKI appears to be independent of the method of preparation or the number and age of the fruits consumed.[1,2] The clinical presentation may range from mild to severe symptoms. If toxicity occurs after the first meal, this does not indicate subsequent meals would produce an adverse reaction. Toxicity may also present after many symptom-free meals.[3] Symptoms may occur immediately or as late as 36 hours after consumption.[1] A history of acute djenkolism doesn't produce immunity or hypersensitivity to subsequent consumption of djenkol beans.[4] In this case report, a previously healthy adult male developed AKI after the consumption of djenkol beans which resolved with rehydration with normal saline and alkalinisation of urine with sodium bicarbonate.

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CASE REPORT

A 28-year-old healthy man was referred from a private general practitioner to our tertiary hospital with difficulty in passing urine, vomiting, bilateral loin and suprapubic pain after the consumption of 20 pieces of "jering" (Figure 1: Djenkol beans, scientific name: *Archidendron Jiringa*) a few hours ago. He also experienced post-voiding dribbling, dysuria and hematuria.

Physical examination was unremarkable. On admission, a blood renal panel showed a creatinine of 274 μmol/L, urea of 6.5 mmol/L, with sodium of 137 mmol/L and potassium of 4.1 mmol/L. A full blood count showed a haemoglobin of 14.5 g/dl, total white cell count was 16,300/μl and platelet was 204,000/μl. Liver function test was normal. Urinalysis confirmed urine protein level of 30.0g/L, red blood cells of 250 cells/μL, leucocyte of 10 WBC/μL and nitrate negative. An ultrasound revealed bilateral mild hydronephrosis and proximal hydroureter; and tiny calculi in both kidneys. A subsequent CT urography revealed mild left hydronephrosis and proximal hydroureter. Urine culture was negative for growth after 48 hours.

He was managed conservatively with rehydration using normal saline and alkalinisation of urine with sodium bicarbonate over 24 hours. A urinary catheter was inserted for continuous bladder drainage. He was also given ural sachet and cefuroxime antibiotic intravenously (IV) 1.5g stat follow by regular dose of 750mg three times a day for 7 days. IV tramadol 50mg was given for analgesia and he was encouraged to take oral fluids.

Figure 1: Djenkol beans, scientific name: *Archidendron Jiringa*. (Click to enlarge)

After 7 days, the patient's symptoms including hematuria resolved. He was discharged in view of a reducing trend of blood Creatinine levels. Upon discharge, the blood urea was 3.4 mmol/L, sodium was 138 mmol/L, potassium was 3.8 mmol/L and the creatinine was 77 μmol/L.

DISCUSSION

Epidemiologic studies revealed that acute tubular necrosis (ATN) due to community-acquired infections are the commonest cause of AKI in the tropical region while cardiogenic shock, industrial accidents, drugs, trauma and renal transplantation rejection are the common causes in the developed world.[4] The burden of AKI is further worsened in the tropical regions by some of the herbal medicines used by traditional healers.[5] Djenkol beans are commonly known as krakos (Cambodia), jering (Malaysia), niang-yai (Thailand), jenkol, genkol, yiniking, yi-ring, ma-niang, cha-niang, niang, kra-niang.[6] Djenkolism is a condition characterised by acute kidney injury following ingestion of djenkol beans. It is not commonly encountered but is an important etiology of AKI amongst natives of Southeast Asia. Djenkol beans are eaten raw at meal times to purify the blood.[7] It is also a local snack in Southeast Asia, sold all year round in the markets and are consumed raw, roasted or fried.[6] Despite being available during most of the year, some reports states that djenkolism has a seasonal incidence with the peak incidence being between September and January, which corresponds with the rainy season and the blossom time of djenkol beans.[1] Typically, a pungent odour is detected in the breath and urine after consuming djenkol beans.[8]

Most people are able to consume

djenkol beans without ill effects, however AKI occurs in a small proportion of the population. The pathogenesis of djenkolism is still not well understood. Experiments in rats and mice had been inconclusive, but the pathological findings are suggestive of ATN. The principal toxin has been identified as djenkolic acid, a sulphur-containing non-protein amino acid. The current hypothesis is ATN secondary to obstruction in the renal tubules due to djenkolic acid crystals.[9] However, this has been difficult to prove due to: 1) acid crystals were not found in all animal models (histologic preparation may dissolve the crystals) and 2) renal biopsies are rarely performed on patients with acute djenkolism (one case report of human renal biopsy demonstrated findings of ATN).[8,10]

Bunawan et al reported male predominance (70%) and common presenting signs and symptoms being colicky abdominal pain (70%), dysuria (66%), oliguria (59%) and hematuria (55%).[11] Needle-shaped crystals were inconsistently reported in the urine analysis. Creatinine elevation ranged between 1.7 -14.1 mg/dL. Treatment was mainly rehydration and alkalinisation of urine. In few instances, dialysis, surgery, urethral and bladder irrigation or urethral stenting was required.

