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INFORMATION

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DIAGNOSTIC ACCURACY OF XPERT® MTB / RIF COMPARED TO MICROSCOPY-BASED METHODS FOR DIAGNOSING TUBERCULOUS LYMPHADENITIS FROM FINE NEEDLE ASPIRATES AT THE PORT MORESBY GENERAL HOSPITAL, PAPUA NEW GUINEA

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Running title: Diagnostic accuracy of Xpert assay.

ABSTRACT:

Data on the accuracy of Xpert® MTB/RIF (Xpert) assay in detecting TB in lymph node aspirates in Papua New Guinea (PNG) is scanty. This study evaluated Xpert performance in diagnosing tuberculous lymphadenitis (TBLN) using lymph node needle aspirates at the Port Moresby General Hospital (PMGH). The objective of the study was to compare Xpert accuracy to acid fast bacilli (AFB) microscopy, cytomorphology, a composite reference test (CRS) and culture. A total of 107 eligible subjects were recruited out of 1080 clinic attendees. Results showed Xpert detected significantly more cases of TBLN than AFB microscopy (66 vs 35; p=0.001). Compared to AFB microscopy Xpert had a sensitivity of 45.4% (95% CI 33.1-58.1), specificity of 87.8% (95% CI 73.8-95.9), positive predictive value (PPV) of 85.7% (95% CI 71.6-93.4) and negative predictive value (NPV) of 50.0%% (95% CI 43.8-56.1). There was no difference between Xpert and cytomorphology (66 vs 60; p=0.5). Compared to cytomorphology Xpert had a sensitivity of 71.6% (95% CI 58.5-82.5), specificity of 51.1% (95% CI 35.7-66.3), PPV of 66.1% (95% CI 58.2-73.2) and NPV of 57.5% (95% CI 45.2-68.9). There was no difference between Xpert and CRS (66 vs 71; p=0.6). Compared to CRS Xpert had a sensitivity of 76.0% (95% CI 64.4-85.3), specificity of 66.6% (95% CI 49.0-81.4), PPV of 81.8% (95% CI 73.5-87.9) and NPV of 58.4% (95% CI 46.7-69.4). Culture was completed on 24 subjects with positive isolates in 14 giving a culture yield of 58.3%. Of the 24 subjects, Xpert was positive in 21 subjects. There was no difference between Xpert and culture (21 vs 14; p=0.8). Compared to culture Xpert had a sensitivity of 100.0% (95% CI 76.8-100.0), specificity of 30.0% (95% CI 6.6-65.2), PPV of 66.6% (95% CI 57.1-75) and NPV of 100.0%. The results suggest Xpert is more sensitive than AFB microscopy but comparable to cytomorphology and CRS for TBLN diagnosis in the PNG context. Xpert can be used for diagnosing TBLN at PMGH.

Keywords: Extrapulmonary tuberculosis, lymph node aspirate, acid fast bacilli microscopy

INTRODUCTION:

Sensitivity and specificity of microscopy are variable with the form of extra-pulmonary TB (EPTB) [1]. Although Papua New Guinea (PNG) national guidelines recommend microscopy of fine needle aspirates (FNA) for laboratory confirmation of tuberculous lymphadenitis (TBLN), in practice diagnosis is made based on clinical features. This may be due to inadequate laboratory infrastructure, shortage of pathologists and trained medical laboratory personals to do cytology microscopy [2,3,4].

Microscopic diagnosis of TBLN at Port Moresby General Hospital (PMGH) is based on identification of cytomorphological features of granulomatous inflammation (epitheloid cells, caseous necrosis, granulomas) using May-Grunwald-Giemsa (MGG) stain with or without stainable acid-fast bacilli (AFB) by Ziehl-Neelsen (ZN) stain. Xpert ® MTB/RIF (Xpert) testing for mycobacterium tuberculosis (MTB) complex is available in 22 public health facilities throughout PNG and used for testing respiratory specimens. World Health Organisation (WHO) published guidelines for Xpert testing of non-respiratory samples but these recommendations are conditional and the test has to be evaluated against local settings for optimum use [5,6].

Denkinger et al [1] conducted a systemic review and meta-analysis of 18 studies that evaluated Xpert diagnostic accuracy against culture from tissues of FNA specimens in detecting TB in lymph node and found Xpert to have sensitivity range between 50% and 100% [1]. When Xpert was evaluated against a composite reference standard for TBLN pooled sensitivity was 81.2% [1]. Xpert was also found to perform better than existing methods of diagnosing EPTB using other samples such as pleural fluid or cerebrospinal fluid [1]. As a result, WHO recommends Xpert over conventional tests for TB diagnosis in lymph node and other non-respiratory specimens to exclude TB in a very sick child [1]. With the objective of nationwide implementation of Xpert testing of FNA aspirates, this pilot study at PMGH was done to evaluate the diagnostic performance of Xpert (index test) with existing microscopy-based methods (reference tests) of TBLN diagnosis.

METHODOLOGY:

Study population, setting and sampling procedure:

Port Moresby General Hospital is the largest and only tertiary referral hospital in PNG with approximately 600 beds.

The Pathology Department conducts a twice weekly FNA clinic that processes a maximum of 30 patients per clinic day. Patients that were clinically suspected of TBLN by the treating physicians referred for FNA between November 2014 and August 2015 were recruited via the weekly FNA clinics. Every third consecutive patient meeting the eligible criteria was invited to participate in the study. Both inpatient and outpatient attendees were recruited. Each patient was clinically examined and interviewed using a standardised questionnaire piloted before the study implementation.

Ethical approval was obtained from the Medical Research Advisory Council of Papua New Guinea

National Department of Health (MRAC file No: 54-6-2, November 11, 2014).

Inclusion criteria:

Patients with lymph node enlargement of the cervical, axillary and inguinal regions with a clinical suspicion of TBLN were included. Both male and female patients in all age groups were eligible for the study. Inclusion criteria for culture were (1) rifampicin resistance detection by Xpert and (2) study sample selected for external quality assurance testing. External quality assurance sample selection was done per standard operating procedure for MTB at the PNG National Department (NDoH) Central Public Laboratory (CPHL) housed within PMGH. Cultures were done at the Queensland Mycobacterium Reference Laboratory (QMRL), Brisbane, Australia.

Lymph node sampling and processing:

Nodes of more than two centimetres were sampled using aspiration and nodes less than two centimetres were sampled using non-aspiration technique. A 22 or 23-gauge hypodermic needle attached to a 10ml syringe was used with the aspiration technique. A 2ml vacuum pressure was created in the syringe after inserting the needle into the chosen sampling site and the needle moved back and forth using a rapid steady motion without completely withdrawing the needle.

Sampling was stopped when sample (blood, pus or mixture of both) was visible at the hub of the needle. In the non-aspiration method, a 22 or 23-gauge needle was passed into the enlarged gland without the syringe and sampling was by moving the needle back and forth using a rapid steady motion without completely withdrawing the needle. Aspiration was drawn into the needle by capillary action. One or two passes were done for each study subject. Two smears were made for each study subject. One slide was stained with MGG stain and the other with ZN stain. Remainder of the sample in the needle was rinsed with 2ml physiological saline in a sterile container. The saline-aspirate mixture was sent to CPHL and used in the Xpert assay. Samples testing positive for rifampicin resistance on Xpert or if selected as part of CPHL external quality program were sent to QMRL in Brisbane Australia for culture. Standard personal protection equipment was worn and biosafety procedures were followed at all times.

Index and reference tests:

Xpert was defined as the index test. The reference tests were (1) AFB microscopy, (2) cytomorphology (epithelioid cells, granulomas, caseous necrosis), (3) composite reference standard (CRS) comprising AFB and cytomorphology and (4) culture. Positive AFB was defined as stainable AFB by ZN stain and positive cytomorphology by MGG was defined as presence of cytomorphological features of granulomatous inflammation. Positive CRS was defined as positive AFB and/or positive cytomorphology. Positive Xpert was defined as MTB detection (positive or borderline positive).

Data were tabulated in Microsoft Excel sheet and transferred to SPSS® for analysis. Demographic and clinical characteristics were recorded to describe the study population. Accuracy of the index test was described using sensitivity, specificity, positive predictive value (PPV) and

negative predictive value (NPV) using 2x2 tables. T-test was used for statistical significance testing between index test and reference tests with level of significance set at p value less than 0.05.

RESULTS:

Total of 1080 patients attended the FNA clinic during the study period and 107 consenting eligible subjects were recruited for the study. The mean age was 26 (±14) years. There were 45 (42.1%) males and 62 (57.9%) females with a male to female ratio of 0.7. Xpert detected MTB in 66/107 (61.6%) subjects. Acid fast bacilli was positive in 35/107 (32.7%) subjects, cytomorphology showed 60/107 (56.1%) positive cases and CRS was positive in 71/107 (66.3%) subjects (Table 1.0).

Xpert compared with AFB microscopy as the reference test showed Xpert detected significantly more MTB than AFB microscopy (66 positive cases versus 35 positive cases; p=0.001). Compared to AFB microscopy Xpert had a sensitivity of 45.4% (95% CI 33.1-58.1), specificity of 87.8% (95% CI 73.8-95.9), PPV of 85.7% (95% CI 71.6-93.4) and NPV of 50.0% (95% CI 43.8-56.1) (Table 2.0).

There was no difference between Xpert and cytomorphology (66 positive cases versus 60 positive cases; p=0.5). The sensitivity, specificity, PPV and NPV of Xpert using cytomorphology as reference test were 71.6% (95% CI 58.5-82.5), 51.1% (95% CI 35.7-66.3), 66.1% (95% CI 58.2-73.2) and 57.5% (95% CI 45.2-68.9) respectively (Table 2.0).

There was no difference between Xpert and CRS as the reference test (66 positive cases versus 71 positive cases; p=0.6). Compared to CRS Xpert had a sensitivity of 76.0% (95% CI 64.4-85.3) and specificity of 66.6% (95% CI 49-81.4). The PPV and NPV of Xpert were 81.8% (95% CI 73.5-87.9) and 58.5% (95% CI 46.7-69.4) respectively (Table 2.0).

Culture was completed on 24 subjects with positive isolates in 14/24 (58.3%) cases (Table 3.0). Xpert detected MTB in all culture positive samples (14/14, 100.0%). Of the culture negative samples Xpert was positive in 7/10 (70.0%).

Sensitivity and specificity of Xpert using culture as reference test were 100.0% (95% CI 76.8-100) and 30.0% (95% CI 6.6-65.2) respectively. The PPV was 66.6% (95% CI 57.1-75) and NPV was 100.0%. There was no difference between Xpert (21/24, 87.5%) and culture (14/24, 58.3% [21 versus 14; p=0.8]). Of the 14 isolates, nine (9/14; 64.2%) were resistant to at least one drug tested. Six of the nine resistant isolates (6/9; 66.6%) were MDR-TB. Mono-resistant rate was 33.3% (3/9).

Table 1: AFB microscopy, cytomorphology, Xpert, culture and CRS test results

	AFB	Cytomorphology	Xpert	Culture*	CRS**
Positive	35/107	60/107	66/107	14/24	71/107
Negative	72/107	47/107	41/107	10/24	36/107

*Only 24 samples were sent for culture. **CRS = composite reference standard.

Table 2. Diagnostic accuracy of Xpert compared with microscopy-based methods for TBLN diagnosis at PMGH.

Xpert vs various reference tests	Sensitivity (%)		Specificity (%)		Predictive value (%)	
	(n**/N***)	95% CI	(n**/N***)	95% CI	Positive (95% CI)	Negative (95% CI)
Xpert vs AFB	45.4 (30/35)	33.1-58.1	87.8 (36/72)	73.8-95.9	85.7 (71.6-93.4)	50.0 (43.8-56.1)
Xpert vs cytomorphology	71.6 (44/60)	58.5-82.5	51.1 (23/45)	35.7-66.3	66.1 (58.2-73.2)	57.5 (45.2-68.9)
Xpert vs CRS*	76.0 (54/71)	64.4-85.3	66.6 (24/36)	49.0-81.4	81.8 (73.5-87.9)	58.5 (46.7-69.4)
Xpert vs culture	100.0 (14/14)	76.8-100.0	30.0 (3/10)	6.6-65.2	66.6 (57.1-75.0)	100.0

*CRS = composite reference standard, composite of positive cytomorphology and AFB microscopy. **n = positive cases using Xpert. ***N = positive cases using respective reference tests.

Table 3: Drug susceptibility test results of resistant isolates (Total isolates = 14, Total resistant isolates = 9)

Drugs Tested	Resistant rate
Amikacin	0
Streptomycin	0
Isoniazid 0.1 mg/L	2/9 (33.3%)
Isoniazid 0.4 mg/L	6/9 (66.6%)
Ofloxacin	1/9 (11.1%)
Rifampicin	9/9 (100%)
Ethambutol	4/9 (44.4%)
Pyrazinamide	2/9 (22.2%)
Ethionamide	3/9 (33.3%)
Kanamycin	0
Capreomycin	0
Cycloserine	0
*PAS (para-aminosalicylic acid)	0

DISCUSSION:

Sensitivity of Xpert in our study ranged between 45.4% and 100% varying with the reference test. Xpert had 100% sensitivity with culture as reference test, compared to CRS Xpert had 76% sensitivity, 71.6% sensitivity compared to cytomorphology and 45.4% sensitivity with AFB microscopy. Other studies evaluating Xpert in diagnosing TBLN have used other composite reference tests or culture as the reference tests and obtained sensitivities ranging between 59% and 96.1% [7,8,9,10,11,12]. The sensitivity of Xpert is also variable with gross appearance of the aspirate with sensitivities between 73% and 87% where presence of pus was associated with a higher sensitivity rate [8,9,10,11,12].