Similarly, Vachvanichsanong and Lebel reported a history of consuming djenkol beans causes an increased risk of hematuria in children by four times although the amount consumed was not a factor. However, there is no relationship between consumption of djenkol beans and other urine abnormalities, including pyuria and crystaluria.[12]

In our case, the most probable cause of AKI would be associated with the recent history of ingestion of djenkol beans. History, physical examination and investigations had ruled out other pre-renal causes for the AKI. The symptoms are also supportive of djenkolism. Imaging studies supported an obstructive pathology and urine culture ruled out an infection.

CONCLUSION

There are few reports of djenkolism in the medical literature but as treating doctors, we have to be aware of this condition as it is uncommon and remains an important cause of AKI in Southeast Asia. Knowledge of its clinical presentation, suggested pathophysiology and principles of therapy are relevant for healthcare professionals in the Southeast Asian region.

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DOUBLE VISION: A SYMPTOM OF AN OVERLOOKED ORBITAL WALL FRACTURE.

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ABSTRACT

Double vision or diplopia following trauma is almost always related to orbital wall fracture particularly orbital floor fracture. Albeit other possibilities leading to diplopia including, orbital haemorrhage, muscular oedema or haemorrhage, cranial nerve palsy or entrapment of muscle or soft tissue, orbital floor fracture ought to be ruled out primarily. Orbital floor fracture should be suspected more so when diplopia is accompanied with enophthalmos and hypoglobus. Clinical examination and radiographic examination are normally sufficient in diagnosing this entity. Herein, we report an overlooked orbital floor fracture and its management in a young girl who presented to the emergency department post trauma complaining of multiple facial laceration with double vision.

Keywords: Diplopia, enopthalmos, orbital fractures, traffic accidents.

INTRODUCTION

Orbital floor fracture a common traumatic lesion of the craniofacial complex, is often missed by the attending physicians especially when it's accompanied by other facial trauma. Presence of diplopia or double vision following trauma should raise suspicion towards orbital floor fracture as imaging is usually carried out immediately to confirm this entity. Herein, we are reporting a case of a missed orbital floor fracture with a positive radiographic finding which at the time of initial presentation was not detected by the attending Accident and Emergency Department physician. Timely diagnosis and prompt treatment will prevent unnecessary morbidities and possibility of future costly medical litigation. We recommend referral of all cases with diplopia following facio-orbital trauma suspected of orbital floor fracture for Oculoplastic consultation at the Ophthalmology Department.

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CASE REPORT

A 22-year-old medical student with past medical history of bronchial asthma was brought to Accident and Emergency Department, Hospital Universiti Sains Malaysia, Kelantan following motor-vehicle-accident whereby she was a back seat passenger in the car. Patient did not wear her seat-belt and she was thrown forward when the car she was in col-

lided with the back of the car in front. Her face hit the car seat in front causing her spectacles to break. She admitted that there was no loss of consciousness, no nausea or vomiting. There was also no blurring of vision, floaters or foreign body sensation over both her eyes. Primary survey was noted to be clear. During secondary survey, multiple superficial laceration wound were noted over the face which was sutured under local anesthesia. Patient was placed in the observation ward during which she complained of double vision on the right eye especially upon upward gaze. She was informed by the attending doctor that her facial radiograph done was normal and she was eventually discharged home.

The symptoms of right diplopia persisted and she had difficulty reading and concentrating during lessons. According to the patient, 1 week after the accident she was referred to the ophthalmology department for her diplopia. A computer tomography (CT) scan of the orbit was ordered and she was given a one-month appointment. Patient also was referred to the Plastic and Reconstructive Surgery clinic for follow up of her tender facial wounds from the accident.

Upon inspection at the Plastic and Reconstructive Surgery clinic, well-healed scars were noted over the right eyebrow and right lower lid. Right-sided enophtalmos was also noted. There was no restriction in the ocular movement however according to patient, diplopia was noted upon upward gaze. Upon palpation, there was no step deformity or crepitation over the right inferior orbital wall. Upon reviewing the initial radiograph of skull following her trauma, the 30 degree occipito-mento view radiograph done revealed comminuted fracture of the right inferior orbital wall (Figure 1). CT scan which was ordered by the Ophthalmology team 1 week after the accident demonstrated comminuted fracture of right inferior orbital wall (Figure 2).