The specificity of Xpert in this study ranged between 30% and 87.8% varying with the reference test. Xpert had specificity of 87.8% compared to AFB microscopy, 66.6% specificity with CRS as reference test, cytomorphology 51.1% specificity and compared to culture Xpert specificity was 30%. Our specificity results are lower than similar studies that reported specificities between 88.9% and 100% [6,8,9,10,11,12]. The difference in specificities between these studies and our results are most likely due to the limited number of aspirates sent for culture in our study. Of the 10 culture negative cases, seven were positive on Xpert of which six had cytomorphological features of TBLN and two were AFB positive.

Laboratory confirmation of TBLN at PMGH is based on AFB microscopy and identifying cytological features of granulomatous inflammation. These methods are labour intensive, require trained cytologists and have long result turn-around time. This practice is the same in most resource limited settings [4,14,15,16,17]. Although microscopy of FNA is cheap and suitable in resource limited settings, cytomorphological features are non-specific and lack sensitivity without demonstration of AFB [18,19,20]. Presence of caseous necrosis and neutrophils is associated with high AFB positivity [14]. There is also positive association between pus aspirates, granulomas, presence of neutrophils or necrosis and AFB detection and it has been suggested that AFB must be demonstrated and other causes of granulomatous inflammation be excluded before making a diagnosis of TBLN [18,19,20]. In PNG where there is shortage of pathologists, laboratory medical scientists can be trained to do needle aspiration of enlarged lymph nodes and process FNA aspirates for Xpert diagnosis of TBLN. This may improve result turn-around time allowing earlier commencement of TB treatment. Xpert also has the advantage of detecting drug resistant TB and allows clinicians to consider second line drugs while culture results are pending, particularly in sick children where obtaining respiratory samples is challenging. Fine needle aspirate smear and cytological microscopy for TBLN diagnosis in PNG is limited to pathologists but the shortage of pathologists hinders nationwide implementation of FNA cytology for TBLN diagnosis. Although Xpert is currently available in 22 sites in PNG making it possible to diagnose TBLN in health facilities that has no resident pathologist, a cheaper alternative

would be to train medical laboratory scientist in PNG to perform FNA cytomorphological analysis for diagnosis of TBLN.

This study reports a mycobacterial culture yield of 58.3% (14/24). Culture yield of MTB from FNA is reported to be between 42% and 83% [13]. Positive HIV status is also associated with a higher yield from FNA aspirates in adults [13]. The present study's design did not permit gathering information of the HIV status of subjects. Factors contributing to negative culture results may have included inadequate volume (one patient) or TB treatment for more than two weeks (two patients). Although physiological saline was used for emulsifying the FNA prior to shipment for culture, the yield is higher in MTB specific transport mediums [13]. Prolonged storage (more than 10 days at CPHL) of specimen prior to shipment to Australia may have resulted in reduced number of viable bacilli negatively affecting culture results as suggested by some studies [6].

Studies on multi-drug resistant TB (MDR-TB) in PNG have reported rates of 4.6% and 26% [21,22]. A large population-based survey in PNG has shown that the national MDR-TB rate is 2.7% in new cases and 19.1% in previously treated cases [23]. Of the 14 isolates, nine (9/14; 64.2%) were resistant isolates showing resistance to at least one drug with rifampicin mono-resistant rate of 33.3% (3/9) and MDR-TB rate of 66.6% (6/9). The high rate is preliminary and indicates the need for a larger sample size study looking at the resistant pattern of MTB isolates from subjects with TBLN at PMGH. The present study's drug susceptibility test results are consistent with Aia et al [23] who showed that MTB drug resistance in PNG is heterogenous [23]. The MDR-TB isolates showed resistance to rifampicin (9/9, 100%), ethambutol (4/9, 44.4%) and pyrazinamide (n=2, 14%). This pattern is similar to other published data on MTB drug resistance in PNG [21,22,24]. Whereas other studies in PNG reported isolates showing resistance to streptomycin, capreomycin and para-aminosalicylic acid [21,22], isolates in this study did not show resistance to these drugs. The observed differences may be due to different strains causing TBLN as suggested by a study in Ethiopia [25].

The use of Xpert in PNG is limited to testing respiratory specimens with the exception of cerebrospinal fluid [2]. The results of the present study provided evidence to re-evaluate this recommendation in PNG and contributed to the development of a laboratory algorithm for processing of FNA specimens. The newly developed laboratory algorithm has been included in the revised PNG national guidelines for the testing of EPTB specimens. This study further demonstrates that Xpert processing of FNA aspirates can be tailored to local settings in other high TB-burden countries.

There are limitations to our study. Although culture is the preferred reference standard, only 24 samples were sent for culture and reflect the realities of the study setting. We did follow up cases to determine clinical outcome. A follow up prospective study that includes monitoring of clinical outcomes can help show impact of Xpert on the management of drug resistant TBLN in PNG. The use of 2ml physiological saline may be inadequate and or may not be the ideal transport medium for

MTB culture. A MTB specific solution may have produced higher culture yields.

CONCLUSIONS:

The results suggest Xpert is comparable to cytomorphology and a microscopy-based composite reference test (CRS) for TBLN diagnosis at PMGH. The sensitivity and specificity of Xpert compared to existing microscopy methods for diagnosing TBLN is acceptable in the PNG context. Most importantly, Xpert can be implemented nationally to provide laboratory confirmation of TBLN and offer the added advantage of detecting MDR-TB, particularly in children.

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REFERENCES:

1. Denkinger CM, Schumacher SG, Boehme CC, Dendukuri N, Pai M, Steingart KR. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systemic review and meta-analysis. European Respiratory Journal 44:435-446. 2014; DOI: 10.1183/09031936.00007814.
2. Papua New Guinea National Department of Health. PNG National Tuberculosis Management Guidelines 2013.
3. McMaster P, Ezeilo N, Vince D. A retrospective review of paediatric lymph node tuberculosis in Port Moresby, Papua New Guinea. Annals of Tropical Paediatrics 2000; 20(3):223-6.
4. McMaster P, Ezeilo N, Freisen H, Pomat N, Vince D. Ten-year experience with paediatric lymph node tuberculosis in Port Moresby. Journal of Tropical Pediatrics 2001; 47(3):160-164.
5. World Health Organisation. Xpert MTB/RIF implementation manual. Technical and operational 'how to' practical considerations 2014.
6. Tadesse M, Abedele G, Abdissa K, Aragaw D, Abdella K, Bekele A, Bezabih M, Apers L, Jong BC, Ringouts L. GeneXpert MTB/RIF assay for the diagnosis of tuberculous lymphadenitis on concentrated fine needle aspirates in high tuberculous burden settings. Plos One 2015;10(9):e0137471. Doi: 10.1371/journal.pone.0137471.
7. Kirwan DE, Cardenas MK, Gilman RH. Rapid implementation of new TB diagnostic tests: Is it too soon for a global roll-out of Xpert MTB/RIF? American Journal of Tropical Medicine and Hygiene 2012; 87(2):197-201.
8. Malbruny A, Marrec G.L, Courageux K, Leclercq R, Cattoir V. Rapid and efficient detection of Mycobacterium tuberculosis in respiratory and non-respiratory samples. International Journal of Tuberculosis and Lung Disease 2011; 15(4):553-555.
9. Ligthelm LJ, Nicol MP, Hoek KGP, Jacobson R, van Helden PD, Marais BJ, Waren RM, Wright CA. Xpert MTB/RIF for rapid diagnosis of

tuberculous lymphadenitis from fine-needle-aspiration biopsy specimens. Journal of Clinical Microbiology. 2011; 49(11):3967-3970.

10. Kim Y, Kwak N, Seong M-W, Kim E-C, Yoo C-G, Kim Y, Han S, Yim J-J. Accuracy of the Xpert MTB/RIF assay for the diagnosis of extra-pulmonary tuberculosis in South Korea. International Journal of Tuberculosis and Lung Disease 2015; 19(1):81-86.
11. Scott LE, Beylis N, Nicol N, Nkuna G, Molapo S, Berrie L, Duse A, Stevens WS. Diagnostic accuracy of Xpert MTB/RIF for extrapulmonary tuberculosis specimens: Establishing a laboratory testing algorithm for South Africa. Journal of Clinical Microbiology 2014; 52(6):1818-1823.
12. Dhasmana D, Ross C, Bradley C, Connell D, George P, Singanayagam A, Jepson A, Craig C, Wright C, Molyneaux P, Wickremasinghe M, Lalvani A, Cooke G, Kon O. Performance of Xpert MTB/RIF in the diagnosis of tuberculosis mediasternal lymphadenopathy by endobronchial ultrasound. Annals of the American Thoracic Society 2014; 11(3):392-396.
13. Razack R, Louw M, Wright C.A. Diagnostic yield of fine needle aspiration biopsy in HIV infected adults with suspected mycobacterial lymphadenitis. South African Medical Journal 2014; 104(1):27-28.DOI:10.7196/SAMJ.7492.
14. Prasson D. Acid-fast bacilli in fine needle aspiration smears from tuberculous lymph nodes. Where to look for them. Acta Cytologica 1999; 44(3):297-300.
15. Wright CA, Hesseling AC, Bamford C, Burgess SM, Warren R, Marais BJ. Fine-needle aspiration biopsy: a first-line diagnostic procedure in paediatric tuberculosis suspects with peripheral lymphadenopathy. International Journal of Tuberculosis and Lung Disease. 2009; 13(11):1373-1379.
16. Fanny ML, Beyam N, Chrustome G, Zandanga G, Yango F, Manirakiza A, Rigouts L, Pierre-Audigier C, Gicquel B, Bobossi G. Fine-needle aspiration for diagnosis of tuberculous lymphadenitis in children in Bangui, Central African Republic. BMC Pediatrics. 2012; 12:191.
17. Marais B, Wright C, Gie R, Hesseling A, Enarson D, Beyers N. Tuberculosis lymphadenitis as a cause of persistant cervical lymphadenopathy in children from a tuberculosis-endemic area. The Pediatric Infectious Disease J. 2006; 25(2):142-146.
18. Sen R, Marwah N, Gupta KB, Marwah S, Arora R, Jain K. Cytomorphologic patterns in tuberculous lymphadenitis. Indian Journal of Tuberculosis 1999; 46:125-127.
19. Rana S, Sharma P, Kalhan S, Singh P, Gill MK, Kumar A. Scholars Journal of Applied Medical Sciences. 2015; 3(3G):1547-1552.
20. Hemalatha A, Shruti PS, Kumar MU, Bhaskaran A. Cytomorphological patterns of tubercular lymphadenitis revisited. Annals of Medical and Health Sciences Research 2014; 4(3):393-396.
21. Ley, SD, Harino P, Kilagi V, Kamus R, Carter R, Coulter C, Pandey S, Fieldmann J, Balif M, Siba PM, Phuanukoonnon S, Gagneux S, Beck

HP. Diversity of Mycobacterium tuberculosis and drug resistance in different provinces of PNG. BMC Microbiology, 2014; 14:307.

22. Simpson G, C. Coulter, J. Weston, T. Knight, R. Carter, S. Vincent, L. Robertus, and A. Konstantinos. Resistance patterns of multidrug-resistant tuberculosis in Western Province, Papua New Guinea [Notes from the field]. The International Journal of Tuberculosis and Lung Disease15; (4): 2011; 551-552.
23. Aia P, Kal M, Lavu E, John LN, Johnson K, Coulter C, Ershova J, Tosas O, Zignol M, Ahmadova S, Islam T. The burden of drug-resistant tuberculosis in Papua New Guinea: Results of a large population-based survey. PLOSONE.DOI:2016; 10.1371/journal.pone.0149806.
24. Ballif M, Harino P, Ley S, Coscolla M, Niemann S, Carter R, Coulter C, Borrel S, Siba P, Phuanukoonnon S, Ganeux S, Beck HP. Drug resistance-conferring mutations in Mycobacterium tuberculosis from Madang, Papua New Guinea. BMC Microbiology. 2012; 12: 191.
25. Biadglegne F, Merker M, Sack U, Rodloff A.C, Niemann S. Tuberculous Lymphadenitis in Ethiopia Predominantly Caused by Strains Belonging to the Delhi/CAS Lineage and Newly Identified Ethiopian Clades of the Mycobacterium Tuberculosis Complex. PloS One 9(10), 2015 :e0137865

ANTI-NOCICEPTIVE EFFECT OF GLYCYRRHIZA GLABRA ROOT EXTRACT ON CHRONIC CONSTRICTION INJURY OF SCIATIC NERVE INDUCED NEUROPATHIC PAIN AND SOME SELECTED INFLAMMATORY BIOMARKERS IN EXPERIMENTAL ANIMALS

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Running title: Glycyrrhiza glabra root extract elicits anti-nociceptive effect on rat induced neuropathic pain

ABSTRACT:

Multiple causes of neuropathic pain have been identified and its incidence is likely to increase owing to the ageing global population. Glycyrrhiza glabra (licorice) is a medicinal plant known to be a highly efficacious medicinal herb with several pharmacological effects. Few researchers have demonstrated anti-nociceptive activity of licorice acute pain. The aim of this study was to investigate the anti-nociceptive effect of prepared aqueous extract of Glycyrrhiza glabra root administration on chronic constriction injury (CCI) of sciatic nerve induced neuropathic pain and some selected inflammatory biomarkers in adult male wistar rats. Seven groups of 5 rats per group were used. Groups 1 and 2 were controls. Administration started in groups 3, 4, and 5 three days after surgery and continued for 18 days. Group 3 received 10mg/kg of Imipramine. Groups 4 and 5 received 75mg/kg and 150mg/kg of licorice respectively. Groups 6 and 7 received 75mg/kg and 150mg/kg respectively for 10 days before surgery. Paw withdrawal thresholds were assessed using hot plate method on days 3, 7, 14, and 21. On day 21, plasma level of tumor necrotic factor (TNF-α) and C-reactive protein (CRP) were determined using appropriate ELISA kits. There was significant change in pain threshold in the extract treated ameliorative groups when compared with the control and the ameliorative reference drug. TNF-alpha and CRP concentrations were significantly reduced in groups 6 and 7, compared with groups 1, 2 and 3. In conclusion, anti-nociceptive activity of licorice and its effect on TNF-α, and CRP are dose dependent and administration before surgery was more effective.

Keywords: Glycyrrhiza glabra; Pain threshold; Tumor Necrotic Factor (TNF-α); C-reactive protein

INTRODUCTION:

Chronic pain is a debilitating condition that commonly impairs activities of daily living and health-related quality of life, and its prevalence is around 7–8% in the world population [1]. Neuropathic pain is also usually associated with increased drug prescriptions and visits to health care providers [2]. Some individuals experience distinct set of symptoms, such as, burning and electrical-like sensations, and pain resulting from non-painful stimulations (such as light touching); these symptoms usually persist and have a propensity to become chronic and less responsive to pain medications [3]. In spite of rigorous research over the last 30 years, the nature of neuropathic pain is still not clear [4]. These controversies include debate on the nature of neuropathic pain, whether such pain is peripheral or central in origin, and whether its etiology is inflammatory or non-inflammatory [5]. Increasing evidence has provided better understanding of the roles of both immune and pro-inflammatory mediators (e.g., the interleukins, TNF-α, complement components, ATP and the chemokines) in the mechanisms of both peripheral and central neuropathic pain [6]. Conversely, medicinal plants and the active principles sequestered from them are of vast importance to researchers in their fight against diseases [7]. Licorice obtained from the dry roots and rhizomes of licorice plant have been shown to be widely used in clinical prescriptions [7]. The pharmaceutical importance of licorice however lies in its capacity to yield a great variety of secondary substances. According to recent studies, the most important bioactive compounds in licorice are triterpenes, flavonoids and polysaccharides [8]. These compounds are reported to have biological activities such as: antitumor [9], anti-microbial [10], antiviral [11], anti-inflammatory [12], anti-diabetic [13], immunoregulatory [14], hepatoprotective [15], neuro-protective activities [16] and adrenal cortical hormone kind functions [17].

Previous study by Bhandage et al. [18] demonstrated effect of Glycyrrhiza glabra on acute pain using different models of pain assessment. From their results, the extract had anti-nociceptive activity via central and peripheral mechanisms. However, there are few studies that have investigated the use of this extract on chronic pain.

The aim of the present study was to further investigate the anti-nociceptive effect of the extract on chronic constriction injury of sciatic nerve model of neuropathic pain in rats and to assess some selected inflammatory biomarkers.

METHODOLOGY:

Extract Preparation:

Licorice root powder was purchased from Amazon and was sold by Herbs and Crops Overseas, India with batch no: LRP-2017/02. Portion of the powder (50 g) was mixed with

100 ml of sterile distilled water in a flask with occasional shaking. The extract was then filtered through a muslin cloth for coarse residue and finally through Whatman No. 1 filters paper and kept in an airtight amber colored container [19].

Animals:

Thirty - five male 6 week-old Wistar rats bought at the Animal House of College of Medicine, Ekiti State University, (weight 200 ± 20 g) were used for the study. The rats were housed and maintained in standard conditions of light, feeding and temperature in the Animal House of College of Medicine, Ekiti State University. The study was conducted in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals [20]. Rats had unrestricted access to standard rat chow and tap water. After one week of acclimatization, the animals were randomly assigned to one of the following experimental groups (n = 5 per group), ligated and were treated accordingly.

Group I: received distilled water (10ml/kg, orally) daily; designated as non - ligated vehicle-treated.

Group II: received distilled water (10ml/kg, orally) daily; designated as ligated vehicle-treated

Group III: received imipramine (40mg/kg, orally); designated as reference drug treated.

Group IV: received licorice extract (75mg/kg, orally); post-surgery; designated as low dose treated (LDL).

Group V: received licorice extract (150mg/kg, orally); post-surgery; designated as high dose treated (HDL).

Group VI: received low dose of licorice extract (75mg/kg, orally); pre surgery; designated low dose pre-treated (LDLp).

Group VII: received high dose of licorice extract (150mg/kg, orally); pre surgery; designated high dose pre-treated (HDLp).

Administrations of treatment began in group III, IV, and V three days after surgery and continued for 18 days. Group VI and VII received treatment for 10 days before surgery and treatment continued three days after surgery for another 18 days. All vehicle and licorice were administered parenterally. Blood plasma levels of tumor necrotic factor (TNF-α) and C-reactive protein (CRP) were also determined using an ELISA kit on day 21.

Nerve injury pain model:

Chronic constriction injury (CCI) of sciatic nerve was used to assess neuropathic pain according to the method described by Bennett and Xie's [21]. The rats were anesthetized using sodium pentobarbital via intraperitoneal (i.p.) administration. Neuropathic pain was thereafter induced by chronic constriction (CCI) of sciatic nerve using a chromic suture. The suture was tightly tied around the sciatic nerve located in

the right hand paw side making a diameter of approximately 0.33 to 0.50mm.

Hot plate latency test:

This procedure was carried out in accordance with the technique used by Eddy and Leimbach [22] as modified subsequently in another study by Gupta et al. [23]. Baseline values for hot plate latency test were obtained prior to surgery. Thermal hyperalgesia was assessed by placing animals on a hot plate (maintained at 55^0c) on the 3rd, 7th, 14th and 21st day after partial sciatic nerve ligation. The latency of first sign of jumping off or paw licking by the animals from the hot plate to avoid thermal pain was taken as an index of pain threshold. A cut off time of 30 secs was maintained. At no time was an animal allowed to stay on the hot plate for more than 30 secs to avoid tissue damage. The mean of the latencies of the animals on the hot plate was determined.

Determination of biochemical parameters:

At the end of the treatment period, the rats were anaesthetized using a mixture of 25% (w/v) urethane and 1% (w/v) alpha chloralose (5ml/kg; i.p., BDH chemicals Ltd., Poole, England). Blood samples were obtained from cannulated carotid artery into heparinized centrifuge tubes. Plasma was extracted by centrifugation at 3000 rpm for 15min. Plasma level of tumor necrotic factor (TNF-α) and C-reactive protein (CRP) were determined by using an Enzyme Immunoassay (EIA) kit from Randox laboratory Ltd. Co (Antrim, UK).

Statistical analysis:

All data are expressed as means ± standard error of the mean (SEM) for 5 rats per group. Statistical group analysis was performed with graph pad (Prism 7) statistical software. Test of variance was done using ANOVA, followed by Tukey's multiple comparisons test and Bonferroni's multiple comparisons test. Statistically significant differences were accepted at $p < 0.05$. Ethical Approval Protocol number: EKSU/A67/2018/02/009

RESULTS:

Hot plate latency Test:

Thermal threshold of ipsilateral hind paw of animals across the groups were shown in table 1. Group VI and VII demonstrated significant increase in pain threshold when compared with group I in which other groups maintained close range values at the baseline, after which the surgeries were performed on groups II through to group VII. On day 3 post-surgery, the animals in group I demonstrated a significant pain threshold difference when compared with all other groups. Conversely, groups V, VI and VII demonstrated significant difference in pain threshold when compared with group II. On day 7, group VII demonstrated a slight significant difference in pain threshold when compared with group I, likewise, groups III, V, and VII also demonstrated significant difference in pain

threshold when compared with group II. Furthermore, on day 14, groups V, VI and VII also demonstrated increased significant difference in pain threshold compared with group II. Finally, on day 21, groups VI and VII demonstrated increased significant difference when compared with groups I and II, similarly, group VII demonstrated increased significant difference in pain threshold when compared with groups III.

Tumor Necrotic Factor (TNF-α)

Changes in serum concentration level of TNF-α among the groups was shown in table 2. There was significant increase in change in concentration in groups II and group III when compared with group I, while group VI and group VII showed a significant decrease in change in concentration when compared with group I, II and III. Conversely, group V showed significant decrease in concentration when compared to group II and III.

C - reactive protein (CRP):

Changes in serum CRP concentration across the groups are shown in table 3. There was a significant increase in change in CRP concentration in groups III and IV when compared with group I. The results showed significant decrease in change in CRP concentration in groups VI and VII when compared with groups I, II, III, IV, and V. Similarly, group V showed a significant decrease in change in CRP concentration when compared with groups III and IV.

Table 1: Effect of licorice extract on pain threshold during CCI induced neuropathy in male Wistar rats.

	Pain threshold (Seconds)				
Rat groups	**Base line**	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Control	7.3 ± 0.4	6.5± 0.35	7 ± 0.6	4.6 ± 0.5	3.3 ± 0.2
Control ligated	8.4 ± 0.3	10.9 ± 0.8a	3.8 ± 0.53	4 ± 0.2	2.5 ± 0.2
Imip treated	7 ± 0.7	13.1 ±0.5a	6.7 ± 0.5b	5.1 ± 0.7	3.8 ± 0.2
LDL treated	7.9 ± 0.78	10.9 ± 0.8a	4.5 ±0.45	4.6 ± 0.7	3.6 ± 0.3
HDL treated	8 ± 0.63	13.3 ± 1.7a,b	6.9 ± 0.6b,d	5.1 ± 0.4b	4.4 ± 0.3
LDLp treated	9.6 ± 0.2a,b	14.8 ± 0.6a,b	5.7 ± 0.3	5.7 ± 0.2 b	5.7 ± 0.3a,b
HDLp treated	1.7 ± 0.7a,b,c,d,e	16.3 ± 0.6a,b,c	8.5 ± 0.7a,b,c	6.8 ± 0.3 b	7 ± 0.1a,b,c

Data expressed are means ± SEM, n = 5.

Data were analysed by two-way ANOVA followed by Turkey's multiple post hoc test.

a,b,c,d,e, $p < 0.05$ vs Control, Control ligated, Imipramine treated, LDL treated and HDL treated respectively.

Key: Imipramine (Imip); Low dose licorice (LDL); High dose Licorice (HDL); Low does Licorice pre-treated (LDLp); High dose Licorice pre-treated (HDLp)

Table 2: Effect of licorice extract on serum TNF-α during CCI induced neuropathy in male Wistar rats

Rat groups	Serum TNF-α (ng/ml)
	Mean ± SEM
Control	443.3 ± 14.2
Control Ligated	557.7 ± 7.3a
Imipramine Treated	555.3 ± 18.4a
LDL treated	518.2 ± 16.6
HDL treated	401.3 ± 18.6b,c
LDLp treated	329.2 ± 9.6a,b,c
HDLp treated	285.1 ± 11.2a,b.c

Data expressed are means ± SEM, n = 5
Data were analysed by one-way ANOVA followed by Turkey's multiple post hoc test.
a,b,c, p<0.05 vs Control, Control ligated, Imipramine treated.
Key: Imipramine (Imip); Low dose licorice (LDL); High dose Licorice (HDL); Low does Licorice pre-treated (LDLp); High dose Licorice pre-treated (HDLp).

Table 3: Effect of licorice extract on serum CRP during CCI induced neuropathy in male Wistar rats

Rat groups	Serum CRP (ng/ml)
	Mean±SEM
Control	158.7±6.02
Control Ligated	206.6±7.29
Imipramine Treated	254.6±6.94a
LDL treated	255.8±5.96a
HDL treated	182.2±6.85c,d
LDLp treated	122.4±6.57a,b,c,d,e
HDLp treated	107.6±4.86a,b,c,d,e

Data expressed are means ± SEM, n = 5
Data were analysed by one-way ANOVA followed by Turkey's multiple post hoc test.
a,b,c,d,e, p<0.05 vs Control, Control ligated, Imipramine treated, LDL treated and HDL treated.
Key: Imipramine (Imip); Low dose licorice (LDL); High dose Licorice (HDL); Low does Licorice pre-treated (LDLp); High dose Licorice pre-treated (HDLp)

DISCUSSION:

This study investigates the anti-nociceptive effect of aqueous roots of Glycyrrhiza glabra in CCI induced neuropathic pain in male Wistar rats. Our study demonstrated that the aqueous extract increased pain threshold significantly in the licorice pre-treated ameliorative groups with most effect on group pre-treated with high dose of licorice as shown in table 1. At baseline, there was great significant increase in response

in both pre-treated licorice groups compared with all others across the group before the surgery demonstrating the analgesic role of licorice in these groups. Thermal painful stimuli are well known to be selective to centrally but not peripherally acting analgesic drugs [18]. Furthermore, on day 3 after the chronic constriction injury (CCI) of the sciatic nerve, the result of this study demonstrated a generalized increased in pain threshold across the groups except the group that was not subjected to surgery. The higher magnitude of pain threshold noticed in high dose licorice pre-treated group is suggestive of a cumulative effect of sensation lost in the limb and the anti-nociceptive effect of licorice. On day 7, all the animals across every group except group I (non-ligated vehicle) demonstrated the presence of neuropathic pain resulting from CCI procedure performed on them, as their pain thresholds were all reduced compared with that of day 3.

The result of this study also showed that the high dose licorice pre-treated (group VII) demonstrated best pain control over all the days when compared with low dose licorice pre-treated (group VI) and reference drug group (group III). However, ligated vehicle treated demonstrated worst response to pain stimuli. Furthermore, there was downward trend in the pain sensitivity (threshold) across all groups, suggesting the effect of receptor sensitization to pain stimuli over the days.

TNF-α plays a role in the peripheral mediation of neuropathic pain [24]; study by Tonini et al [25] reported that neuropathic pain is associated with massive release of TNF-α in serum, this is evident in this study where the ligated vehicle (group II) had the highest serum concentration of TNF-α as seen in table 2. This study also shows that, both vehicle treated (groups I and II), reference drug treated (group III) and the group that received low dose extract after surgery (group IV) had a significant increased change in TNF-α concentration in the serum compared with the high dose treated group after surgery (group V) and both pre-surgery licorice treated groups (groups VI and VII). The result obtained in this study is similar to the trend of result obtained by Yang et al. [7] that reported anti-inflammatory property of this extract in different models of inflammation as well as its ability to reduce the level of pro-inflammatory cytokines. However, in our current study the findings show that the effect of licorice was dose and duration dependent.

Previous study has shown a correlation between TNF-α production and the concentration of CRP [26]. TNF-α induces a dose-dependent secretion of CRP in hepatocytes [27]. Conversely, elevated CRP levels in atheroma also leads to the induction of TNF-α production by macrophages [28].