Figure 1: 30 degree occipito-mento view radiograph displaying comminuted fracture (black arrow) of the right infe-

Patient subsequently underwent right inferior orbital wall exploration and reconstruction with calvaria bone graft. Intraoperatively, sub ciliary approach was used to access the inferior orbital wall. The defect of the fracture site was 3.0 x 1.0 cm which was 1.5cm away from the inferior orbital rim. Autologous calvaria bone graft was harvested from the patient at the same setting and used to reconstruct the fractured inferior orbital floor (Figure 3). Post-operatively, patient was well and there was no further diplopia at 2 weeks after surgery. Upon her follow-up, patient had no complaints of diplopia, blurring of vision or any other complaints till date.

DISCUSSION

Amongst different types of facial fracture, orbital fracture comprises of 10-20% of cases.[1]

Figure 2: CT orbit performed 1 week after the accident showing comminuted fracture (black arrow) of right inferior orbital wall. (Click to enlarge)

Figure 3: Calvaria bone graft harvested at the same setting to reconstruct the fracture site.

Orbital floor fracture is known to be the most common fracture site of the orbital fracture due to the lack of central support.[3] In about 50% of cases, orbital floor fracture is accompanied by medial wall fracture. Soft tissue including muscle and fat entrapment usually accompanies orbital floor fracture. In our patient, there was no entrapment noted.

Two main theories has been postulated on the mechanism of orbital floor fracture.[4] Of the most plausible theory is the 'hydraulic theory' whereby a blow to the orbital rim will create pressure gradient which will be transmitted to the weakest part leading to disintegration of the part of the bone. The second theory is the 'buckling theory' which claims that the increase pressure following trauma causes compression of the inferior orbital rim and subsequently buckling of the floor.

Ideally, complete history taking of all patients' with facial trauma should include mechanism of injury. Blow-out fracture is suspected with a history of eye being struck by an object larger than diameter of orbital rim.[5] Patient with orbital fractures have clinical presentation which varies according to presence of ocular trauma and its location. Common presenting symptoms include pain with motility, diplopia with limitation of motion, hypaesthesia and trismus. As for the signs, it includes ecchymosis, crepitus, bone step-off, ptosis, enopthalmos and strabismus. Occasionally, infraorbital nerve injury may lead to hypesthesia, dysesthesia or hyperalgesia.[5] Presence of diplopia in our patient may have been overlooked by the attending physician as double vision post trauma may also be due to muscular edema or haemorrhage which resolves spontaneously and also as the patient was not wearing her glasses.

Ideally a CT scan with an axial and coronal view and thin cuts (2-3mm) is the gold standard in diagnosing facial skeletal fracture, particularly orbital floor fracture, since such fracture can be missed by the undiscerning eye if only the usual 30 degree occipito-mento view radiograph is taken, as shown by our case.[5] CT scan enables the determination of size and type of fracture, which aids in diagnosing and surgical planning for the patient. Plain radiography is useful in diagnosing facial fracture especially when urgent computer tomography CT scan is not indicated or available in the Accident and Emergency Department. The occipito-mental view radiograph done for our patient demonstrated comminuted fracture of the inferior orbital floor but was missed by the attending Accident and Emergency Department physician.

There are three general guidelines for surgical intervention, which includes: presence of diplopia with a positive forced duction test and positive radiological imaging, presence of enophthalmos greater than 2mm two weeks post trauma and lastly, fracture involving one half or more of the orbital floor causing cosmetic and functional deformity.[6] However, management ought to be tailored based on patient's symptoms and condition.

Surgical intervention is best done two weeks following trauma as more accurate visual examination can be carried out and during this period facial oedema would have subsid-

ed. Delay in surgery especially with a missed or overlooked orbital floor fracture leads to fibrosis, scarring, contracture and unsatisfactory union. Surgical intervention has its fair share of complications including persistent diplopia, loss of vision, traumatic optic neuropathy, over-correction or under correction of enopthalmos, lower eyelid retraction, extrusion of the orbital implant, infraorbital nerve damage, orbital congestion and infection. Complete eye examination is required post-operatively to assess patient's vision. Our patient successfully underwent orbital floor exploration and reconstruction with autologous calvaria bone graft which has resolved her diplopia.