CRP is mainly classed as an acute marker of inflammation which exists in two different

isoforms and the levels are known to increase dramatically in response to injury, infection, and inflammation [29]. Our present study demonstrated that the pre-treated licorice groups (group VI and VII) had significantly reduced change in CRP concentration in the serum compared with all other groups. This may suggest that the long duration use of licorice not only plays an anti-inflammatory role but also cause better injury healing of the nerve process as noticed in this study. However, further studies are needed to assess the wound and nerve healing potential of licorice.

In conclusion, anti-nociceptive activity of Glycyrrhiza glabra (licorice) and its effect on TNF-α, and C-reactive protein (CRP) during CCI induced neuropathic pain is dependent on dose and duration.

Conflict of Interest:

The authors declare no financial or other conflicts of interests in the design and interpretation of study results.

REFERENCE:

1. Bouhassira D., Lanteri-Minet M., Attal N., Laurent B., Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. Pain. 2008; 136: 380–7.
2. Attal N., Lanteri-Minet M., Laurent B., Fermanian J., Bouhassira D. The specific disease burden of neuropathic pain: results of a French nationwide survey, Pain. 2011; 152: 2836–2843.
3. Finnerup N.B., Haroutounian S., Kamerman P., Baron R., Bennett D.L., Bouhassira D., Cruccu G., Freeman R., Hansson P., Nurmikko T., Raja S.N., Rice A.S., Serra J., Smith B.H., Treede R.D., JensenT.S.Neuropathic pain: an updated grading system for research and clinical practice, Pain. 2016; 157: 1599–1606
4. Henry J. L. Pathophysiology of chronic pain in a health policy perspective. 2008 pg. 59-66
5. Becerra L., Morris S., Bazes S., Gostic R., Sherman S., Gostic J., Pendse G., Moulton E., Scrivani S., Keith D., Chizh B.,Borsook D. Trigeminal neuropathic pain alters responses in CNS circuits to mechanical (brush) and thermal (cold and heat) stimuli. J Neurosci. 2006; 26:10646–57.
6. Moalem G., Tracey D.J. Immune and inflammatory mechanisms in neuropathic pain. Brain. Res. Rev. 2006; 51:240-264.
7. Yang R., Yuan B., Ma Y., Zhou S., and Liu Y. The anti-inflammatory activity of licorice, a widely used Chinese herb, Pharmaceutical Bio 2017;55(1):5-18.
8. Zhu Z.H., Tao W.W., Li J.P., Guo S., Qian D.W., Shang E.X., Su S.L., Duan J.A. Rapid determination of flavonoids in licorice and comparison of three licorice species. J Sep Sci. 2016; 39:473–482.
9. Li S., Zhu J.H., Cao L.P., Sun Q., Liu H.D., Li W.D., Li J.S., Hang C.H. Growth inhibitory in vitro effects of glycyrrhizic acid in U251 glioblastoma cell line. Neurol Sci. 2014; 35:1115–1120.
10. Long D.R., Mead J., Hendricks J.M., Hardy M.E., VoyichJ.M. Glycyrrhetinic acid inhibits methicillin-resistant Staphylococcus aureus survival and

attenuates virulence gene expression. Antimicrob Agents Chemother. 2013; 57:241–247.
11. Feng Y.C., Wang K.C., Chiang L.C., Shieh D.E., Yen M.H., San C.J. Water extract of licorice had anti-viral activity against human respiratory syncytial virus in human respiratory tract cell lines. J Ethnopharmacol. 2013; 148:466–473.
12. Chandrasekaran C.V., Deepak H.B., Thiyagarajan P., Kathiresan S., SangliG.K., Deepak M., Agarwal A. Dual inhibitory effect of Glycyrrhizaglabra (GutGardTM) on COX and LOX products. Phytomedicine. 2011; 18:278–284.
13. Li J., Lee Y.S., Choi J.S., Sung H.Y., Kim J.K., Lim S.S., Kang Y.H. Roasted licorice extracts dampen high glucose-induced mesangial hyperplasia and matrix deposition through blocking Akt activation and TGF-beta signaling. Phytomedicine. 2010; 17:800–810.
14. Li X.B., He X.J., Liu B., Xu L., Lu C., Zhao H.Y., NiuX.Y., Chen S.L., Lu A.P. Maturation of murine bone marrow-derived dendritic cells induced by Radix Glycyrrhizae polysaccharide. Molecules. 2012; 17:6557–6568.
15. Abe K., Ikeda T., Wake K., Sato T., Sato T., Inoue H. Glycyrrhizin prevents of lipopolysaccharide/d-galactosamine-induced liver injury through down-regulation of matrix metalloproteinase-9 in mice. J Pharm Pharmacol. 2008; 60:91–97.
16. Michel H.E., Tadros M.G., Abdel-Naim A.B., KhalifaA.E. Prepulse inhibition (PPI) disrupting effects of Glycyrrhizaglabra extract in mice: a possible role of monoamines. Neurosci Lett. 2013; 544:110–114.
17. Raikkonen K., SecklJ.R., Heinonen K., Pyhala R., Feldt K., Jones A., PesonenA.K., Phillips D.I., Lahti J., JavenpaaA.L., Eriksson J.G., Matthews K.A., StrandbergT.E., Kajantie E. Maternal prenatal licorice consumption alters hypothalamic-pituitary-adrenocortical axis function in children. Psychoneuroendocrinology. 2010; 35:1587–1593.
18. Bhandage A., Shevkar K., Undale V. Evaluation of Antinociceptive Activity of Roots of Glycyrrhiza Glabra Linn. Journal of Pharmacy Research 2009; 2(5):803-807.
19. Ajagannanavar SL., Battur H., Shamarao S., Sivakumar V., Patil PU., Shanavas P. Effect of aqueous and alcoholic licorice (glycyrrhiza glabra) root extract against streptococcus mutans and lactobacillus acidophilus in comparison to chlorhexidine: an in vitro study. J Int Oral Health 2014; 6:29-34.
20. National Research Council. (2011). Guide for the Care and Use of Laboratory Animals; Korean Edition. Washington, DC: The National Academies Press.
21. Bennett G.J. and Xie, Y.K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain. 1988; 33:87-107
22. Eddy N.B. and Leimbach D. Synthetic analgesic II diathianyldiathienylbutylamines. J. Pharmacol. Expe. Ther. 1953; 107: 385.
23. Gupta M., Mazumder U.K., Kumar R.S., Gomathi P., Rajeshwar Y., KakotiB.B., SelvenV.T. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from Bauhinia racemosa stem bark in

animal models. Journal of Ethnopharmacology. 2005; 98: 267.

24. Leung L., Cahill C.M. TNF-α and neuropathic pain - a review. Journal of Neuroinflammation. 2010; 7:27
25. Tonini G., Santini D., Vincenzi B., Borzomati D., Dicuonzo G., La Cesa A., Onori N., Coppola R. Oxaliplatin may induce cytokine-release syndrome in colorectal cancer patients. J BiolRegulHomeost Agents. 2002; 16:105-109.
26. Sproston N.R. Ashworth J.J. Role of C - reactive protein at Sites of Inflammation and Infection. Front. Immunol. 2018; 9:754. doi: 10.3389/fimmu.2018.00754
27. Calabró P., Willerson J.T., Yeh E.T. Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. Circulation. 2003; 108 (16): 1930–2. doi:10.1161/01.CIR.0000096055. 62724.C5
28. Han K.H., Hong K.H., Park J.H., Ko J., Kang D.H., Choi K.J., Hong M.K., Park S.W., Park S.J. C-reactive protein promotes monocyte chemoattractant protein-1-mediated chemotaxis through upregulating CC chemokine receptor 2 expression in human monocytes. Circulation 2004; 109: 2566–71.
29. Braig D., Nero T.L., Koch H.G., Kaiser B., Wang X., Thiele J.R., Morton C.J., Zeller J., Kiefer J., Potempa L.A., MellettN.A., Miles L.A., Du X.J., Meikle P.J., Huber-Lang M., Stark G.B., Parker M.W., Peter K., Eisenhardt S.U. Transitional changes in the CRP structure lead to the exposure of pro-inflammatory binding sites. Nat Commun. 2017; 8:14188. doi:10.1038/ncomms14188.

EXPOSURE TO ATRAZINE ELICITS MORPHO-PHYSIOLOGICAL AND TESTICULAR DYSFUNTIONS IN ADULT MALE RATS

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Running Title: Atrazine affects morpho-physiology and testicular functions

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ABSTRACT:
Some of the environmental toxicants acting as endocrine disruptors have been associated with health hazards in human and wildlife by modulating hormonal actions. The widely used herbicide; atrazine (ATZ) is a potent endocrine and testicular disruptor. However, studies on it remain largely inconclusive especially whether the effects are reversible or permanent. We therefore designed this study to evaluate the histological and hormonal changes associated with differential ATZ exposure. Twenty (20) adult male Wistar rats were divided into 4 groups (5 rats per group) control and three experimental groups. Control received the vehicle; the 3 groups received ATZ, 38.5, 77.0 and 154.0 mg/kg bw/day for 30 days respectively. The effects of Atrazine were assessed through histopathological observation, spermatozoa quality examination and reproductive hormone levels. Results showed that irrespective of the ATZ dose, there was significant decrease in weight, severe pathological changes in testicular tissue, decrease in the quality of semen and altered luteinizing hormone (LH) of the rats. Taken together, our findings showed that ATZ exposure could lead to poor reproductive ability in male Wistar rats.
Keywords: Atrazine, endocrine disruptor, testes, reproductive toxicity, Luteinising hormone

INTRODUCTION:

Globally, there is a growing concern among the scientific community, policy makers as well as general public about the adverse impact on health, in general, and reproductive potential, in particular, of a wide range of chemicals released in the environment as herbicides [1, 2]. Some of these environmental toxicants strongly act as endocrine disruptors with the potential to alter hormonal action within the body. One of such chemicals is Atrazine (ATZ). Most of these toxicants have been banned in some developed countries for agricultural and household purposes due to continuous revelation of their side effects [2, 3]. They are, however, still being used in developing countries because of their low cost, easy availability as well as the absence of safer and cheaper alternatives. These chemicals are entering into animal and human body through various means and their chronic exposure is associated with serious detrimental effects on the body system [4, 5].

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), an active component found in herbicides commonly used in agriculture worldwide, has been considered a potent endocrine disruptor and cause adverse effects on the male genital system [5, 6, 7]. The importance of endocrine disruptors in males reflects the growing body of evidence highlighting the close relationship between these compounds and the increase in male reproductive disturbances of many vertebrates, including humans [5]. Reproductive problems linked to atrazine exposure include de-masculinization and feminization in fish, amphibians and reptiles [5], loss of ovarian germ cells [6], testicular degeneration in amphibians [8], structural disruption of testes in fish [9], crocodilians [10], birds [11], and rodents [12], reduction in sperm count and motility [13], weight reduction of the rat prostate and seminal vesicle [14], as well as delayed sexual maturation [15]. These reproductive defects have been associated with disrupted hormonal activity [16,17] and oxidative stress [18].

One of the several important unanswered questions is whether the effects of ATZ in the testis are primary or secondary to changes in relation to other segments of the male reproductive tract. More so, considering that ATZ [15] continues to be broadly used in large scale in agriculture across the world [4, 19], is easily disseminated in the environment, and causes adverse effects on male reproduction by acting as an endocrine disruptive agent [20], it is paramount to further investigate tissue and hormonal alterations induced by this herbicide.

Therefore, this study was designed to investigate effects of different doses of atrazine on morpho-physiological and testicular functions in adult male Wistar rats.

METHODOLOGY:

Atrazine (ATZ) in the commercial product cotrazine 80 WP (purity, 80% wet table powder) was obtained from Nantong Foreign Trade Meheco Corporation (China). All other reagents were analytical grade chemicals.

A total of 20 adult male Wistar rats, weighing between 180g to 200g and acquired from the Animal House of the Department of Biochemistry, University of Ilorin, Nigeria, were used in this study. The animals were maintained at 12 hours light:12 hours dark cycle and were supplied with water and feed ad libitum. After one-week acclimatization, the rats were randomly assigned into four groups comprising of the control group (A) and 3 treatment groups (B – D) with 5 rats per group. The treated groups received ATZ comprising of the following dosage [5]: Group B: 38.5 mg/kg bw/day (low dose); Group C: 77.0 mg/kg bw/day (average dose); Group D: 154.0 mg/kg bw/day (high dose). ATZ was dissolved in the vehicle (distilled water, 1.0 mL kg-1) and orally administered to the treated groups at the dosage stated. The control group was administered the vehicle (1.0 mL kg-1 bw). The treatments lasted for 30 days. At the end of the experiment, the rats were humanely euthanized and their testes and epididymis were carefully dissected out and weighed. Blood was collected from the apex of the heart by cardiac puncture and stored in heparinized bottles. The testis was harvested and fixed for histological analysis.

Experimental procedures involving the animals and their care were conducted in conformity with International, National and Institutional guidelines for the care of laboratory animals in Biomedical Research and use of Laboratory Animals in Biomedical Research as promulgated by the University of Ilorin Ethical Review Committee.

Histo-pathological evaluation of testis: The testes were fixed in bouin's [21]. After 24 hours, the testes were washed and maintained in 70% ethanol. Samples were then embedded in paraffin and Sectioned with rotary microtome. The tissue sections of the testes were then stained with hematoxylin and eosin (H&E). The histological slides were viewed under light microscope and the photomicrographs of the desired sections were made at different magnifications with LCD camera of the microscope for further observations.

Assay of hormones: The plasma obtained from the blood was used for assay of Luteinizing hormone (LH) and Follicle stimulating hormone (FSH). These were done using the appropriate ELISA kits, according to the manufacturer's protocol.

Semen analysis: After the right caudal epididymis was excised, sperm was obtained

using a modification of the method previously described [22]. The testis from each rat were carefully exposed and removed, they were trimmed free of the epididymis and adjoining tissues. From each separated epididymis, the caudal part was removed and placed in a beaker containing 1.0 ml of normal saline solution. Each section was quickly cut off with scissors and left for a few minutes to liberate its spermatozoa into the saline solution. Sperm motility, concentration and progressive motility were determined. Semen drops were placed on the slide with two drops of normal saline. The slide was covered with a cover slip and examined under microscope using X40 objective for sperm motility. Sperm count was done under the microscope using improved heamocytometer.

Sperm morphology: The sperm cells were evaluated with the aid of light microscope at X100 magnification. Caudal sperm cells were taken from the original dilution for motility and diluted 1:20 with 10% neutral formalin. Five hundred sperm cells from the sample were scored for morphological abnormalities [23]. In wet preparation using phase contrast optics, spermatozoa were categorized. In this study a spermatozoa was considered abnormal morphologically if it had one or more tail, rudimentary tail, round head and detached head, neck and middle piece defects.

Statistical analysis: The data were analyzed by one-way analysis of variance (ANOVA), followed by Bonferroni post hoc test using Graph pad prism version 5.0 software. Results were presented as mean ± standard error mean (SEM). Values of $p \leq 0.05$ were considered to be statistically significant.

RESULTS:

The body weights were significantly reduced ($p<0.05$) in all the treated groups compared with the control group (Table 1). Sperm count was significantly reduced in ATZ – exposed rats irrespective of the dosage, although it was more prominent in the high ATZ-treated groups. Percentage sperm motility, morphology and life-death ratio were significantly reducedin the treated groups as well, compared with the control group (Table 2).No pathological changes were detected in the testes of rats in the control group. The testes of the low dose ATZ-treated rats showed mild pathological lesions represented by the depletion of the nuclei, accumulation of fluid in the cells and cellular degeneration. For the rats exposed to the average dose of ATZ, their testes displayed moderate pathological changes represented by the destruction and degeneration of the connective layers of the leydig cells, the damage to the seminiferous tubule was moderate, spermatogenesis was still present, but the number of cells decreased per unit of area compared with the control group.

For the rats exposed to the high dose of ATZ, their testes displayed severe pathological changes, represented by tubular and cellular degeneration of the seminiferous tubule, a disruption of normal spermatogenic cell organization with visible holes among the cells inside the tubules, and the total number of germ cells inside the tubules significantly decreased and the spermatocytes were connected to the lumen indicating cell disorganization (Figure 1).

There was a significant decrease in the level of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the treated groups compared with the control (Table 3).

Likewise the LH/FSH ratios were significantly reduced in the treated groups compared with the control group (Table 3).

Table 1: Effects of Atrazine exposure on body weight in adult male rats

	Control	Experimental groups		
	A	B	C	D
Initial weight (g)	219.3 ± 1.5	222.0± 3.2	219.5 ± 1.4	218.8 ± 4.2
Final weight (g)	269.1 ± 4.0	215.2 ±7.7*	182.0 ± 5.9*	163.0 ± 8.2*
Weight change (g)	49.8± 1.7	-6.8± 2.0*	-37.5± 2.4*	-55.8± 3.2*

Data are expressed as mean ±S.E.M. (n=5). Data were analysed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.05 vs. A).

Table 2: Effects of Atrazine exposure on sperm parameters in adult male rats

	A	B	C	D
	Control	Experimental groups		
Sperm count (x10^6/ml)	89.20±2.20	67.83±0.70*	58.90±1.89*	53.70±1.13*
Sperm motility (%)	92.50±2.80	82.63±3.50	61.23±1.71*	50.34±2.34*
Sperm morphology (%)	93.20±3.34	78.26±2.45*	59.42±1.49*	68.19±3.45*
Sperm life/death ratio (%)	81.50±2.81*	65.05±3.56*	58.64±3.73*	47.39±4.47*

Data are expressed as mean ±S.E.M. (n=5). Data were analysed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.05 vs. A).

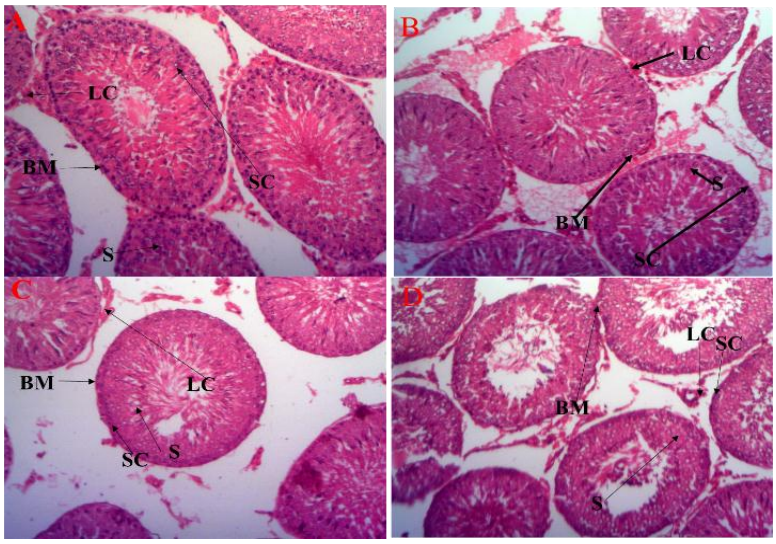

Figure 1. Histology of testicular tissue showing the effect of Atrazine exposure on the testis of adult male rats. The testes of the low dose ATZ-treated rats showed mild pathological lesions represented by the depletion of the nuclei, accumulation of fluid in the cells and cellular degeneration. For the rats exposed to the average dose of ATZ, their testes displayed moderate pathological changes represented by the destruction and degeneration of the connective layers of the leydig cells, the damage to the seminiferous tubule was moderate, spermatogenesis was still present, but the number of cells decreased per unit of area compared with the control group. For the rats exposed to the high dose of ATZ, their testes displayed severe pathological changes, represented by tubular and cellular degeneration of the seminiferous tubule, a disruption of normal spermatogenic cell organization with visible holes among the cells inside the tubules, and the total number of germ cells inside the tubules decreased dramatically and the spermatocytes were connected to the lumen indicating cell disorganization. (H&E paraffin stain; ×40). BM (Basement membrane), LC (Ledig cell), SC (spermatogenic cell), S (seminiferous tubule).

Table 3: Effects of Atrazine exposure on luteinizing (LH) hormone and follicle stimulating hormone (FSH) in adult male rats

	A	B	C	D
	Control	Experimental groups		
FSH (ng/ml)	9.05±1.22	6.01±0.93*	5.95±0.63*	5.39±0.75*
LH (ng/ml)	61.90±4.35	42.61±3.23*	43.04±3.01*	37.14±4.34*
FSH/LH ratio	0.156±0.002	0.140±0.001*	0.138±0.001*	0.138±0.001*

Data are expressed as mean ±S.E.M. (n=5). Data were analysed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.05 vs. A).

DISCUSSION:

The current study investigated the effects of different doses of ATZ on sperm count, motility, morphology and circulating gonadotropic hormones in male Wistar rats. Importantly, it shows the effects of ATZ exposure even in the

smallest dose used in the present study. Our results show noticeable histopathological changes and reduced sperm viability characteristics in the three experimental groups compared to the control group.

We observed a significant reduction in body weight following exposure to ATZ in all the treated groups when compared with control group.This is in line with previous reports that ATZ exposure leads to a reduction in rat body weight [24, 25]. Loss of body weight has been associated with reduction in food intake after exposure to ATZ [26]. We cannot rule out this possibility and this happens to be one of the limitations of this study as the food intake was not monitored. However, other studies have shown that irrespective of the weight change, ATZ has direct effect on the testis and androgen biosynthesis, a factor that can also interfere with the body mass [26, 27]. There was significant reduction in LH and FSH levels in the treated rats compared with the control. This is in consonance with a previous report [27]. This of course will affect the normal reproductive functions. However, the effect of ATZ on circulating gonadotropic hormones in the present study is not dose dependent. In the semen analysis, we observed significant reduction in sperm count, percentage sperm motility, morphology and life-death ratio in the treated groups compared with the control group. The observed alterations in the sperm characteristics are in line with previous reports [8, 15]. Furthermore, histopathological examination shows that high doses of ATZ could influence the seminiferous epithelium. For rats administered average and high doses of ATZ the arrangement of cells was irregular and disordered, and intracellular connections, e.g. gap junctions, were not compact, which indicated that ATZ could pass blood-testis barrier and disturb the junction between Sertoli cells and germ cells. Thus it can be suggested that following exposure to ATZ, leydig cells would degenerate. Thus disrupts testicular function.

CONCLUSION:

The present study demonstrates that ATZ, irrespective of the dose causes morpho-physiological and testicular dysfunctions with correspondent reduction in sperm quality and circulating gonadotropic hormones.

REFERENCE:

1. Pocar P, Brevini TA, Fischer B, Gandolfi F. The impact of endocrine disruptors on oocyte competence. Reproduction 2003; 125:313–25.
2. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocrinol Rev. 2009; 30:293–341.
3. Bethsass J, Colangelo A. European Union bans atrazine, while the United States negotiates continued use. Int

Journal ofOccup Environ Health 2006; 12:260-267.

4. Murray TJ, Lea RG, Abramovich DR, Abramovich DR, Haites NE, Fowler PA. Endocrine disrupting chemicals: effects on human male reproductive health.EarlyPregnancy2001; 5:80–112.
5. Hayes TB, Khoury V, Narayan A, Nazir M, Park A, Brown T, Adame L, Chan E, Buchholz D, Stueve T, Gallipeau S. Atrazine induces complete feminization and chemical castration in male African clawed frogs (Xenopuslaevis). Proceedings of the National Academy of Sciences of the United States of America 2010; 107:4612-7.
6. Song Y, Jia ZC, Chen JY, Hu JX1, Zhang LS. Toxic effects of atrazine on reproductive system of male. Biomed Environ Sci. 2014; 9:281-28.
7. Potter SJ, Kumar DL, DeFalco T. Origin and Differentiation of Androgen-Producing Cells in Gonads.Results and problems in cell differentiation. Results Probl Cell Differ. 2016; 58:101-34.
8. Wirbisky SE, Weber GE, Sepulveda MS, Lin TL, Jannasch AS, Freeman JL. An embryonic atrazine exposure results in reproductive dysfunction in adult zebrafish and morphological alterations in their offspring. Scientific reports 2016; 6:21337-16.
9. Sai L, Dong Z, Li L, Guo Q, Jia Q, Xie L, Bo C, Liu Y, Qu B, Li X, Shao H, Ng JC, PengC.Gene expression profiles in testis of developing male Xenopuslaevis damaged by chronic exposure of atrazine. Chemosphere 2016; 159:145-52.
10. Spano L, Tyler CR, Aerle V, Devos P, Mandiki SN, Silvestre F, Thomé JP, Kestemont P. Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (Carassiusauratus). Aquatic toxicology 2004; 66:369-79.
11. Rey F, Gonzalez M, Zayas MA, Stoker C, Durando M, Luque EH, Muñoz-de-Toro M. Prenatal exposure to pesticides disrupts testicular histoarchitecture and alters testosterone levels in male Caiman latirostris. General and comparative endocrinology 2009; 162:286-92.
12. Friedmann AS. Atrazine inhibition of testosterone production in rat males following peripubertal exposure. Reproductive toxicol 2002; 16:275-9.
13. Abarikwu SO, Adesiyan AC, Oyeloja TO, Oyeyemi MO, Farombi EO. Changes in sperm characteristics and induction of oxidative stress in the testis and epididymis of experimental rats by a herbicide, atrazine. Archives of environmental contamination and toxicology 2010; 58:874-82.
14. Stoker TE, Laws SC, Guidici DL, Cooper RL. The effect of atrazine on puberty in male wistar rats: an evaluation in the protocol for the assessment of pubertal development and thyroid function. Toxicological sciences: an official journal of the Society of Toxicology 2000; 58:50-9.
15. ForadorCD, Zimmerman AD, Hinds LR, Zuloaga KL, Breckenridge CB, Handa RJ. Atrazine inhibits pulsatile gonadotropin-releasing hormone (GnRH) release without altering GnRH messenger RNA or protein levels in the female rat. BiolReprod. 2013; 88:1–7.
16. Rampogu S, Vardhani N, SSNP Ekke, Gajula RG. Dubious action of atrazine as an endocrine disrupting agent. An In Silico approach. J Cancer Clin Trials 2017; 2:133-138.

17. Yuan B, Liang S, Jin YX, Zhang MJ, Zhang JB, Kim NH. Toxic effects of atrazine on porcine oocytes and possible mechanisms of action. PLoS ONE 2017; 12(6): e0179861.
18. Breckenridge CB, Sawhney CP, Tisdel MO, Simpkins JW, Yi KD, C.D. Foradori CD, Handa RJ. Effect of Age, Duration of Exposure, and Dose of Atrazine on Sexual Maturation and the Luteinizing Hormone Surge in the Female Sprague-Dawley Rat. Birth defects research Part B, Developmental and reproductive toxicology 2015; 104:204-17.
19. Pogrmic K, Fa S, Dakic V, Kaisarevic S, Kovacevic R. Atrazine oral exposure of peripubertal male rats downregulatessteroidogenesis gene expression in Leydig cells. Toxicological sciences: an official journal of the Society of Toxicology 2009; 111:189-97.
20. Tillitt DE, Papoulias DM, Whyte JJ, Richter CA. Atrazine reduces reproduction in fathead min now (Pimephalespromelas). Aquatic toxicology 2010; 99:149-59.
21. Oyewopo AO, Olaniyi SK, Oyewopo CI, Jimoh AT. Radiofrequency electromagnetic radiation from cell phone causes defective testicular function in male Wistar rats. Andrologia 2017; 49.
22. Yokoi K, Uthus EO, Nielsen FH. Nickel deficiency diminishes sperm quantity and movement in rats. Biol Trace Elem Res. 2003; 93:141-53.
23. Atessahin A, Karahan I, Yılmaz S, Ceribasi AO, Karaoglu A. Effects of Lycopene on cisplatin and gentamicin induced oxidative stres in blood and liver in rats. Firat University Medical Journal of Health Sciences 2006; 20:39-43.
24. Victor-Costa AB, Bandeira SM, Oliveira AG, Mahecha GA, Oliveira CA. Changes in testicular morphology and steroidogenesis in adult rats exposed to Atrazine. Reproductive toxicology 2010; 29:323-31.
25. Jin Y, Wang L, Fu Z. Oral exposure to atrazine modulates hormone synthesis and the transcription of steroidogenic genes in male peripubertal mice. General and comparative endocrinology 2013; 184:120-7.
26. Stoker TE, Guidici DL, Laws SC, Cooper RL. The effects of atrazinemetabolites on puberty and thyroid function in the male Wistar rat. Toxicologicalsciences: an official journal of the Society of Toxicology 2002; 67:198-206.
27. Foradori CD, Hinds LR, Hanneman WH, Handa RJ. Effects of atrazine and its withdrawal on gonadotropin-releasing hormone neuroendocrinefunction in the adult female Wistar rat. BiolReprod. 2009; 81:1099–1105.