CONCLUSION

Despite the pathognomic findings exhibited by our patient which highly suggest orbital floor fracture, yet it was overlooked by the attending physician and the radiograph done was also overlooked. This case report emphasizes the importance of awareness and high clinical suspicion amongst attending physicians especially the Accident and Emergency Department physician in diagnosing this entity. We would like to recommend that all cases with double vision post trauma are referred to the Ophthalmology department for evaluation and further management.

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(***Refer to page 205***)

Answer: Right superficial femoral puncture from dog bite

The incidence of vascular injury following dog bite has been reported to be about 11.6%, majority of which occurs in the extremities (9.3%).[1] The puncture mark on the skin can be small and in most cases sealed by the time the victim present for medical attention. Any history of initial bleeding particularly of arterial spurt immediately after the dog bite should warrant a careful examination of the wound and even an angiogram if the suspicion of vascular injury is high, although only 28% of routine angiograms of suspected vascular injury following dog bite demonstrated significant arterial vascular injuries.[2]

Delay in seeking treatment as in this case or a missed diagnosis of vascular injury resulting from a dog bite, may lead to the formation of a pseudoaneurysm (Figure 1) as in this case or more commonly an occlusion of the vessel. Infection risk is in the order of 3-17%.[3]

Management of any dog attacked victims must include an initial primary survey for any immediate life threatening injuries, and all wounds should be examined carefully for any skin loss, joint penetration, and tendon, nerve or vascular injuries. Treatment will include thorough cleansing of all wounds and wound debridement performed if required.[4] Tetanus injection should be given. However, only 10.5% of vascular injury requires an immediate operation to repair the punctured vessel. Antibiotics should be given only on indication such as hand wounds, puncture deep wounds and in immune compromised patients.[3] For this case, the patient underwent urgent repair of the punctured right distal SFA and excision of the pseudoaneurysm successfully. He was also given tetanus shot and covered with antibiotics cefuroxime and metronidazole for 1 week.

Figure 1: Right femoral angiogram showing contrast media filling up a large pseudoaneurysm sac. All SFA and profunda femoris vessels are patent with good distal filling.

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Footnote: This article was first published on www.bimjonline.com as an Image of the Week.

(*Refer to page 206*)

Answer: Right cervical rib compressing the subclavian vessels.

A cervical rib is an accessory rib arising from the C7 vertebral body (Figure 1_a), which is often an incidental finding on chest x-ray. It is a rare condition, occurring in less than 1% of the population but can vary from 0.58% in a Malaysian population to as 6.2% in Turkish population.[1] It can however, be highly significant clinically, with patients presenting with symptoms of neurovascular compromised due to compression on the neurovascular bundle. Cervical rib is one cause of 'thoracic outlet syndrome' is a result of direct impingement on the subclavian vessels and or brachial plexus.

Compression on the brachial plexus causing neurological symptoms, such as paraesthesia is more common than ischaemia due to vascular involvement.[2] Neurogenic due to vascular involvement. Neurogenic symptoms usually arise following neck trauma in 80% of patients with cervical ribs, either following work-related injuries or repetitive stress or road traffic accidents.[3] In 20% of patients with cervical ribs, symptoms appeared spontaneously.

Indications for surgical resection of the accessory rib are disabling pain, paraesthesia and failure of conservative treatment. Surgery involves resection of the accessory rib with or without excising the first rib. Failure rate of surgical treatment is high in those with work-related injuries (42%) compared with those arising following RTA (26%) or spontaneously (18%).[3] Surgical success rate is also higher if both cervical and first ribs are resected together.

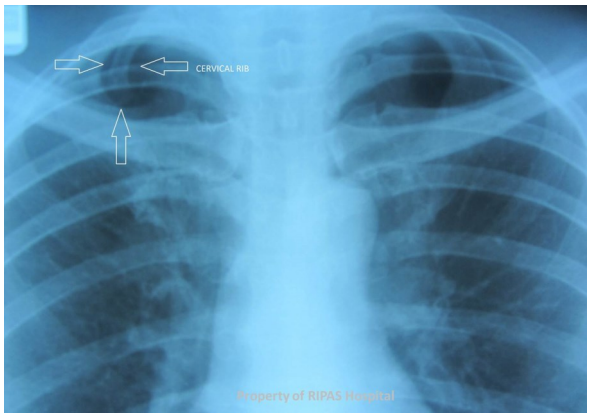

Figure 1_a: Annotated Chest X-ray (Figure 1, page 206) pointing at the cervical rib.

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