PERCEIVED VERSUS ACTUAL RISK OF DIABETES MELLITUS AMONG UNDERGRADUATES IN OSOGBO, NIGERIA

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ABSTRACT:
This study determined actual and perceived risk of Diabetes Mellitus (DM) among undergraduates in Osogbo in Southwestern Nigeria. It was a descriptive cross sectional explorative study conducted among 250 newly admitted undergraduates of Osun state University selected using multi-staged sampling method. Data on perceived and actual risk to DM were collected using pre tested self-administered semi-structured questionnaire. Data was analyzed using the SPSS software. Mean age of respondents was 21.0± 2.4 years. Of the 250 students 230(92.0%) have heard about DM, 37(14.8%) were overweight and 9(3.6%) were obese. Only 11(4.4%) felt they were at risk of DM, 237(94.8%) said they were not at risk, 225(90.0%) said they can never have DM. Based on the six selected risk factors, only 64(25.6%) were at no risk, 137(54.8%) had single while 49(19.6%) were at multiple risk of DM. Of the 237 who claimed or perceived they were not at risk, only 64 (27.0%) were actually at no risk. A statistically significant relationship was found between actual DM risk and having heard about DM ($p<0.05$). Having heard about DM was the major predictor of congruent actual and perceived risk among studied respondents. It was concluded that incongruence between perceived and actual risk to DM exists among University undergraduates studied, an indication to step up awareness programmes about DM.

Keywords: Diabetes, Perceived and actual risk, western lifestyles, undergraduates.

INTRODUCTION:

The reported epidemiological transition from infectious to non-communicable diseases (NCDs), and the growing prevalence of these chronic disabling diseases have portrayed Diabetes Mellitus (DM) as a significant problem of public health importance. As at the end of 2013, about 382m people worldwide and estimated 20m sub Saharan Africans had DM, and this figure is expected to rise to 592m and 41.4m respectively by 2035 [1]. Majority of those affected are in the low income developing nations [2], where it mainly affects the young and the economically productive age groups [3]. Nigeria has the highest number of people with DM with an estimated 3.9 million

people [4]. The disease is characterized by chronic hyperglycemia and impaired metabolism related to carbohydrates, lipids, and proteins caused primarily by insufficient secretion of insulin.

Adoption of lifestyles such as cigarette smoking, alcoholism and eating junk foods, coupled with sedentary lifestyles may have increased the risk of youths developing DM among other non-communicable diseases through behavioural means. The rise of DM among young adults has substantially increased over the past ten years in Nigeria, especially as the rise of obesity continues to reach new heights, and as youths grow into adulthood and older [5-7].

University students generally exhibit poor risk perception to DM, and this disease is of less concern to them for now [8[.The Health Belief Model hinging health behaviour on several social factors and for at-risk persons to modify their intention to perform the behavior fits into the issue of risk to DM [9].

Since perceived risk is a correlate of knowledge, attitude and the potential efforts a client would take at going for screening and curtailing an ongoing health problems [10], it is important to determine perceived risk among these vulnerable group in order to inform policy and programmatic efforts and decisions.

The objective of this study was to determine and compare actual and perceived risk of DM among undergraduates in Osogbo in Southwestern Nigeria

METHODOLOGY:

Osogbo is the capital of Osun state in Southwestern Nigeria. The State University runs a multi campus system with the main campus and 3 of the 7 faculties in Osogbo. The prevalence of DM among youths either in Osogbo or the entire State was not known as a result of poor surveillance data. Newly admitted students are expected to pass through basic health screening at the University health services.

This was a descriptive cross sectional study. The study population consisted of newly admitted undergraduates who came for pre-admission screening exercise at the University Health Center. Only registered students of the University were recruited into the study.

The sample size was estimated using the Leslie Fischer's formular for calculation of sample size for population less than 10,000 [11]. The perceived risk prevalence of 0.5 was used. Although a sample size of 234 was obtained, it was increased to 250 to account for attrition.

Multi stage sampling method was used to select the students. In stage I, two out of 3 faculties at the main campus were selected using simple random sampling employing

simple balloting. In stage 2, three departments per faculty were randomly selected. In stage 3 in a department, a class was selected using simple random sampling employing simple balloting. In Stage 4, eligible students were selected in a class using systematic sampling of one in 3 students after obtaining a sample frame (list of students) who were present on that day as the students sat in class preparing for a lecture; and this continued until allocated questionnaires were exhausted.

A pretested semi-structured self-administered questionnaire was used in data collection. This was coordinated by two trained research assistants. Two nurses also administered a checklist used in collecting anthropometric measurements from the respondents.

With the initial zero calibration of the weighing scale, the weight was taken in kilograms when the respondent was standing upright and the 2 arms by the side and with the shoes off and no loads on him or her. Height was taken using the standard laboratory stadiometer and a properly calibrated tape rule. The weight and height were used to calculate the Body Mass Index (BMI) for each of the respondents.

The research ethics committee of Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital Osogbo gave consent for this study. Each student filled a written consent form prior to data collection and after the essence of the procedure and research had been communicated to them and consent obtained.

Data was analyzed using the SPSS software version 17.0 after data cleaning. Validity of data entered was ensured through random checks, and double entry. Frequency tables and a chart were generated. Perceived risk was assessed verbally by asking respondents whether or not they are at risk of having DM. Actual risk was assessed by considering six risk factors to DM namely: obesity or overweight (from BMI calculation), smoking, alcohol, no regular exercises (i.e daily brisk walk of about 30 minutes per day), eating junk foods and having a first level relative having DM. While each of these risks scored +1 mark if present, the cumulative (total) score was used to categorize risk into None (Zero risk), single (+1 risk), double (+2 risks) and more than 2(multiple risks). The chi squared test was used in demonstrating relationship between categorical variables of interest while binary logistic regression was used in doing further analysis related to the actual risk of respondents. P values were considered significant at values equal or less than 0.05 for all inferential statistics.

RESULTS:

The mean age of the 250 respondents was 21.0±2.4 years with 154(61.6%) of them existing in the 20-24 year age group,

163(65.2%) were females, only 14(5.6%) were ever married, 144 (57.6%) grew up in the urban city and 82 (32.8%) in the semi urban locations (Table 1).

Among the respondents 92.0% (230/250) have heard about DM, 11.2% (28/250) of them could give correct description or definition of DM. Only 4.4% (11/250) of total respondents (which is equivalent to 4.8% (11/230) of those who have heard about DM), said they were at risk of having DM (perceived risk); while 94.8% (237/250) of total respondents said they were not at risk (Table 2). Furthermore, 90.0% (225/250) of the respondents said they can never have DM while 15.2% (38/250) felt that their friends were at higher risk than them; 38.8% (97/250) believed that changing lifestyle was responsible for high DM occurrence.

The common risk factors identified in the current study were sedentary lifestyle, eating fat and junk foods, a positive family history of DM and to a lesser extent, smoking and alcohol. The BMI results show that 14.8% (37/250) of respondents were overweight while 3.6% (9/250)) were obese (Table 2).

Going by the pooled or cumulative total score of risk factors, our results showed that 25.6% (64/250) of respondents had no DM risk factors, 54.8% (137/250) had single risk factor, 19.6% (49/250) had multiple risk factors, 18.0% had double while 1.6% had more than double risk factors of DM. Thus, only 27.0% (64/237) of those who claimed or perceived they were not at risk were actually not at risk (actual risk) of DM.

Table 3 showed association between risk categories and some variables of interest. A statistically significant relationship was found between actual DM risk and having heard about DM ($p<0.05$), but no relationship was found with age, gender, perceived risk to DM and BMI status of the respondents ($p>0.05$). While there was no significant difference in actual risk perceived relative to age and gender differences on binary logistic regression, respondents who have heard about DM were four times more likely to have perceived their actual risk of DM compared to those who have not heard about DM (OR 0.25, 95%CI 0.096-0.622 and p- 0.002). Likewise, respondents who perceived themselves to be at risk of DM were 1.7 times more likely to have a congruent actual risk score compared to those who did not perceived themselves to be at risk, though this observation was not statistically significant (OR 1.70, 95%CI 0.482-6.026 and p 0.210).

Table 1: socio-demographic data of respondents

Variables	Number (%)
Age (years):	
15-19	78 (31.2)
20-24	154 (61.6)
25-29	18 (7.2)
Gender:	
Male	87 (34.8)
Female	163 (65.2)
Marital status:	
Single	236 (94.4)
Married	14 (5.6)
Ethnicity:	
Yoruba	206 (82.4)
Ibo	21 (8.4)
Hausa	2 (0.8)
Others	21 (8.4)
Religion:	
Christian	145 (58.0)
Islamic	102 (40.8)
Traditional	1 (0.4)
Others	2 (0.8)
Location where respondents grew up	
Local	24 (9.6)
Semi-urban	82 (32.8)
Urban	144 (57.6)

Table 2: awareness and risk of DM (n=250)

Variables	Number (%)
Have heard about DM	
Yes	230(92.0)
No	20(8.0)
Could give correct definition of DM	
Yes	28(11.2)
No	222(88.8)
I am at risk of having DM	
Yes	11(4.4)
No	237(94.8)
Don't know	2(0.8)
I can never have DM	
Yes	225(90.0)
No	25(10.0)
My friends are at higher risk than me	
Yes	38(15.2)
No	198(79.2)
Don't know	14(5.6)
Changing lifestyle is responsible for high DM occurrence	
Yes	97(38.8)
No	153(61.2)
Common DM risk (multiple responses)	
Alcohol	12(4.8)
Smoking	11(5.6)
No regular exercise	147(57.6)
Junk foods	97(38.8)
First level relative having DM	30(12.0)
BMI categories	
Normal	204(81.6)
Overweight	37(14.8)
Obese	9(3.6)

Table 3: association between risk categories and some variables of interest

Bi-variate analysis

Variables	Actual risk		X^2/F test	P value
	No risk (f / %)	>1 risk (f / %)		
Age (years)				
15-19	22 (28.2)	56(71.8)	2.52	0.866
20-24	40 (26.0)	114(74.0		
25-29	2 (12.5)	16(87.5)		
Gender:				
Male	23(26.4)	64(73.6)	0.532	0.912
Female	41(25.2)	122(74.8)		
Heard about DM				
Yes	53(23.0)	177(77.0)	12.507	0.006
No	11 (55.0)	9(45.0)		
I am at risk of DM (perceived risk)				
Yes	4 (36.4)	7(63.6)	2.825	0.419
No	60(25.3)	179(74.7)		

Binary logistic regression (with 'actual risk')

	Odds Ratio	95% CI		P value
		Lower	Upper	
Age in years ((reference category= <20 years))	1.21	0.665	2.223	0.263
Sex (reference category =female)	1.07	0.590	1.935	0.410
Heard about DM (reference category=no risk)	0.25	0.096	0.622	0.002
I am at risk of DM (reference category=no)	1.70	0.482	6.026	0.210

DISCUSSIONS:

Majority of the respondents have heard about DM, this awareness may not directly translate into good knowledge for the studied population moreover only about one-tenth of them could give a correct description or definition of DM. These awareness figures are higher when compared to other studies done amongst University students [12, 13]. This could be due to the fact that youths or students give full

concentration to their education, and regard DM as future life events that should not be given priority now. To further support this thinking of youths, another study [8] found that University students believed that the issue of DM is presently of less concerns to them and should be reserved for the future.

In our study, only very few (4.4%) perceived themselves to be at risk of DM while majority said they can never have DM, about less than one-fifth however perceived their friends to be at risk. In a study among similar age group, forty-eight percent of participants perceived themselves at minimal risk for developing type II diabetes [14]. In yet another study conducted in 2008, 32% of University students perceived themselves at risk for developing diabetes [8].

The comparable very low figure of risk perception found in our study is an indication to the fact that little would be done among study population to prevent DM, and this portray danger to the development of voluntary DM screening habit desired among youths and the general population. In Nigeria for example, most NGOs had focused on HIV and other infectious diseases. The author is not aware of any non-governmental organization (NGO) doing significant work or enlightenment campaign or screening exercises that could improve knowledge of DM or other Non-communicable diseases among youths.

Common risk factors identified were smoking, alcohol, sedentary lifestyle, positive family history. Few (about 14.8%) were overweight while 3.6% were obese. This pattern supports some other studies [15].About two-fifth of studied respondents attributing DM to adoption of lifestyles, supports another study sharing same belief; and in which three quarter believed lifestyle was associated with diabetes onset [15].

Going by pooled or cumulative total risk factors, up to about three-quarters were actually at risk of DM with about half having at least a single risk factor. This supports another Nigerian study on Diabetic risk factors [14].

Comparing the various category of risk exhibited by our respondents with their 4.4% perceived risk, there is obviously a disparity between extent of perceived risk and actual risk of DM. This supports similar other studies [15, 16] suggesting that the sample population could have underestimated their level of risk. This calls for concerted and sustained efforts of all stakeholders towards improving awareness and in-depth knowledge about DM among University students and youths generally.

These efforts capable of encouraging positive behavioural change, attitude and perception of risk of DM and screening could be organized by the University health services, community health services and Governments among other stakeholders.

CONCLUSION:

Incongruence between perceived and actual risk of DM exists among University undergraduate students. There is a need for improved and sustained public health education targeted at these future leaders and economically productive age group in order to bring about better lifestyles devoid of risk, improved attitude and perception of risk of DM screening, prevention and control.

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Conflict of interest: None

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REFERENCES:

1. International Diabetes Federation. Diabetes Atlas. 6th ed. Brussels: International Diabetes Federation; 2013. Last accessed on 2019 Jan 21. https://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf.
2. Shaw JE, Sicree RA, Zimmet PZ, Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2009;87:4-14
3. Joses MK, Hama BS, Luis GS and Saidou PB. Economic burden of Diabetes in the WHO African region. BMC International health and Human rights, 2009;9:6
4. World Health Assembly. Follow-up to the Political Declaration of the High-level Meeting of the General Assembly on the Prevention and Control of Non-communicable Diseases. Geneva: World Health Organization; 2013.
5. Sabir A, Ohwovoriole A, Isezuo S, Fasanmade O, Abubakar S, Iwuala S. Type 2 diabetes mellitus and its risk factors among the rural Fulanis of Northern Nigeria. Ann Afr Med. 2013 Oct-Dec;12(4):217-22
6. Hurtado-Ortiz, MT, Santos S and Reynosa A Health behaviors and health status of at-risk Latino students for diabetes. American Journal of Health Studies, 2011; 26(4), 196-207.
7. Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS and Koplan JP. The continuing epidemics of obesity and diabetes in the United States. Journal of American Medical Association, 2001; 286, 1195-2000.
8. Dong-Chul S, Torabi MR, Kaigang L, John PM, Woodcox SG and Perera B. Perceived susceptibility to diabetes and attitudes towards preventing diabetes among college students at a large mid-western university. American Journal of Health Studies, 2008; 23, 143-150.
9. Glanz K, Rimer BK and Viswanath K. Health behavior and health education: Theory, research, and practice (4th ed.). San Francisco: Jossey-Bass. 2008
10. Hochbaum G. M. (1958). Public participation in medical screening programs: A socio-psychological study (Public Health Service Publication No. 572). Washington, DC: Government Printing Office.
11. Araoye MO. Research Methodology with Statistics for Health and Social Sciences.

Ilorin, Nigeria: Nathadex Publishers; 2004:117-120

12. Al-Sarayra Land Khalidi R. Awareness and knowledge about diabetes mellitus among students at Al-Balqa' Applied University. Pak. J.Nutr., 2012; 11: 1023-1028.
13. Tawalbeh L and Gharaibeh B. Diabetes Knowledge among University Nursing Students in the North of Jordan, Pakistan J of Nutrition, 2014; 13 (12): 728-734
14. Shodunke, RA. "Sampled University Students' Perceived Risk of Diabetes & Self-reported Diabetes Risk Factors". Theses, Dissertations, and Other Capstone Projects. Paper 316. Minnesota State University, 2014
15. Smith ML, Dickerson JB, Sosa ET, J McKyer EL, Ory MG. College students' perceived disease risk versus actual prevalence rates. Am J Health Behav.2012; 36(1):96-106.
16. Bray M, Shapnick H, Mackie R and Silvers WM. "Difference between perceived and actual cardiovascular disease risk factors in a college aged student population, "International Journal of Exercise Science: Conference Proceedings, 2015; 8 (3):. http://digitalcommons.wku.edu/ijesab/vol8/iss3/28. Accessed 11th January 2016.

CASE REPORT:

CO-EXISTENCE OF GRAVES' DISEASE, BRACHIAL ARTERY PSEUDOANEURYSM AND RADIAL NEUROPATHY IN AN ADOLESCENT GIRL

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ABSTRACT:
Brachial artery aneurysm is rare but could be potentially both life-threatening and limb-threatening. It may lead to peripheral nerve injuries which cause disability and affect everyday function, both domestic and economic. We present a rare case of pre-menarcheal adolescent girl with Graves' disease co-existing with radial neuropathy due to radial nerve compression by a giant brachial artery pseudoaneurysm. The patient was managed surgically with aneurysmal resection and saphenous vein graft interpositioning. Prompt diagnosis and institution of appropriate surgical repair to prevent adverse outcome was imperative.

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Keywords: Adolescence, Graves' disease, pseudoaneurysm, radial neuropathy, wrist drop

Running title: Co-existence of Graves' disease, brachial artery pseudoaneurysm and radial neuropathy

INTRODUCTION:

Hyperthyroidism due to Graves' disease is a common endocrine disorder in adolescence with a female preponderance. Often, it is treated with oral anti-thyroid medications such as carbimazole (or its active metabolite methimazole). The usual side effects of this medication are allergic-skin reactions and agranulocytosis but antineutrophil cytoplasmic antibody (ANCA) vasculitis is a rare side effect [1]. In 2008, Mehindratta et al, [2] reported the first case of ANCA positive vasculitis complicating carbimazole therapy in a 20-year old Indian man. The risk of carbimazole-induced ANCA positive vasculitis is higher in females than males [1].

Pseudoaneurysm is a collection of blood formed as a result of vascular injury and

retained in tissues surrounding the breached vessel. The circulating blood is contained in a cavity surrounded by adjacent tissues, fascia and thrombus but not by normal arterial wall components [3]. Although most pseudoaneurysms result from penetrating injury, minor blunt trauma may cause pseudoaneurysm in individuals who are prone to haemorrhage [4,5]. Besides trauma, infection, polyarteritis nodosa and congenital arterial defects are other known causes of upper extremity pseudoaneurysm [6]. It can present as a new thrill or bruit, pulsatile haematoma, or marked pain or tenderness. A giant pseudoaneurysm can cause peripheral venous oedema and may compress the adjacent neurologic structures, causing sensory and motor deficits [7]. The first symptoms of upper extremity aneurysms can be nerve injury or adjacent nerve compression [8]. The potential associated complications of upper extremity pseudoaneurysm are local pain, local skin ischaemia, rupture and distal embolization with loss of hand and fingers [9,10]. Thus, early diagnosis and prompt surgical repair are essential to avoid complications.

Peripheral nerve injuries are of great economic importance because of their effect on quality of life. The radial nerve is one of the most frequently injured peripheral nerves because of its close anatomical relationship with the humerus. The radial nerve is composed of branches of the C5 through T1 nerve roots and arises from the posterior chord of the brachial plexus within the axilla. Emerging from the axilla and in the arm, the radial nerve gives off three cutaneous sensory branches, namely posterior cutaneous and lower lateral cutaneous nerves of the arm. The third, posterior cutaneous nerve of forearm is responsible for sensation over a large area of the forearm [11].Thereafter the radial nerve runs over the dorsal surface of the humerus in the spiral groove, supplying the extensor muscles of the forearm and fingers. Proximal to the elbow, it bifurcates into a sensory superficial radial nerve (SRN) and a motor posterior interosseous nerve (PIN) branch [11,12].The sensory component (SRN) travels in the forearm over the radial bone, supplying most of the dorsal surface of the hand [11].

As a rule, pattern of clinical involvement is dependent on level of injury [11].A radial nerve injury above the elbow may present with extensor weakness of the elbow, wrist and finger accompanied by sensory disturbance along PIN and SRN distribution [13,14]. Sensory loss over the posterior arm, forearm and the posterior lateral hand and thumb indicate a radial nerve lesion above the spiral groove [15].

Wrist drop is the most common presentation of radial nerve palsy which can occur because of external compression secondary to a giant pseudoaneurysm [7].

CASE REPORT:

We report a case of a 15-year-old pre-menarcheal Nigerian girl who presented with anterior neck swelling of 4 years duration, protrusion of both eyes of 3 years duration, painful swelling of left arm of one week duration. The size of neck swelling progressively increased but it is not associated with pain. There is no family history of thyroid disorder. About one year after onset of neck swelling, the eyeballs were noticed be to more prominent, revealing an increased area of the sclera. No history of eye discharge or redness. One week before presentation the patient developed painful progressive swelling of the left arm and this was followed three days later by loss of sensation and paralysis of the forearm. This prompted parents to seek medical help. One year ago, she was commenced on carbimazole because of the anterior neck swelling. Her appetite has remained good. She is the youngest of 3 surviving children; two younger siblings died from unknown cause. Her family was displaced from Borno State, Northeast Nigeria to internally-displaced-person's camp in Edo State as a result of the Boko Haram terrorist insurgency. She denied any history of trauma to the left arm, even while they were fleeing from the terrorist group who invaded their homes five months earlier. She was in class 2 in Junior Secondary School with good academic performance. The whereabouts of her father is currently unknown. At present, the mother has no source of livelihood. Permission to report this case was obtained from the patient and her mother.

On examination she was afebrile (37.4^0C), not pale or jaundiced and there was no peripheral lymphadenopathy. No vasculitic rash was seen. Anthropometric measurements showed weight 37kg (5^{th} percentile), height 149cm (5^{th} percentile) and body mass index 16.8kg/m^2 (10^{th} percentile). Her sexual maturity rating was Tanner stage 2 [16]. She had bilateral exophthalmos with uniform swelling in the anterior triangle of the neck. The neck swelling moves with deglutition, smooth, non-tender and no bruit. Examination of the left upper limb revealed uniform swelling of the forearm with wrist drop and weakness at the elbow joint. Sensory examination of the radial nerve with pin prick and light touch testing of the posterior arm, forearm, posterior lateral hand and thumb revealed sensory loss in these areas. Figure 1 shows the left radial wrist drop with burns in the left wrist region (burns due to contact with hot pot as a result of sensory loss). She had a resting right radial pulse rate of 120 beats/ minute (tachycardia), regular, full volume. The left radial pulse volume was small. Her blood pressure was elevated (150/100mmHg) with hyperactive precordium. The apex beat was in the 5th left intercostal space (LICS), mid-clavicular line (MCL). The heart sounds were normal. Examination of the other body systems was unremarkable. The peripheral blood film

showed anisocytosis, microcytosis, severe hypochromasia, neutrophilic hyper-segmentation with toxic granulations and thrombocytosis. Complete blood count showed a total white cell count of 22.0 x 103/µl, lymphocytes 8.9%, granulocytes 84.1%, Haematocrit 39.3%, platelets 324 x 103/µl. Thyroid scan showed diffuse enlargement of thyroid lobes, multiple anechoic lesions within both lobes (worse in the right) and mild displacement of vascular structures. Serum urea and electrolyte results showed no abnormality. The electrocardiogram showed sinus tachycardia and the echocardiogram revealed a structurally normal heart. The thyroid function tests (TFT) results are summarized in Table 1.

Table 1: Summary of thyroid function test results

Laboratory parameter	Results	Comments
Total serum T3	10ng/ml	High
Total serum T4	247mmol/L	Very high
TSH	0.05µU/ml	Very low

The initial diagnostic consideration was Graves' disease with cellulitis of the left arm. The patient was commenced on IV Amoxicillin-Clavulinic acid, Genticin and oral Labetalol and Propanolol with continuation of Carbamizole. The paediatric endocrinology team reviewed the patient and considered Multiple Endocrine Neoplasia (MEN) type 2A with metastasis to left arm. The investigations requested for were serum calcium, phosphate, parathyroid hormone (PTH), free para-metaneprine level, magnetic resonance imaging (MRI) of adrenal glands and fine needle aspiration of thyroid gland. These investigations could not be carried out because of financial constraints. The result of serum calcium concentration obtained later was normal. By the 6th day on admission, the swelling and pain in the left arm worsened and ultrasound scan of the left upper limb was requested. The scan revealed a complex mass at the medial aspect of left arm measuring 4.52 x 4.51cm with both solid and cystic components. Cystic components appear vascular but dilated in the proximal part tapering into a tubular structure in the distal part. Within the cystic component, there was a high turbulence with an associated extensive soft tissue swelling in this region. The diagnosis was modified to Graves' disease co-existing with left brachial artery pseudoaneurysm. At this point, the Cardiothoracic surgical team was invited and a Doppler ultrasound was

performed. The Doppler ultrasound findings were as follows: (i) Aneurysm measuring 32 x 44.9 x 40mm located at the proximal part of the left brachial artery with its thinnest wall thickness of 4.70mm; (ii) Organized thrombus cresenteric in shape measuring about 37.2 x 18.4mm with 54.7mm- luminal patency; (iii) Thrombus is at the distal end of the aneurysm and occludes the draining brachial artery; and (iv) Multiple collateral channels are seen which re-enters the draining brachial artery increased peak systolic velocity of brachial artery of about 82.8cm/s. Having confirmed the diagnosis, aneurysmal resection together with salphenous vein graft interpositioning was performed. At surgery, the following were found: (i) Huge false aneurysm of the proximal left brachial artery, filled with clots, necrotic tissue and pus; (ii) aneurysmal sac measuring about 10x 8cm (iv) 16cm gap between both ends of the artery;(iv) surrounding soft tissue oedema and cellulitis (Figure 2).

At surgery, the great saphenous vein of the left leg was harvested up to the knee, reversed and anastomosed to the proximal and distal ends of the brachial artery. Visible and palpable arterial pulsations were noted post repair. She was transfused with two pints of blood (One pint intra- and post-operatively, respectively).The surgical wound healed satisfactorily but sensory loss was still present at discharge. She is being followed up in the outpatient clinics.

Fig. 1: A 15-year-old girl with left wrist drop and burns (arrow) at the left wrist region.

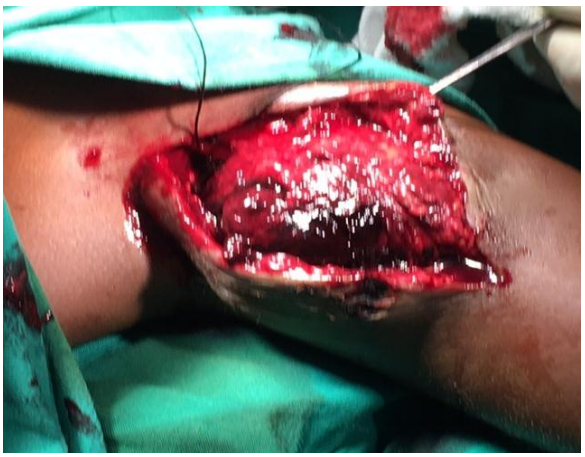

Fig. 2: Exploration of the pseudoaneurysmal sac during surgery

DISCUSSION:

In the index case, the cause of the brachial artery pseudoaneurysm is not clear. Although most pseudoaneurysms result from penetrating injury, minor blunt trauma may cause pseudoaneurysm in individuals who are prone to haemorrhage [4,5]. In our patient, history of trauma was negative. Is it possible that the patient may have sustained a minor blunt trauma without noticing it? Alternatively, could the index case have carbimazole-induced antineutrophil cytoplasmic antibody positive vasculitis which is known to be more frequent in females [1]. Our patient is a female. We could not investigate for antineutrophil cytoplasmic antibody (ANCA) because of inadequate laboratory facilities in our centre.

With regard to the radial nerve injury, the pattern of clinical involvement is dependent on level of injury [11]. The index case had extensor weakness of the elbow, wrist and fingers, suggesting radial nerve injury above the elbow [13,14]. In addition, sensory loss over the posterior arm, forearm and the posterior lateral hand and thumb were present. This distribution of sensory loss indicates a radial nerve lesion above the spiral groove [15].

At surgery, the pseudoaneurysmal sac was found to be located at the proximal part of the left brachial artery, in keeping with the pattern of clinical involvement in our patient. Wrist drop is the most common presentation of radial nerve palsy which can occur because of external compression secondary to a giant pseudoaneurysm [7] as is the case in our patient. Evaluation of the patient at one of her follow-up visits (12 weeks post repair), revealed that the patient was just beginning to regain ability to extend interphalangeal joints. The sensory loss was still present, accounting for the burns she sustained at home from contact with hot pot. These findings suggest a slow regain of function. In most cases of compressive radial neuropathy, the type of injury is a neuropraxia which does not involve damage to the axon [15].

Neuropraxia is classified as a transient conduction block of motor or sensory function without neuronal degeneration [15]. Within this context, radial nerve injury caused by brachial artery pseudoaneurysm is expected to be a neuropraxia. As such, regain of function is expected within a few weeks after surgical repair [15].

The reason for the delay in regain of function in our patient is not clear. The presence of an infected pseudoaneurysmal sac in our patient may have contributed to the slow regain of function by altering the usual nature of the nerve injury and subsequently, the process of regain of function. An alternative explanation is that peripheral nerves are embedded in epineural tissues which are different in each individual [13]. Therefore, apparently identical nerve injury type may be associated with different rates of regain of function.

In conclusion, prompt diagnosis and institution of appropriate surgical repair to prevent adverse outcome was imperative.

REFERENCES:

1. Gunton JE, Stiel J, Clifton-Bligh P, Wilmshurst E, McElduff A. Prevalence of positive antineutrophil cytoplasmic antibody (ANCA) in patients receiving antithyroid medication. Eur J Endocrinol 2000; 142:587-596.
2. Mehindratta MM, Pandey S, Kushwaha RS, Mehndiratta P. Carbimazole induced ANCA positive vasculitis. J Assoc Physicians India 2008; 56:801-803.
3. Hall HA, Minc S, Babrowski T. Peripheral artery aneurysms. Surg Clin N Am 2013; 93(4):911-923.
4. Johnston KW, Rutherford RB, Tilson MD, Shah DM, Hilier L, Stanley JC. Suggested standards for reporting on arterial aneurysms. Subcommittee on Reporting Standards for Arterial Aneurysm, Ad Hoc Committee on Reporting Standards, Society for Vascular Surgery and North American Chapter, International Society for Cardiovascular Surgery. J Vasc Surg 1991; 13:452-458.
5. Kumar S, Agnilotri SK, Kharima SK. Brachial artery pseudoaneurysm following blood donation [Letter]. Transfusion 1995; 35(9):791.
6. Yetkin U, Gurbuz A. Post-traumatic pseudoaneurysm of brachial artery and its surgical treatment. Tex Heart Inst J 2003; 30:293-297.
7. Robbs JV, Naidoo KS. Nerve compression injuries due to traumatic false aneurysm. Ann Surg 1984; 200(1):80-82.
8. Ergungor MF, Kars HZ, Yalin R. Median neuralgia caused by brachial pseudoaneurysm. Neurosurgery 1989; 24(6):924-925.
9. Nugud OO, Hedges AR. Axillary artery pseudoaneurysm. Int J Clin Pract 2001; 5:494-499.
10. Singh A, Kumar V, Tandon S. Brachial artery pseudoaneurysm causing median nerve compression: A case report. J Med Sci Clin Res 2015; 3(5):5742-5745.
11. McNamara B. Clinical anatomy of the radial nerve: Anatomy Primer. ACNR 2003; 3:28-29.
12. Kimbrough DA, Metha K, Wissman RD. Case of the season: Saturday Night Palsy. Semin Roentgenol 2013; 48:108-110.
13. Dŭz B, Solmaz L Civelek E, Onal MB, Pusat S, Daneymez M. Analysis of proximal radial nerve injury in the arm. Neurol India 2010; 58:230-234.
14. Posner MA. Compressive neuropathies of the median and radial nerves at the elbow. Clin Sports Med 1990; 9:343-363.
15. Han BR, Cho YJ, Yang JS, Kang JH, Choi HJ. Clinical features of wrist drop caused by compressive radial neuropathy and its anatomical considerations. J Korean Neurosurg Soc 2014; 55(3):148-151.
16. Nakamoto JM, Franklin SL, Geffner ME. Puberty. In: Kappy MS, Allen DB, Geffner ME (Eds) Pediatric Practice: Endocrinology, McGraw Hill Company Inc. New York, 2010: 257-298.

LETTER TO THE EDITOR:

HIGH PREVALENCE OF MALNUTRITION AMONG GERIATRIC AND PALLIATIVE INPATIENTS: THE IMPORTANCE OF MALNUTRITION SCREENING

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Short Running Title: Malnutrition geriatrics and palliative

Dear Editor,

The prevalence of malnutrition in hospital inpatients is high, ranging from 38% to 83% [1]. Malnutrition is associated with increased morbidity, mortality, prolonged hospitalisation and risk of infection [2, 3]. The use of malnutrition screening tools in hospitals has been shown to facilitate nutrition support to reduce malnutrition and its detrimental effects [4, 5].

In Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital, an 880 bedded tertiary hospital in Brunei, the dietetics service reviews all inpatients with diabetes mellitus, as well as referrals from the primary team. In May 2016, the Malnutrition Universal Screening Tool (MUST) was implemented for inpatients admitted under geriatrics and palliative medicine. This screening initiative aimed to ensure that older inpatients or patients with advanced non-communicable diseases are screened for malnutrition. Patients identified with MUST score greater than two (deemed high risk for malnutrition) were referred to dietitians for assessment to consider nutritional support, such as supplementation, food fortification or enteral feed-

ing. We report findings from a retrospective review of MUST, which illustrate the importance of malnutrition screening in geriatrics and palliative inpatients.

This retrospective review of MUST include inpatients admitted under geriatrics and palliative medicine in RIPAS hospital between May to June 2016. The MUST form includes, height, weight, body mass index (BMI), weight loss, acute disease status and the MUST score. The MUST score is calculated from recorded BMI, weight loss and presence of acute disease with no nutritional intake for 5 days, categorising patients into low, medium or high risk of malnutrition.

Variables were collected from manual forms or electronic records. Data was entered and analysed using Microsoft Excel for Windows. Descriptive statistics were used to characterise the samples. Two-tailed paired t-tests were used to determine differences between malnutrition risks between patient groups.

There were 138 patients, consisting of 67 (48.6%) geriatrics and 71 (51.4%) palliative patients. Screening for malnutrition was performed for 86 (62.3%) of the 138 patients. This consisted of 48.8% (42/86) geriatrics (13 male, 29 female) patients and 51.2% (44/86) palliative (20 male, 24 female) patients. The 52 (37.7%) of the 138 patients excluded were those already seen by dietitians. They include 25 geriatrics and 27 palliative patients.

Mean age of screened patients was 71 ± 14.9 years. Mean weight was 44.9kg ± 12.7kg. Patients with moderate to high risk of malnutrition (MUST score 2 or more) was 45.2% and 72.7% for geriatrics and palliative respectively. There was no statistically significant difference in malnutrition status between genders.

Among the geriatrics patients screened, 11.9% (5/42) had BMI score 1 (BMI 18.5-20 kg/m2) and 31% (13/42) had BMI score 2 (BMI <18.5kg/m2). For palliative patients, 6.8% (3/44) had BMI score 1, and 20.5% (9/44) had BMI score 2. There was a significantly higher proportion of geriatrics patients with BMI score 2 compared to palliative patients (p=0.005).

In terms of weight loss, 7.1% (3/42) of geriatric inpatients had weight loss score 1 (5-10% weight loss in past 6 months), and 2.4% (1/42 patients) had weight loss score 2 (>10% in past 6 months). Among palliative patients, 15.9% (7/44) had weight loss score 1, while 50% (22/44) had weight loss score 2. There was a significantly higher proportion of palliative patients with weight loss score 2 compared to geriatrics inpatients (p=0.000). There were no differences in malnutrition status between genders for geriatrics (p=0.165) or palliative inpatients (p=0.419).

This retrospective review of MUST screening assessments identified a high risk of malnutrition among geriatrics and palliative inpatients. More than half of the geriatrics inpatients had BMI below 20 kg/m2 while approximately two-thirds of palliative inpatients had more than 5% weight loss in the previous six months. In older people, physiological changes to taste and smell, loss of dentition, decreased mobility and reduced eyesight may affect food preparation and oral consumption [6, 7]. Polypharmacy, cognitive decline, social isolation and depression may also contribute to malnutrition [6]. For palliative inpatients, weight loss is associated with a poor prognosis [8]. Weight loss is common among palliative patients due to multiple reasons involving the malignant process and treatment [9].

The European Society for Parenteral and Enteral Nutrition (ESPEN) recommends use of MUST for nutritional screening to identify nutritional issues for inpatients [10]. Early intervention for malnutrition with oral nutritional supplements and dietary counselling of these patients results in increased dietary intake and improved quality of life [4, 5, 10]. Therefore, introduction of malnutrition screening hospital-wide will be beneficial for inpatients requiring attention to improve their nutritional status.

In summary, malnutrition was prevalent among geriatrics and palliative inpatients in our hospital, with predominant issues in low BMI and weight loss respectively. The use of MUST was valuable in identifying patients at risk of malnutrition

REFERENCES:

1. O'Leary-Kelley C, Puntillo, K, Barr J, Stott N, Douglas M. Nutritional adequacy in patients receiving mechanical ventilation who are fed enterally. Am J Crit Care 2005; 14 (3): 222-231.
2. Saka B, Kaya O, Ozturk G, Erten N, Karan M. Malnutrition in the elderly and its relationship with other geriatric syndromes. Clin Nutr 2010; 29(6):745-748. doi: 10.1016/j.clnu.2010.04.006
3. Visvanathan R, Macintosh C, Callary M, Penhall R, Horowitz M, Chapman I. The Nutritional Status of 250 Older Australian Recipients of Domiciliary Care Services and Its Association with Outcomes at 12 Months. J Am Geriatr Soc 2003; 51(7): 1007-1011. doi: 10.1046/j.1365-2389.2003.51317.x
4. van Bokhorst-de van der Schueren M, Guaitoli P, Jansma E, de Vet H. Nutrition screening tools: Does one size fit all? A systematic review of screening tools for the hospital setting. Clin Nutr 2014; 33(1): 39-58.
5. Cereda E, Pedrolli C, Zagami A, Vanotti A, Piffer S, Opizzi A, Rondanelli M, Caccialanza R. Nutritional screening and mortality in newly institutionalised elderly: A comparison between the Geriatric Nutritional Risk Index and the Mini Nutritional Assessment. Clin Nutr 2011;30 (6):793-798.doi: 10.1016/j.clnu.2011.04.006

6. Nieuwenhuizen W, Weenen H, Rigby P, Hetherington M. Older adults and patients in need of nutritional support: Review of current treatment options and factors influencing nutritional intake. Clin Nutr 2010; 29 (2): 160-169.
7. Johansson Y, Bachrach-Lindström M, Carstensen J, Ek A. Malnutrition in a home-living older population: prevalence, incidence and risk factors. A prospective study. J Clinical Nurs 2009; 18 (9): 1354-1364.
8. National Cancer Institute (NCI) Nutrition in Cancer Care–for health professionals, 2015; Available at: http://www.cancer.gov/about-cancer/treatment/side-effects/appetite-loss/nutrition-hp-pdq
9. Hopkinson J, Wright D, Corner J. Exploring the experience of weight loss in people with advanced cancer. J Adv Nurs 2006; 54(3): 304-312.
10. Volkert D, Berner Y, Berry E, Cederholm T, Coti Bertrand P, Milne A, Palmblad J, Schneider S, Sobotka L, Stanga Z; DGEM (German Society for Nutritional Medicine), Lenzen-Grossimlinghaus R, Krys U, Pirlich M, Herbst B, Schütz T, Schröer W, Weinrebe W, Ockenga J, Lochs H; ESPEN (European Society for Parenteral and Enteral Nutrition). ESPEN Guidelines on Enteral Nutrition: Geriatrics. Clin Nutr 2006; 25 (2): 330-360.

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Acknowledgements:
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Book:

Gillett JE. The health of women in Papua New Guinea. PNGIMR: Kristen Press, 1991

Chapter in a Book:

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Published proceedings paper:

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